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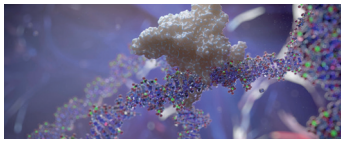

ICP-MS/MS Applications and New Elemental Impurity Methods and Guidelines

In this issue of the Agilent ICP-MS Journal, we continue to celebrate 10 years since the launch of the first triple quadrupole ICP-MS. User-contributed articles from geochronology and life science research feature novel applications that have been enabled by ICP-MS/MS.

We also report on updated guidelines for elemental impurity analysis of pharmaceutical products, a new ASTM method for cannabis, and new EU regulations on the elemental content of fertilizing products.

Figure 1. Agilent 8900 ICP-QQQ coupled to an Agilent 1260 HPLC for speciation analysis and protein mass purity measurements.

In Situ Strontium Isotope Analysis and Rubidium-Strontium Geochronology by LA-ICP-MS/MS

Dr. Alicia Cruz-Uribe, Edward Sturgis Grew Associate Professor of Petrology & Mineralogy, University of Maine, USA

Isotope analysis for geochronology

Geochronology is the study of the age of geological materials such as rocks, minerals, meteorites, sediments, and fossils. Constraining the age of rocks and minerals and how they formed is important for understanding the history and evolution of the Earth. Geochronology is also useful for mineral prospecting surveys and identifying commercial ore bodies. Of the analytical tools employed by geochronologists, radiometric dating using isotope ratios might be considered among the most essential.

Radiometric dating uses the fact that some naturally occurring elements have unstable isotopes that undergo radioactive decay to form a different isotope or element. The rate of decay is constant for a given isotope, so the ratio of the original (parent) isotope to the new (daughter) isotope can be used to calculate how long ago the material was formed. Depending on the element, the rate of decay can be from a few thousand years (for example, ^{14}C has a half-life of ~5730 years) to many billions of years. So, different isotopes are used as appropriate for the age of the material being studied.

Geochronology typically uses isotope systems with long half-lives to examine geological timescales, for example, uranium-lead, uranium-thorium, samarium-neodymium, lutetium-hafnium, and rubidium-strontium.

Rb-Sr dating is based on the radioactive decay (by beta emission) of ^{87}Rb to ^{87}Sr, with a half-life of about 49 billion years (*1*). Older rocks have progressively less of the parent isotope (^{87}Rb) and more of the daughter isotope (^{87}Sr). For a material with uniform age but different proportions of Rb and Sr, the $^{87}Rb/^{86}Sr$ to $^{87}Sr/^{86}Sr$ ratios are plotted on an isochron. The slope of the isochron is related to the age, as shown in Figure 1.

Obtaining the most accurate age requires high precision isotope ratio measurement, so a simultaneous technique such as thermal ionization mass spectrometry (TIMS) or

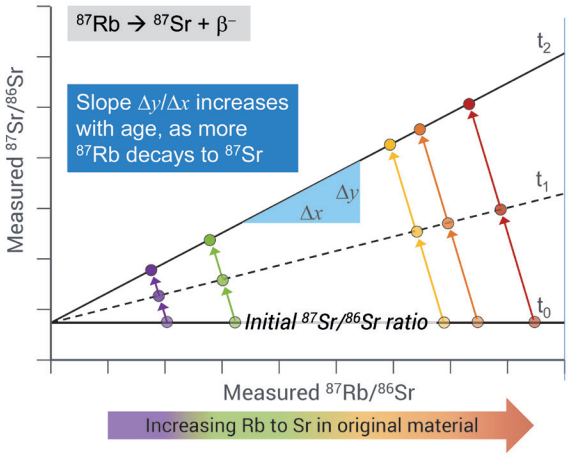

Figure 1. Principle of radiometric dating using Rb-Sr isochron.

multicollector (MC) ICP-MS is typically used. But these techniques cannot discriminate between isotopes of different elements at the same mass (such as ^{87}Rb and ^{87}Sr). So, the elements must first be separated by ion exchange, which is a slow, labor-intensive process.

Neither single quadrupole ICP-MS nor high resolution (HR) sector field ICP-MS can resolve ^{87}Rb from ^{87}Sr on the mass scale, as the mass difference between the overlapping isotopes is too small. The exact mass of ^{87}Rb is 86.90918053 u, and ^{87}Sr is 86.90887750 u, so mass resolution ($M/\Delta M$) of about 287,000 would be required to separate them. This resolution is far beyond the capability of commercial HR-ICP-MS systems, which have a maximum resolution of about 10,000.

Triple quadrupole – or tandem – ICP-MS (ICP-MS/MS) provides a potential solution. The MS/MS configuration allows reactive collision/reaction cell gases to be used, such that ^{87}Rb and ^{87}Sr can be chemically resolved, rather than mass resolved. The method uses the different reaction chemistry of the Rb and Sr ions with the chosen cell gas. Sulfur hexafluoride (SF_6) is a good candidate as it reacts quickly with Sr^+ (to form SrF^+), whereas Rb^+ is nonreactive with SF_6.

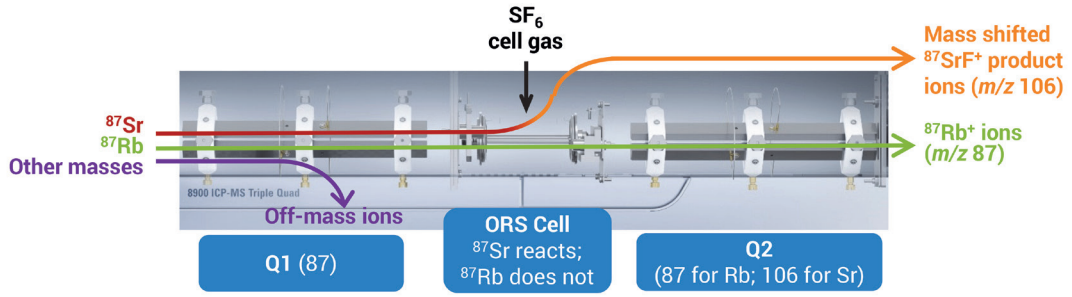

Figure 2. Reaction schematic showing separation of ^{87}Rb from ^{87}Sr using SF_6 cell gas. Reference isotope ^{86}Sr is also measured as SrF^+ (at *m/z* 105).

The reaction schematic for Rb-Sr is shown in Figure 2. Note that ^{87}Sr and ^{86}Sr are both measured as SrF^+ reaction product ions (at *m/z* 106 and 105, respectively). Rb isotopes are measured as Rb^+ (at *m/z* 85 and 87) in the same analysis.

LA-ICP-MS/MS for *in situ* isotope ratio analysis

Laser ablation (LA) ICP-MS of geological thin sections offers enormous benefits for geochemistry, eliminating the requirement to crush and dissolve the sample. LA-ICP-MS also provides information on the distribution of elements within mineral grains. Combining LA with the ICP-MS/MS SF_6 cell gas method enables direct, *in situ* Rb-Sr dating. Quadrupole LA-ICP-MS gives isotope ratio precision about 10x poorer than TIMS or MC-ICP-MS but requires minimal sample preparation and has much shorter analysis time of about 1 minute per analysis.

Figure 3 shows the good agreement between $^{87}Sr/^{86}Sr$ isotope ratios measured using LA-ICP-MS/MS and MC-ICP-MS (*1*) in apatite (calcium phosphate) minerals. The LA-ICP-MS/MS data were measured using a spot size of 50–75 µm, 2–2.5 J/cm² energy, and 12 Hz repetition rate.

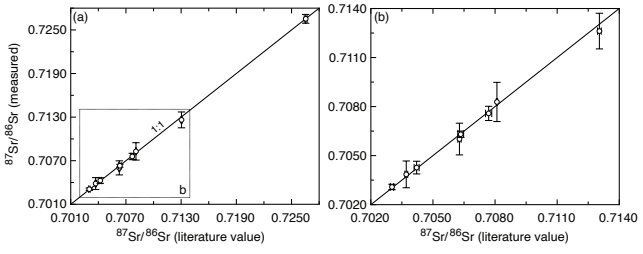

Figure 3. Comparison of $^{87}Sr/^{86}Sr$ isotope ratios by LA-ICP-MS/MS and MC-ICP-MS (*2*). 1:1 line shows that the techniques give consistent Sr ratios. Error bars indicate uncertainty of 2 SD.

LA-ICP-MS/MS was used to measure several well-characterized minerals including LaPosta Biotite.

Figure 4 shows the Rb-Sr isochron obtained using LA-ICP-MS/MS, confirming excellent agreement with the known age of 93.8 +/- 2.5 Ma for the sample (*3*).

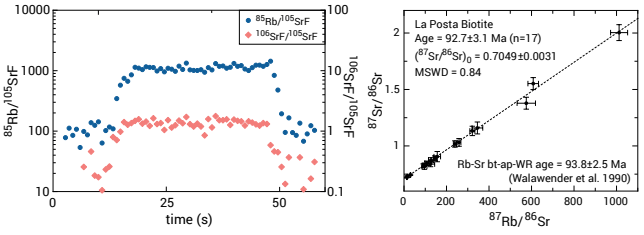

Figure 4. Left: Time resolved signals for isotope ratios measured by LA-ICP-MS/MS. Right: LA-ICP-MS/MS Rb-Sr isochron for LaPosta Biotite. LA parameters were 50 µm spot size at 2.5 J/cm² and 10 Hz.

Conclusion

ICP-MS/MS coupled to laser ablation provides a simple and fast way to perform isotopic analysis without the need for prior chemical separation. *In situ* LA-ICP-MS/MS analysis provides micron-scale resolution and enables dating of a wider range of rock-forming minerals (i.e., micas and feldspars) that record processes in a diverse array of rock types throughout geologic history.

More information

Go to Spectroscopy Webcasts (on24.com) and look for the on-demand webinar on In situ Sr isotope analysis and Rb-Sr geochronology by LA-ICP-MS/MS.

References

1. Villa, I.M., *et al. Geochim. Cosmochim. Acta*, **2015**, 164, 382–385
2. Yang, Y-H., *et al, Chem. Geol.*, **2014**, 385, 35–55
3. Walawender, M., *et al, GSA Memoir*, **1990**, 174, 1–18

Absolute Quantification of Proteins by Capillary LC-ICP-MS/MS for Mass Purity Certification of Standards

Francisco Calderon-Celis, Alicia Jiménez Nosti, Julia Ruiz Allica, Ana Soldado, Laura Cid Barrio, Jorge Ruiz Encinar, Department of Physical and Analytical Chemistry, University of Oviedo, Spain

Protein analysis by ICP-MS/MS

The launch of the Agilent 8800 ICP-QQQ in 2012 brought true tandem mass spectrometry (MS/MS) to the field of inorganic analytical chemistry. MS/MS dramatically improved performance for many ICP-MS applications, including in the life sciences. Researchers soon realized that the technique could enable quantification of biomolecules based on sulfur and phosphorus content.

Existing methods for protein and peptide quantification, such as amino acid analysis, are indirect and labor-intensive. Organic MS techniques, such as electrospray ionization (ESI)-MS/MS provide low detection limits, but ionization and sensitivity are strongly affected by the compound being measured. This makes absolute quantification difficult if species-specific standards are not available. Analysts working in quantitative proteomics would appreciate a simple approach that enables direct, traceable determination of protein purity, without needing compound-specific standards.

ICP-MS uses a high temperature plasma ion source, which gives consistent response for each heteroelement, regardless of the compound the element is contained in. But conventional ICP-MS can only determine proteins if they contain a metal that can be measured at low level by ICP-MS, which limits its applicability. Sulfur is present in the amino acids methionine and cysteine and therefore occurs in almost all eukaryotic proteins. But S (and P) suffer intense spectral overlaps from polyatomic ions formed from N, O, H, and C. These ions arise from the aqueous solvent, air entrainment, and – in the case of LC-ICP-MS – from the organic mobile phase.

Before the introduction of ICP-QQQ, existing ICP-MS instruments – high resolution sector field and single quadrupole with collision/reaction cell – could not resolve the spectral overlaps well enough to enable trace S analysis. ICP-MS was therefore unable to achieve the S detection limits required for protein quantification.

ICP-MS/MS enables reactive cell gases to be used reliably, and trace level S analysis using O_2 cell gas was one of the first ICP-QQQ applications reported (*1*). The method uses O_2 cell gas to move the $^{32}S^+$ ions away from the interferences, forming $^{32}S^{16}O^+$ reaction product ions, which are measured at mass 48. The on-mass interferences (O_2^+, NO^+, NOH^+) do not react with the O_2 cell gas and so remain at mass 32. MS/MS is essential for the analysis, as the additional mass filter (Q1, set to *m/z* 32) prevents any other masses from entering the reaction cell, so potential overlaps on the SO^+ product ions ($^{48}Ca^+$, $^{48}Ti^+$, $^{31}P^{16}O^1H^+$, $^{36}Ar^{12}C^+$, etc.) are eliminated.

When coupled to capillary liquid chromatography (capLC), ICP-MS/MS can achieve femtomole limits of detection for proteins, which is about two orders of magnitude lower than previously possible by ICP-MS.

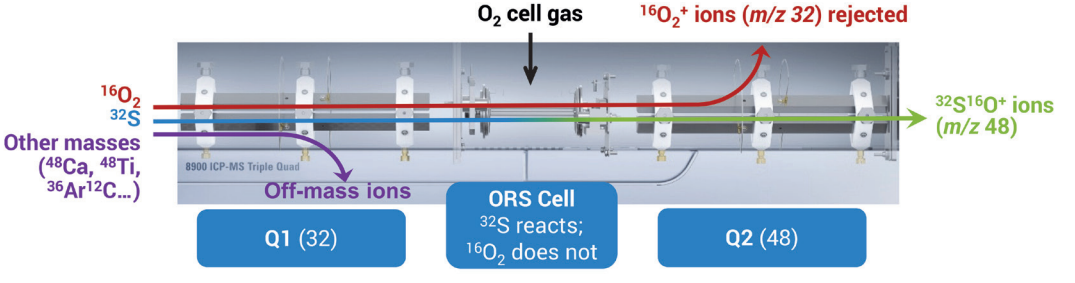

Figure 1. MS/MS mass-shift with O_2 cell gas resolves $^{16}O_2^+$ overlap on $^{32}S^+$. Minor isotopes ^{33}S and ^{34}S measured as SO^+ product ions at *m/z* 49 and 50.

Protein determination by ICP-MS/MS is based on the S signal, which is independent of compound structure, so proteins can be quantified without species-specific standards. ICP-MS/MS can also measure the minor S isotopes, so isotope dilution analysis (IDMS) is possible.

Standardization approaches for protein analysis

In this work (*2*), protein quantification was performed using a capLC-ICP-MS/MS method with a low flow rate, total consumption nebulizer. This is necessary to ensure equal response factors for all compounds, which is required for species-independent quantification. However, the variation in S ionization due to the changing carbon content in the capLC gradient must also be addressed. To compensate for the ionization effect, excess carbon is added to the plasma as CO_2 gas, ensuring the S response is consistent throughout the gradient separation.

Four protein standards, bovine serum albumin (BSA), transferrin, β-casein, and cytochrome C, were analyzed by capLC-ICP-MS/MS to determine protein mass purity. Two standardization approaches were used for quantification:

1. Internal standardization. A known amount of calibrant BOC-L-methionine was added to the samples.
2. External standardization. A certified sulfate standard was injected using flow injection (FI) before the chromatographic separation.

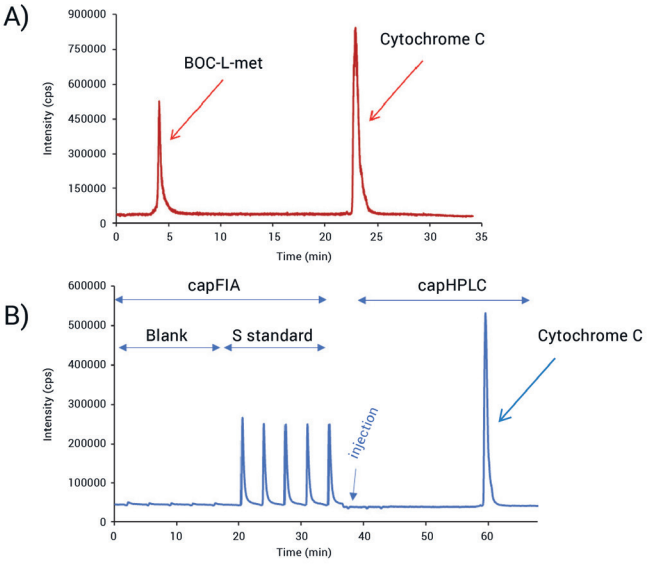

Figure 2. Internal (top) and external (bottom) standardization approaches for cytochrome C protein quantification.

Chromatograms illustrating both standardization approaches are shown in Figure 2. Internal standardization has the benefit that the analysis is completed in one injection. But the calibrant must be selected for compatibility with the capLC method and must be adequately resolved from the target protein. External standardization can use any certified sulfur standard – such as the SO_4^{2-} standard used in this work. Since the external standard is injected using FI, bypassing the column, several replicate injections can be performed before the chromatographic separation, giving more data points for calculation of the precision.

Table 1. ICP-MS/MS results for mass certification of protein standard purity compared to theoretical purity provided by the manufacturer.

	Protein Mass Purity (%)		
Protein	Internal Standard Method	External Standard Method	Theoretical (Manufacturer) Value
BSA	99 ± 2%	97 ± 3%	≥ 98%
Transferrin	95 ± 1%	93 ± 3%	≥ 95%
β-casein	93 ± 6%	94 ± 5%	≥ 98%
Cytochrome C	92 ± 1%	96 ± 4%	≥ 95%

Table 1 shows the purity results obtained using capLC-ICP-MS/MS for all four target proteins. For both standardization methods, the results are in good agreement with the theoretical mass purity of the standards, showing the accuracy of the technique.

Conclusion

CapLC-ICP-MS/MS provides a simple and fast method for accurate, traceable quantification of proteins based on measurement of the sulfur heteroelement. Internal and external standardization approaches both gave precise protein purity results that were in good agreement with the reference purities provided by the manufacturer.

References

1. Fernandez, S. D., *et al, Anal. Chem.*, **2012**, 84, 5851–5857
2. Nosti, A. J., *et al*, Direct and Traceable Mass Purity Certification of Protein Standards using LC-ICP-MS/MS, Agilent publication, 5994-5073EN

Revised Guidelines for Testing Elemental Impurities in Pharmaceutical Products

Ed McCurdy, Agilent Technologies, Inc.

Elemental impurities in drug products

The quality and safety of drug products is controlled by guidelines defined by bodies such as USP (United State Pharmacopeia) and ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use). These guidelines specify maximum levels for contaminants – including elemental impurities – that a patient may be exposed to.

Permitted daily exposure (PDE) limits for elemental impurities are defined in USP<232> and ICH Q3D, which were updated and harmonized in 2017. The harmonized limits, which are risk-based and vary depending on route of exposure, cover a wide range of elements, so labs typically use a multi-element ICP-MS or OES technique.

Revisions to ICH Q3D

After the release of the harmonized guidelines in 2017, the ICH Expert Working Group continued to investigate elemental impurity limits. This led to Q3D Revision 1 (R1) which proposed raising the inhalation PDE limit for cadmium from 2 µg/day to 3 µg/day. Q3D(R1) was recommended for adoption (Step 4) on 22 March 2019.

The ICH working group, addressing a request from industry, was also developing PDEs for elemental impurities in drugs for cutaneous and transcutaneous administration. These limits were defined in Q3D Revision 2 (R2), which also corrected errors in the existing PDEs for Ni, Au, and Ag, as shown in Table 1. Q3D(R2) was adopted by ICH on 26 April 2022 and comes into effect for new products from September 2022. Manufacturers have 36 months after publication to apply the new limits to existing products.

The (R2) limits are all higher than before, so existing Agilent ICP-MS methods will still be applicable for labs working to the new guidelines.

Table 1. Revised limits in ICH Q3D(R2) and USP <232>.

ICH/USP Class, Element	Permitted Daily Exposure (PDE) for different routes of exposure (µg/day)			
	Oral	Parenteral	Inhalational	Cutaneous
Class 1				
Cd - Cadmium	5	2	3 (2)*	20
Pb - Lead	5	5	5	50
As - Arsenic (inorganic)	15	15	2	30
Hg - Mercury (inorganic)	30	3	1	30
Class 2A				
Co - Cobalt	50	5	3	50 (35)***
V - Vanadium	100	10	1	100
Ni - Nickel	200	20	6 (5)**	200 (35)***
Class 2B				
Tl - Thallium	8	8	8	8
Au - Gold	300 (100)**	300 (100)**	3 (1)**	3000
Pd - Palladium	100	10	1	100
Ir - Iridium	100	10	1	100
Os - Osmium	100	10	1	100
Rh - Rhodium	100	10	1	100
Ru - Ruthenium	100	10	1	100
Se - Selenium	150	80	130	800
Ag - Silver	150	15 (10)**	7	150
Pt - Platinum	100	10	1	100
Class 3				
Li - Lithium	550	250	25	2500
Sb - Antimony	1200	90	20	900
Ba - Barium	1400	700	300	7000
Mo - Molybdenum	3000	1500	10	15000
Cu - Copper	3000	300	30	3000
Sn - Tin	6000	600	60	6000
Cr - Chromium	11000	1100	3	11000

Shaded cells indicate where an elemental impurity should be included in the Risk Assessment even if not intentionally added.

* Inhalational PDE for Cd was increased in ICH Q3D(R1), 2019. Original value (in brackets) still applies to USP <232>/<233>

** Some PDEs for Ag, Au, and Ni were increased in ICH Q3D(R2), 2022. Original values (in brackets) still apply to USP <232>/<233>

*** Cutaneous and Transcutaneous Concentration Limit (CTCL, ug/g, in brackets) for sensitizers

More information

ICH Q3D(R2), ICH, 2022 Q3D (R2) Step 5 Elemental impurities (europa.eu), Accessed July 2022.

ASTM Approves ICP-MS Method for Metals in Cannabis. New European Fertilizer Regulations

Jenny Nelson, Craig Jones, Yolande Abdelnour, Andrew Brotherhood, Agilent Technologies, Inc.

Standard test method for analysis of multiple elements in cannabis and hemp samples

ASTM International develops voluntary consensus standards for many industries around the world. In response to the growing interest in cannabis, ASTM Committee D37 on Cannabis was formed in 2017 to develop standards to ensure the quality and safety of cannabis and cannabis-based products.

Agilent ICP-MS applications specialist Jenny Nelson has been a member of ASTM for many years. As the lead technical contact on ASTM sub-committee D37.03, Jenny led the development of a formal method for the analysis of multiple elements in cannabis and hemp using ICP-MS. The method includes a robust microwave sample digestion approach that was developed in collaboration with workers from CEM Corporation.

The ASTM method for Analysis of Multiple Elements in Cannabis Matrices by ICP-MS specifies the priority toxic trace elements, arsenic, cadmium, mercury, and lead. The method can be extended to other elements if required by local regulators, manufacturers, or customers. The new method has been approved and will be available from the ASTM website (www.astm.org/committee/D37) soon.

Webinar on cannabis analysis by ICP-MS

In this Spectroscopy webinar, Jenny Nelson and Craig Jones provide practical tips for labs that analyze cannabis and cannabis products by ICP-MS. Jenny and Craig explain how the Agilent ICP-MS Cannabis Analyzer helps analysts to avoid common issues that can hamper routine analysis. The webinar will be especially useful for labs that are new to the application.

Register for the on-demand webinar at: Four Things You Shouldn't Be Experiencing When Doing Heavy Metals Analysis of Cannabis and Cannabis Products (on24.com)

EU fertilizer regulations apply from July 2022

Food security concerns and population growth are expected to lead to increased agricultural fertilizer use over the next decade. Existing European Union (EU) regulations – (EC) No. 2003/2003 – focus on mineral fertilizers and do not cover organic fertilizers, organo-mineral fertilizers, growing medium, or blended products. To address these limitations, the EU has developed a new Fertilizing Products Regulation (FRP) 2019/1009, which comes into force on 16 July 2022 (*1, 2*).

Monitoring elemental content of fertilizers

The FRP includes guidance on major and nutrient elements, N, P, K, Ca, Mg, Na, S, for labeling purposes. Limits are specified for contaminant elements, Cd, Pb, Ni, As, Cr, Cu, Zn, Hg, and FPR also introduces a new requirement for manufacturers to monitor Cr(VI) and inorganic As (iAs). Standard test methods are being developed and are due to be published in 2024 or 2025 (*2*).

Agilent ICP-MS instruments are ideally suited to the analysis of fertilizing products to comply with the new regulations. Agilent ICP-MS can measure compositional and contaminant elements and, when coupled to HPLC, provides speciation analysis of Cr(VI) and iAs.

References

1. EUR-Lex - 32019R1009 - EN - EUR-Lex (europa.eu)
2. CEN Sectors - CEN-CENELEC (cencenelec.eu)

Meeting report: Post-BNASS Agilent ICP-QQQ birthday celebrations

The UK Biennial National Atomic Spectroscopy Symposium (BNASS) 2022 was held in Manchester in June. Following BNASS, Raimund Wahlen and Andrew Brotherhood of the Agilent UK Atomic Spectroscopy team organized a post-conference meeting at the Royal Northern College of Music to celebrate the 10th anniversary of the first triple quadrupole ICP-MS.

The meeting included fascinating presentations from some of the early ICP-QQQ users and leading researchers in the UK:

- Dr Bridget Gibson from Intertek Sunbury presented some of the applications that she and the metals team run to support the petroleum industry. The lab uses Agilent single and triple quadrupole ICP-MS, GC-ICP-MS, and LC-ICP-MS to support process control analysis, characterization of fuel composition, and identification of contaminants. Agilent ICP-QQQ has extended the analytical capability to include more difficult elements such as Si and S.
- Dr Heidi Goenaga-Infante gave an overview of the work of the UK National Measurement Laboratory (NML) at LGC, Teddington. NML use Agilent ICP-QQQ to provide high accuracy, traceable analysis for certification of high-purity metals, elemental and speciation analysis of candidate CRMs, and to support the development of regulatory standards and RMs for nanoparticles.
- Dr Ben Russell from the Nuclear Metrology Group at the UK's National Physical Laboratory (NPL) gave an insight into the novel nuclear science applications that have been enabled by ICP-MS/MS. Radionuclide analysis is required for applications including nuclear fuel reprocessing, site decommissioning, waste characterization, and development of radiopharmaceuticals. ICP-QQQ provides a unique capability to resolve spectral interferences caused by isobaric overlaps, polyatomic ions, and peak tailing from adjacent masses.

Latest Agilent ICP-MS publications

- **Primer:** 5th Edition Handbook of ICP-QQQ Applications using the Agilent 8800 and 8900, 5991-2802EN
- **Primer:** Updated ICP-QQQ Application Compendium on Measuring Inorganic Impurities in Semiconductor Manufacturing, 5991-9495EN
- **Application note:** Direct and Traceable Mass Purity Certification of Protein Standards using LC-ICP-MS/MS, 5994-5073EN
- **Application note:** Particle Size Analysis of Polystyrene Microplastics by Single Particle (sp)ICP-MS, 5994-4897EN
- **Technical note:** Is Your Agilent Spectrometer as Cool as it Should Be? 5994-4567EN
- **Technical note:** The Principles of ICP Tandem Mass Spectrometry, 5994-4929EN

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