Using Mass Spectrometry to Provide Accurate Chemical and Bio-Measurements

As the UK's designated National Measurement Institute (NMI) for chemical and bio-measurements, LGC has a major role to play in helping to improve the accuracy and reliability of chemical and bio-analytical measurements that are important to the UK's industrial competitiveness and quality of life.

LGC's measurement science is recognised throughout the world and many of our experts represent UK metrology interests through European and international organisations.

The UK National Measurement System (NMS) is responsible for stimulating good measurement practice and enabling business to make accurate and traceable measurements.
The NMS is funded by the National Measurement Office (NMO), an Executive Agency of the Department for Business, Innovation and Skills (BIS).

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The Knowledge Base programmes of the NMS contain projects that use and develop the extensive existing capability and facilities of the UK NMIs. LGC is a lead contractor for one of these programmes; Chemical & Biological Metrology [1]. A key aim of the programme is to provide the technical and organisational infrastructure that helps ensure that measurements made in the UK are valid and comparable with those made in other countries. LGC's activities in the Chemical and Biological Metrology programme focus on the following areas:

- Bioanalysis (e.g. biopharmaceutical manufacture, drug discovery, diagnostics, medical devices, biometrology);
- Inorganic and Speciation Analysis (e.g. detection of counterfeit goods and tracing a sample to its origin, selenium speciation to support food supplements and anti-cancer therapy);
- Organic Analysis (e.g. use of mass spectrometry for the high accuracy determination of trace organic compounds such as clinical environmental toxins); and
- Reference Materials (e.g. sustaining and developing the capability needed to produce high quality certified reference materials).

To deliver the programme LGC utilises a range of analytical techniques including advances in traditional technologies used in chemistry and molecular biology such as imaging mass spectrometry and digital PCR.

In this article, we describe three examples of mass spectrometry based projects delivered under the Chemical and Biological Metrology programme. The first is an example of our core NMI activity in providing traceable reference values and materials to support UK industry to meet legislative requirements. The second example, shows how we are developing this core capability to move into new areas of application, namely in providing traceable values for protein measurements and ultimately to the measurement of the biologically active form of the proteins. Finally, the third example describes a novel and growing application of mass spectrometry to study the spatial distribution of organic compounds and associated elements (imaging MS) within a biological sample. Although timely and important, the application of imaging MS to accurate bio-measurements is still hampered by the lack of reliable quantitative approaches and of calibration standards and reference materials, which are needed for method validation.

REFERENCE METHODS TO SUPPORT CLINICAL MEASUREMENTS

The In Vitro Diagnostic Medical Devices Directive (EC IVDD, 98/79/EC) stipulates that when available, certified reference materials (CRMs) should be used for validation and ongoing quality control. It is therefore necessary that high accuracy, low uncertainty, traceable (to SI base units) CRMs are produced to satisfy the objectives of the directive. To meet this need, LGC has produced a number of CRMs aimed at the clinical sector, namely:

- Creatinine & electrolytes (Li, K, Ca, Mg & Na) [2] in frozen human serum ERM DA250-253 (3 – 50 µg/g);
- Testosterone in frozen human serum ERM DA345 & 346 (0.3 & 6 ng/g);
- Pure theophylline ERM AC803; and
- Digoxin in frozen human serum ERM DA200 & 201 (0.8 & 2 ng/g) plus a pure digoxin ERM AC200.

Work is currently underway to produce a tacrolimus in whole blood CRM and a serum CRM for total Se, Cu and Zn content.

The approach used at LGC for RM production is accredited to ISO Guide 34 and the measurement procedures to ISO/IEC 17025. The measurements are further validated through participation in international comparison studies organised through the Consultative Committee for Amount of Substance (CCQM) [3].

CASE STUDY: STEROIDS IN BIOLOGICAL FLUIDS

LGC has participated in a number of CCQM studies (K63, P68 & K69) concerned with the analysis of steroids in biological fluids. Successful participation further validates the exact matching isotope dilution mass spectrometry approach [4] used at LGC for the provision of reference values to proficiency testing schemes and reference materials.

Although the analytical methodology may differ depending on the measurand, so as to provide the greatest response, precision and selectivity and subsequent confidence, the general approach to the analysis is always the same. For the CCQM K69 study on the analysis of testosterone glucuronide in urine a procedure using ethyl acetate liquid: liquid extraction followed by LC-MS/MS isotope dilution analysis was employed.

Confirmatory analysis using alternative precursor/product ion transmissions and LC-FAIMS-MS/MS was also performed. The K69 study is the first time that LGC has used FAIMS (High-Field Asymmetric Waveform Ion Mobility Mass Spectrometry) as part of the reference measurement analysis. *Figure 1* clearly illustrates how FAIMS can filter out co-eluting isobaric interferences.

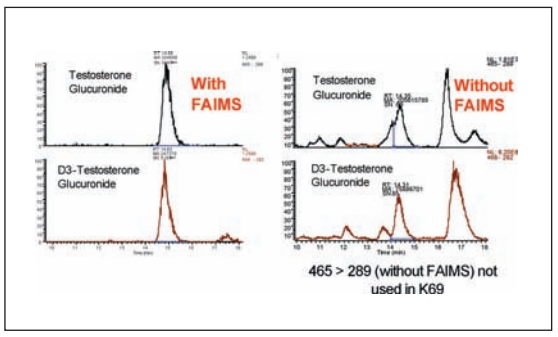

Figure 1. LC-MS/MS Chromatograms of human urine extract – illustrating the benefit of FAIMS to remove isobaric interferences.

Figure 2 shows the excellent agreement between the laboratories participating in another CCQM study, P68 [4,5] on the determination of 19-norandrosterone glucuronide (metabolite of nandrolone) in urine.

The material used in this study, produced by the National Measurement Institute of Australia (NMIA) and funded by the World Anti-Doping Agency (WADA) is now available for WADA accredited laboratories to use in order to validate new methodology and as a quality control material for existing methodology.

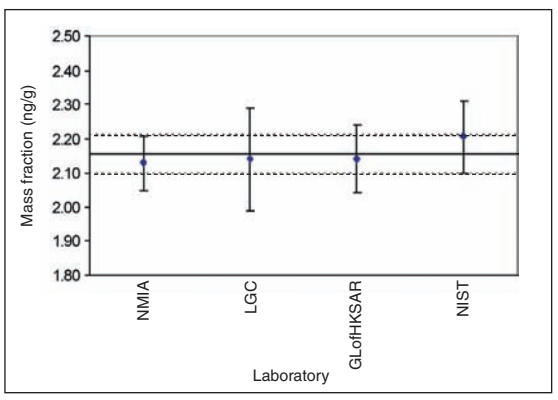

Figure 2. CCQM-P68 participants' measurement results for 19-norandrosterone in urine.

TRACEABILITY FOR PROTEIN MEASUREMENTS

The next example shows how LGC has taken its well established capability for high accuracy analysis, as shown for the clinical reference measurements above and extended them into the field of protein measurements.

LGC is collaborating with other NMIs to develop SI traceable reference materials and methods for the analysis of bio-molecules in complex matrices. This work is of great importance in the clinical and bio-pharmaceutical areas, where requirements of improved measurement confidence have been highlighted.

The developed approach provides SI traceable measurements of a primary sequence of target proteins by applying the principles of isotope dilution mass spectrometry (IDMS) [6] and by using stable isotopically labelled peptides as standards [7].

Reference solutions of peptides are prepared by quantitative amino acid analysis after complete hydrolysis and are used as standards. Accurate quantification of proteins is performed by liquid chromatography coupled with tandem mass spectrometry. Optimisation of the method includes accurate selection of the standard peptides and development of enzymatic digestion protocols to achieve complete release of the peptides of interest from the protein. Examples of proteins which have been quantified in our laboratories or are currently under investigation are lysozyme, alcohol dehydrogenase, bovine carbonic anhydrase, human growth hormone and C-reactive protein [8-10].

While quantification of standard proteins is a well established methodology, standardisation of traceable measurements of proteins in a complex matrix such as plasma is still challenging and a number of robust digestion protocols and chromatography-based sub-fractionation methods are currently under investigation.

In collaboration with other NMIs such as PTB (Germany), IRMM (European Union) and NPL (National Physical Laboratory, UK), we aim to develop SI traceable quantification methods for measuring the structural integrity of proteins. The benefit of being able to define the amount of protein that has the structure of interest in such a manner will have a great impact on diagnosis made by clinicians and treatments received by patients. A hydrogen deuterium exchange method has been developed to provide protein structural information [11]. The protein is treated in deuterated (2D) reagent and the mass changes affected by the exchange of 1H atoms with 2D atoms on the protein surface is used to build a picture of the protein structure. This method allows the interrogation of protein structure under physiologically relevant conditions and provides information on the stability of the protein backbone and its solvent accessibility with resolution at the peptide level. This can be performed in the presence of specified ligands enabling the determination of binding constants and biological activity. The method has been validated using standard proteins such as bovine carbonic anhydrase and has been applied to the analysis of a range of biopharmaceutical products. The potential for this method to provide traceable quantification of the biological activity of proteins and comparison with the performance of commonly used antibody-based assays is currently under investigation.

IMAGING WITH MASS SPECTROMETRY

Finally, in addition to our capability for high accuracy quantification and structural characterisation of proteins, LGC is now looking at the distribution of such chemicals in biological samples by utilising mass spectrometry for imaging.

Mass spectrometry imaging (MSI) is a very useful technique that is able to map and locate the distribution of elemental or molecular information on surfaces. The ability to provide these 'information-rich' images has proved useful in areas such as biomedical research and materials science. SIMS (secondary ion mass spectrometry) and MALDI (matrix assisted laser desorption/ionisation) are established imaging techniques and have been used to map the distribution of a variety of molecules on surfaces. SIMS is ideally suited to the analysis of relatively small molecules (<500u) at extremely high spatial resolution (<200nm) whereas MALDI is more suited to molecules greater than 500u, because of interference problems associated with the use of 'MALDI matrix', and has lower spatial resolution (~100-200µm). SIMS and MALDI imaging analysis of biological samples can be problematic as the analysis needs to be carried out under high vacuum and therefore the sample often needs to be desiccated or frozen. However, with careful sample preparation a number of high-resolution images of biological materials have successfully been obtained [12]. MALDI imaging has been successfully applied to the imaging of biological samples in particular the analysis of peptides [13], proteins [14] and lipids [15]. *Figure 3* shows an example of a MALDI-ToF (time of flight) image of a molecule at m/z = 830 found predominantly within the corpus callosum of a rat brain. MALDI images are typically acquired with raster widths of 100-200µm though higher resolution images (low µm) can be obtained. Other imaging techniques such as LA-ICP-MS (laser ablation - inductively coupled plasma mass spectrometry) and DESI MS (desorption electrospray ionisation mass spectrometry) are also increasing in popularity and are useful as they provide complimentary information (elemental information in the case of LA-ICP-MS) and because they can be carried out under ambient conditions with little or no sample preparation. The spatial resolution of these techniques is similar to that of MALDI (typically 10µm for LA-ICP-MS and >150 µm for DESI MS) and is limited by laser or solvent beam focussing. In most cases MS-based imaging techniques generate images by moving the sample under a laser beam, in the case of MALDI and LA-ICP-MS imaging, an ion beam in the case of SIMS imaging and a charged solvent stream in the case of DESI imaging. *Figure 4* shows the distribution of ^{56}Fe within a rat brain. Iron build up within the brain has been associated with diseases such as Alzheimer's and Parkinson's and important information can be obtained when comparing these images with those obtained by MRI (magnetic resonance imaging). A surface map is reconstructed by extracting ions of interest (each ion is given a colour coding and is then plotted using visualisation software). The reconstructed images are often overlain onto an optical image of the sample so that the various regions of interest can be compared, for example. At LGC we are focusing on some of the underlying issues that are still to be fully addressed such as repeatability, reproducibility and the potential for quantification, to enable analysts to use the techniques with increasing confidence.

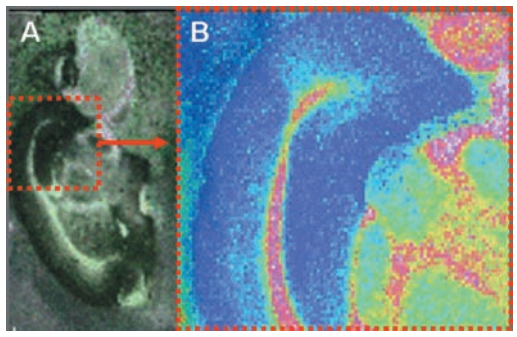

Figure 3. MALDI images of sagittal section through a rat brain. Image A is produced from the most abundant masses observed and was acquired using a 200µm raster. Image B is from the same section and shows the distribution of an unknown ion at m/z 830. This image was acquired using a raster width of 50µm.

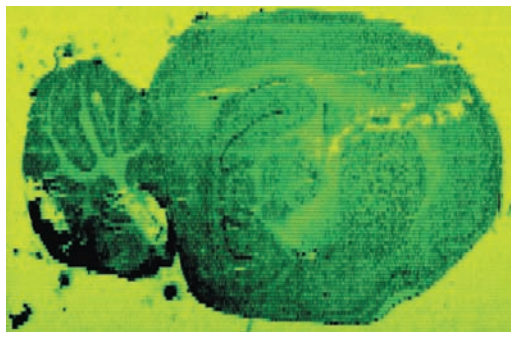

Figure 4. ^{56}Fe measurements in a 20µm rat brain tissue section by LA-ICP-MS.

SUMMARY

The application of leading edge science and development of mass spectrometry techniques has enabled LGC to fulfil its role as a National Measurement Institute as well as supporting the needs of UK industry.

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