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Enhancing reliability of elemental speciation results – quo vadis?

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Environmental context. The majority of publications reporting research in the field of metal speciation devote too little effort to ensuring quality, reliability or traceability of data. This essay discusses the current state of practice and proposes that we adopt a minimum set of standards or benchmarks to which such studies should be held accountable.

While the somewhat clichéd expression appearing in the title has commonly become associated with overviews of topical areas of science, it nevertheless remains appropriate for this essay which aims to briefly explore how confidence in results for elemental speciation and their validation can be enhanced. We thus raise for consideration such issues as: what is being done and what more can we do about improving the quality and reliability of the data from speciation analysis; what are the implications of the progression from solely environmental interests to seeking a broader understanding of speciation's impact on human health and the biochemistry of life; what are the regulatory agencies within these disciplines seeking from speciation analysis; and, finally, to what standards or benchmarks can such studies be held? This essay hopes to provide a forum to discuss these issues, and to formulate, at the least, some generalised approaches on which researchers, peer reviewers, editors and publishers can focus to help ensure a continued expansion of research in this field.

'*When you can measure what you are speaking about and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind*...' (Lord Kelvin (William Thomson), in a lecture to the Institution of Civil Engineers, London, 3 May 1883). Lord Kelvin's comment summarises the importance of undertaking quantitative measurements for the characterisation of samples. The 'science of measurement and its application' is the definition of *metrology*.[1] A comprehensive global infrastructure designed to enhance the reliability of measurements of the amount of substance has emerged over the past fifteen years in analytical chemistry (both general chemistry[2] and clinical chemistry[3]), imbuing the principles of good laboratory practice, laboratory accreditation (to ISO/IEC 17025; cf. ILAC, see http://www.ilac.org/) and results validation through guidelines established by ISO (see http://www.iso.org/iso/home.htm) and other organisations such as Eurachem (see http://eurachem.org/) and CITAC (see http://www.citac.cc/) to bring strength to a '*measured once, accepted everywhere*' precept.

With the above in mind, and before delving into a consideration of elemental speciation, it is instructive to briefly revisit the field of *total* trace element analysis, which has long been established as one of the principle disciplines of analytical chemistry. While these measurements are considered mature from many perspectives, and are frequently mandated through legislated controls, they remain challenging, and strict quality assurance procedures are generally in place to ensure that generated data are reliable. The metrological principles noted above have slowly become integrated into these activities such that it is now rather

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unusual to encounter published method development which has not incorporated some quality assurance and quality control (QA/QC) measure that frequently includes analysis of a Certified Reference Material (CRM), comparison of results from independent techniques, or analysis of a proficiency test sample. Most peer reviewers expect to see such corroboration of results and many journal editors are at least aware of the importance of doing so.

It follows that these concepts should also apply to elemental speciation, underscoring the importance of reliable (quantitative) measurements defining the system under study for the testing of scientific hypotheses. Speciation analysis has been defined[4] as the analytical activity of identifying and/or measuring the quantities of one or more individual chemical species in a sample. In the event that it is not possible to determine the concentration of the different individual chemical species that sum to the total concentration of an element in a given matrix, it is impossible to rigorously determine the speciation. Evident from the above is the endorsement that total element concentration data are still valued. Although it is acknowledged that such data provide limited information, they remain useful in those cases where: a priori knowledge of the speciation *is* known so that total element concentration is sufficient to characterise the sample; when speciation is highly dynamic and there is a need to know an upper limit for a certain species; as a quality check of speciation data (mass balance); when more specific information is unavailable, and when rules and legislation require such data.

Elemental speciation had its origins in supporting fields such as environmental risk assessment, occupational health and hygiene, toxicology, and clinical chemistry and biology, where it was recognised that the chemical, biological and toxicological properties of an element are critically dependent on the form in which the element occurs. As noted by Szpunar et al.,[5] early speciation analysis targeted well defined analytes such as anthropogenic organo-metallic compounds and their degradation products in the environment, typically encompassing alkyl-mercury and -lead compounds, butyltin and phenyltin as well as some organoarsenic and organoselenium species. Because of the reasonable stability of these analytes, and the availability of calibration standards for many of them, coupled with a small array of suitable CRMs developed to support the validation and integrity of the results, this area of speciation analysis advanced to the point where some now consider it to be routine practice.

Despite such rapid progress, validation of the results of speciation analysis pertinent to even such relatively simple measurements is frequently absent. Validation of a method may be viewed as providing a high degree of assurance that a specific process will consistently produce the intended result. Speciation analysts, however, in their pursuit of ingenious technologies that can be applied to these measurements, frequently fail to ensure the credibility of their results. Thus, determination of selenium species in yeast and butyltins in sediments, well known and stable measurands that were believed to be relatively easy to measure, proved to be problematic even amongst laboratories that were considered as 'expert' in such measurements. This revelation only comes to light when controlled inter-comparisons are undertaken, benchmarking is attempted and/or Certified Reference Materials are available for critical evaluations.[6–8] An excellent summary of the critical 'components' comprising sampling, sample preparation, analysis and interpretation of speciation information has been presented by Emons in 2002 and it is fair to state that many of the highlighted pitfalls continue to plague this field.[9] More recently, Francesconi and Sperling highlighted several problem areas and suggested some simple quality criteria that should be applied to research work on speciation analysis.[10]

Validation includes specification of the requirements and determination of the characteristics of the method, a check that the requirements can be fulfilled by using the method and a statement on the validity. In accordance with the general requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2005[11]), a method should contain one of, or a combination of, the following: calibration using reference standards or reference materials; comparison of results achieved with other methods; inter-laboratory comparisons; systematic assessment of the factors influencing the result; assessment of the uncertainty of the result based on scientific understanding of the theoretical principles of the method and practical experience. The validation should be as extensive as is necessary to meet the needs of the given application and the range and accuracy of the values obtainable are all relevant figures of merit (e.g. the uncertainty of the results, detection limit, selectivity of the method, linearity, repeatability and/or reproducibility, robustness against external influences and/or cross-sensitivity against interference from the matrix of the sample or test object), as assessed for the intended use.

Ideally, accurate and reliable measurements that can be compared in both space (i.e. collaborators' or competitors' laboratories) and time are desired, requiring results to be linked to a common stable reference or measurement standard. This is achieved by adoption of the principles of traceability. The International Vocabulary of Metrology[1] defines traceability as 'the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty'. Thus, all chemical measurements should aim at being traceable to SI units (i.e. in principle this is the 'mole' and concentration units should be expressed in mol kg^{-1}). Achieving traceable measurements of amount of substance at the trace level in complex matrices is difficult if not impossible in many cases, because the 'unbroken chain of calibrations' is readily broken when multiple ill-defined physico-chemical manipulations, inherent to speciation analysis (including sampling), are conducted.[12]

In reference to the above, one of the most important aspects of speciation analysis is the issue of preservation. In the ideal world, we would be able to perform speciation analysis in the field. Unfortunately, this is still not possible for most analytes of interest, so even the most sophisticated analytical methods for the determination of an element's speciation are 'useless' if it cannot be assured that the species distribution in the sample remains unchanged between sample collection and analysis. Therefore, choosing the right preservation techniques for the right matrix is obligatory to ensure that the speciation information in the sample remains intact during shipping and storage until the analysis is performed.

It is also recognised that there may be some calibrations that currently cannot be strictly made in SI units (the ideal reference), examples being those arising from recent interest in metal-protein species wherein stoichiometry relating the targeted metal to the 'ligand' may not be entirely clear. In these cases, confidence in measurements must be achieved by establishing traceability to appropriate measurement standards by the use of Certified Reference Materials provided by a competent supplier (for a current list of species specific CRMs for the common elements Hg, Sn, As, Cr, Pb and Se, see refs [13] and [14]) to give a reliable physical or chemical characterisation of

a material, or the use of specified methods and/or consensus standards that are clearly described and agreed upon by all parties concerned along with participation in a suitable program of inter-laboratory comparisons where possible. It is readily apparent that for speciation activities, reference materials are frequently unavailable, validated methods are generally under development and participation in proficiency testing schemes does not necessarily guarantee accurate results, even if alternative (independent) measurement methodologies are at hand.[6] Nevertheless, at the very least, an estimate of the uncertainty should accompany the overall result and include as input all sources arising from every step in the procedure.

Demonstrating traceability of an amount of substance to its true value in a given medium is, therefore, very difficult to achieve in practice, and compromises must be found in terms of the best state-of-the-art analysis techniques.

Despite the demonstrated concepts of species-specific toxicity, hazard and benefit noted earlier, few regulatory agencies currently demand species-specific information. This is at least partially due to the absence of methods that can reliably measure the analytes of interest at current regulatory levels. More generally, international legislation concerning food safety, environment and occupational health is mostly based on total element concentrations, typically dictated by maximum exposure limits or daily intake levels. Only a few regulations refer to molecular species. Most often, only specific contaminants and their 'compounds' are mentioned. There are a few exceptions, these being Cr^{III}/Cr^{VI} and organotin compounds. Hearteningly, a recent global ban on the use of tributyltin for antifouling protection on ship hulls may open the door for future prescribed methodology for detection of this analyte. The slow incorporation of speciation information into legislation impacts development of the methodology, i.e. since the driving force created from legal requirements is missing, development is mainly driven by exploring technological possibilities rather than by market forces and routine analytical laboratories lack the incentive to invest in the necessary technologies. Furthermore, existing rules and legislation forces analytical laboratories to perform total element determinations. Another important issue for speciation analysis is its cost. Although it is reasonable to expect that speciation analysis may save time and money with respect to remediation and risk assessment, it is usually more expensive than routine elemental analyses. It is noteworthy that the US Environmental Protection Agency has now adopted speciated isotope dilution as a methodology for monitoring or complying with the Resource Conservation and Recovery Act hazardous waste regulations.[15] Isotope dilution mass spectrometry, currently categorised as a primary ratio technique,[16] is able to ensure accuracy of results as well as their traceability to the SI through mass metrology. The use of species-specific isotope dilution[17,18] has revolutionised the field of speciation analysis, ideally permitting corrections for extraction yields, species inter-conversions and quantitation based on an ideal internal standard when suitably characterised enriched species are available. Limitations exist as to the degree to which such species inter-conversions can be accurately accounted for in multi-species systems,[19] but this approach should be favoured for elemental speciation as enriched species-specific spikes are currently available for several systems. When no such species-specific methods can be undertaken, nonspecific isotope dilution techniques can still be applied[20] with their attendant metrological benefits.

Elemental speciation efforts are more recently giving way to charting a broader landscape; interest in metal-protein interactions is demanding identification and/or structural characterisation of endogenous biological species and their metabolic interactions which is not only complicated by their trace concentrations and extreme complexity of the matrices, but as well from a complete lack of calibration standards and reference materials. This new discipline, metallomics, proposed by Haraguchi,[21] integrates all research endeavours related to biometals and may be defined as the study of metals and metal species and their interactions, transformations, and functions in biological systems. This gives rise to the metallome, defined as the complete complement of metals and metal moieties in a biological cell, tissue, or system.[22] This new melding of atomic spectrometry and proteomics will thus face even more daunting problems relating to issues of validation and traceability. Currently, the focus of such activities lies with species identification, frequently derived from inference via species retention time in a chromatogram, but sometimes confirmed using purified standards or synthesised products, by application of MS^n techniques and through exact mass MS measurements to some specified degree of bias. Despite such drawbacks and limitations, it is instructive not to lose sight of the criterion of 'fit for purpose', since it is primarily the identities of metal-containing species that are currently of paramount importance for such studies and this can be accomplished using the above noted techniques.

In the context of method validation, one can interpret this as being the process of defining an analytical requirement and confirming that the method under consideration has performance capabilities consistent with what the application requires. Recall that for an analytical result to be fit for its intended purpose it must be sufficiently reliable that any decision based on it can be taken with confidence. Thus, the method performance must be validated and the uncertainty of the result, at a given level of confidence, estimated. This requires that the method should demonstrate that the following have been established: (a) the required tolerances of all measurements undertaken within the method; (b) the forms of the measurand targeted, including speciation; (c) the effect of interferences has been investigated and quantified; and (d) significant sources of error have been elucidated and adequate means of controlling them have been identified.

To conclude, 'classical' speciation targeting such elements as Hg, Pb, Sn, As, Se and Cr, is advancing and legislated methodologies are beginning to appear because the measurands are stable, uniquely identified, and reference materials are available to permit implementation of method validation (both QA and QC activities). Furthermore, efforts to achieve mass balance between total elemental composition and the sum of detected species should readily provide an additional measure of confidence in the results. Some degree of traceability and an uncertainty assessment of the data should be attempted. Published studies in this area should thus now be supported by validated data and peer reviewers and editors alike should be seeking such evidence. The newer field of metallomics, however, will require further time for quantitation techniques to catch up to detection methodologies. Moreover, the actual measurand in many such situations is ill-defined, stoichiometry used for indirect quantitation may not be known with certainty[23,24] and neither calibration standards nor reference materials are available such that traceability and uncertainty propagation are not yet achievable. Nevertheless, fit for purpose measurements remain possible because the aim of such studies frequently lies with simple identification of species and not its quantitation and the absence of traceability and rigorous validation techniques should not impede progress in this

field. CRMs to support such measurements, although not available at present, can be envisaged for the future as the technology for their targeted synthesis exists.

One last remark is appropriate. In order to undertake speciation analysis, the original distribution of chemical species must be either preserved within the sample or the speciation analysis must be performed in situ. The best sample preparation is thus no sample preparation. Several methods are available that can permit this and examples appear in the review article by Feldman et al.[25] in this issue which explores application of speciation methods based on electrochemistry and synchrotron radiation for this purpose. These methods may eventually complement methods based on atomic spectroscopy or mass spectrometry and aid in their validation. In all cases, better knowledge about the chemistry and better control of the methodology is required compared with total element analysis.

Widespread adoption and use of these principles within the speciation community will encourage a culture of more robust physical and chemical sample characterisation that will enable better research, data interpretation and comparison of results. Unfortunately, the time frame demanded for the discussion and consensus agreement on any accepted methods may typically be such that the methodologies in question may already be out of date due to ongoing progress within the field. 'Standards' relating to speciation papers are currently in the same state of development as those in the emerging field of nanotoxicology (see http://characterisationmatters.org/), a discipline in which concerned scientists are encouraging editors and reviewers to pay attention to and demand a minimum quality from such submissions. Internationally, there is a need to provide benchmarking guidelines which will essentially amount to a checklist of minimum criteria for peer reviews. Unfortunately, there lingers an inherent but often misguided trust intrinsic to the current peer review system[26] that leads to the perception that published data are reliable, having implicitly undergone some form of validation by the issuing laboratory; fallaces sunt rerum species – caveat lector!

References

[1] Joint Committee for Guides in Metrology, Working Group 2, *International Vocabulary of Metrology (VIM) – Basic and General Concepts and Associated Terms, 3rd edn* **2008** (Bureau International des Poids et Mesures). Available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2008.pdf [Verified 12 August 2009]

[2] *CCQM: Consultative Committee for Amount of Substance – Metrology in Chemistry* (Bureau International des Poids et Mesures). Available at http://www.bipm.org/en/committees/cc/ccqm/ [Verified 12 August 2009]

[3] *Joint Committee for Traceability in Laboratory Medicine (JCTLM)* (Bureau International des Poids et Mesures). Available at http://www.bipm.org/en/committees/jc/jctlm/ [Verified 12 August 2009]

[4] D. M. Templeton, F. Ariese, R. Cornelis, L.-G. Danielsson, H. Muntau, H. P. van Leeuwen, R. Lobinski, Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000). *Pure Appl. Chem.* **2000**, *72*, 1453. doi:10.1351/PAC200072081453

[5] J. Szpunar, R. Lobinski, A. Prange, Hyphenated techniques for elemental speciation in biological systems. *Appl. Spectrosc.* **2003**, *57*, 102A. doi:10.1366/000370203321558128

[6] Z. Mester, S. N. Willie, J. A. Caruso, M. L. Fernandez, P. Fodor, R. J. Goldschmidt, H. G. Infante, R. Lobinski, et al., Certification of a new selenized yeast reference material (SELM-1) for methionine, selenomethinone and total selenium content and its use in an intercomparison exercise for quantifying these analytes. *Anal. Bioanal. Chem.* **2006**, *385*, 168. doi:10.1007/S00216-006-0338-0

[7] H. Goenaga-Infante, R. Sturgeon, J. Turner, R. Hearn, M. Sargent, P. Maxwell, L. Yang, A. Barzev, et al., Total selenium and selenomethionine in pharmaceutical yeast tablets: assessment of the state of the art of measurement capabilities through international intercomparison CCQM-P86. *Anal. Bioanal. Chem.* **2008**, *390*, 629. doi:10.1007/S00216-007-1654-8

[8] C. S. J. Wolff Briche, R. Wahlen, R. E. Sturgeon, CCQM-P43: Tributyltin and dibutyltin in sediment. *Metrologia* **2006**, *43*(Tech. Suppl.), 08002. doi:10.1088/0026-1394/43/1A/08002

[9] H. Emons, Artefacts and facts about metal(loid)s and their species from analytical procedures in environmental biomonitoring. *Trends Analyt. Chem.* **2002**, *21*, 401. doi:10.1016/S0165-9936(02)00606-4

[10] K. A. Francesconi, M. Sperling, Speciation analysis with HPLC-mass spectrometry: time to take stock. *Analyst* **2005**, *130*, 998. doi:10.1039/B504485P

[11] *ISO/IEC 17025:2005*. Available at http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=39883 [Verified 12 August 2009]

[12] P. Quevauviller, Traceability of environmental chemical analyses: can theory match practice? *Anal. Bioanal. Chem.* **2005**, *382*, 1800. doi:10.1007/S00216-005-3384-0

[13] *Certified Reference Materials for Speciation Analysis* (*EVISA*). Available at http://www.speciation.net/Public/Document/2005/11/18/CRMs_for_Speciation.html [Verified 12 August 2009]

[14] *The International Database for Certified Reference Materials* (*COMAR*). Available at http://www.comar.bam.de/en/ [Verified 12 August 2009]

[15] *Method 6800 Elemental and Speciated Isotope Dilution Mass Spectrometry* (US Environmental Protection Agency). Available at http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/6800.pdf [Verified 12 August 2009]

[16] M. J. T. Milton, T. J. Quinn, Primary methods for the measurement of amount of substance. *Metrologia* **2001**, *38*, 289. doi:10.1088/0026-1394/38/4/1

[17] H. M. Kingston, *Method of Speciated Isotope Dilution Mass Spectrometry, US Patent No 5,414,259, 9 May* **1995**.

[18] J. Meija, Z. Mester, Paradigms in isotope dilution mass spectrometry for elemental speciation analysis. *Anal. Chim. Acta* **2008**, *607*, 115. doi:10.1016/J.ACA.2007.11.050

[19] J. Meija, L. Ouerdane, Z. Mester, Isotope scrambling and error magnification in multiple-spiking isotope dilution. *Anal. Bioanal. Chem.* **2009**, *394*, 199. doi:10.1007/S00216-009-2619-X

[20] L. Rottmann, K. G. Heumann, Development of an on-line isotope dilution technique with HPLC/ICP-MS for the accurate determination of elemental species. *Fresenius J. Anal. Chem.* **1994**, *350*, 221. doi:10.1007/BF00322473

[21] H. Haraguchi, Metallomics as integrated biometal science. *J. Anal. At. Spectrom.* **2004**, *19*, 5. doi:10.1039/B308213J

[22] D. W. Koppenaal, G. M. Hieftje, Metallomics – the future of atomic spectroscopy? *J. Anal. At. Spectrom.* **2007**, *22*, 111. doi:10.1039/B618394H

[23] G. Koellensperger, M. Groeger, D. Zinkl, P. Petzelbauer, S. Hann, Quantification of elemental labeled peptides in cellular uptake studies. *J. Anal. At. Spectrom.* **2009**, *24*, 97. doi:10.1039/B807397J

[24] M. J. Previs, P. VanBuren, K. J. Begin, J. O. Vogoreaux, M. M. LeWinter, D. E. Matthews, Quantification of protein phosphorylation by liquid chromatography–mass spectrometry. *Anal. Chem.* **2008**, *80*, 5864. doi:10.1021/AC800337V

[25] J. Feldman, P. Salaün, E. Lombi, Critical review perspective: elemental speciation analysis methods in environmental chemistry – moving towards methodological integration. *Environ. Chem.* **2009**, *6*, 275. doi:10.1071/EN09018

[26] D. Horrobin, Something rotten at the core of science? *Trends Pharmacol. Sci.* **2001**, *22*, 51. doi:10.1016/S0165-6147(00)01618-7

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