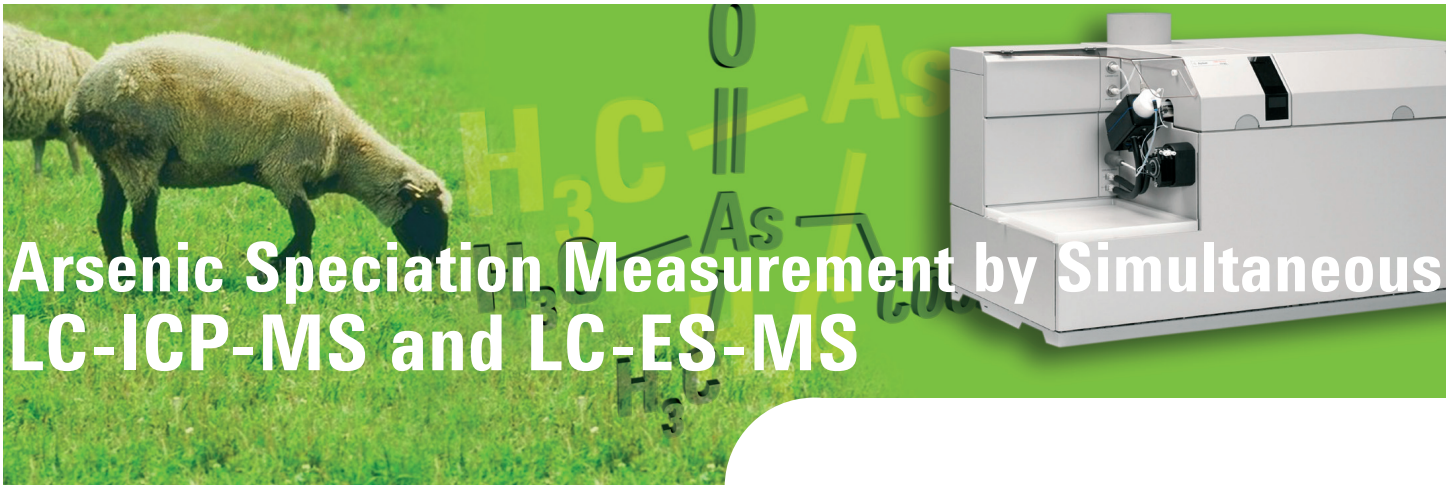

Arsenic Speciation Measurement by Simultaneous LC-ICP-MS and LC-ES-MS

How do the world-record arsenic eaters metabolize arsenosugars? That is one of the main foci of the Trace Element Speciation Laboratories Aberdeen (TESLA) at the University of Aberdeen. Sheep on a small island north of the Scottish Mainland live almost entirely from seaweed, which contains enormous amounts of arsenic in the form of arsenosugars. They eat about 35 mg arsenic daily. Since the toxicity of arsenic in foodstuff depends on its molecular form or species, it is necessary to study the metabolism of arsenic in its many forms.

The initial studies by Prof. Jörg Feldmann and co-workers at the University of Aberdeen showed that most of the arsenosugars are bioavailable and are metabolized to many different arsenic containing species in urine. These studies were done by conventional speciation methods using HPLC-ICP-MS. However, only the main metabolite, dimethylarsinic acid, could be identified by retention time comparison with a standard. Although the group was in possession of more than 15 different arsenic standards, none of them gave exact retention time matches with the 7-8 unknown major metabolites. Fraction collection after anion exchange chromatography and the use of electrospray mass spectrometry (ES-MS) did not result in any successful identification.

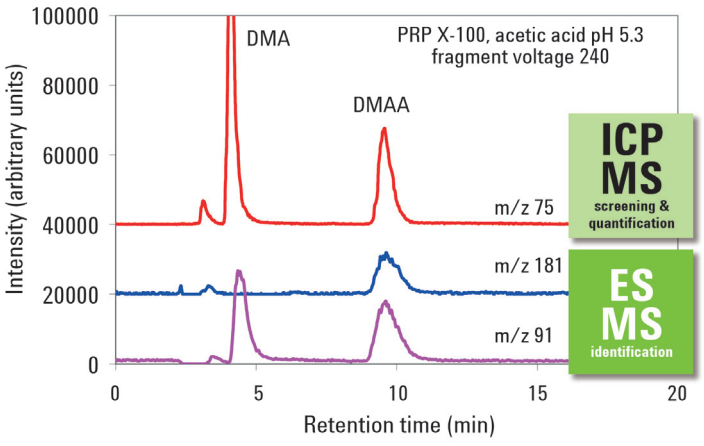

Figure 1. First identification of dimethylarsinoyl acetate (DMAA) using HPLC-ICP-MS/ES-MS. The main metabolite dimethylarsinic acid (DMA) is also shown.

When the group started to use ICP-MS and ES-MS with identical chromatography and they were able to overlay the arsenic peaks from the ICP-MS (m/z 75) with that of certain m/z channels of the ES-MS, co-eluting peaks could easily be identified. The ICP-MS signal gave the window in which an arsenic containing compound must elute, and the ES-MS signal gave the possible molecular mass and fragmentation information.

Breakthrough:

Real advances were made when the HPLC (Agilent 1100 series) was simultaneously coupled online to the ES-qMS (Agilent 1100 series) and the Agilent 7500c ICP-MS: HPLC-ICP-MS/ES-MS. The HPLC is connected to a micro splitter which splits the flow into 75% ES-MS and 25 % ICP-MS. The asymmetric split compensates for the differences in the sensitivity of the two detectors. The peaks and the exact time of the ICP-MS signal (m/z = 75) define the envelope in which molecular fragments from the arsenic metabolites are produced. This reduces the screening to less than 1/50 of the total chromatogram and makes it possible to identify arsenic containing masses in the ESI spectrum. Otherwise (since arsenic is monoisotopic) no identifiable elemental isotope pattern can be recognized among the thousands of masses generated by the ES-MS. It was not long before most of the arsenic metabolites were identified and quantified. Among the metabolites was the first arsenothiol compound found in a biological sample (dimethylarsenothioyl acetic acid, identified by Dr Helle Hansen), and also the new compound dimethylarsinoyl acetate (see Figure 1).

Today the ES-qMS has been replaced by an Agilent ion trap-ES-MS, which has helped Dr Andrea Raab to identify larger molecules containing arsenic such as arseno phytochelatin-3 or arsenic triglutathione in plants. Lately Dr Eva Krupp has utilized the instrumentation for the identification of mercury and organomercury biomolecular species.

References: H.R. Hansen, A. Raab, J. Feldmann, A new metabolite in urine by parallel use of HPLC-ICP-MS and HPLC-ESI-MS, J. Anal. At. Spectrom. 18, 474-479 (2003) H.R. Hansen, R. Pickford, J. Thomas-Oates, M. Jaspars, J. Feldmann, 2-Dimethylarsinothioyl acetic acid identified in a biological sample: the first occurrence of a mammalian arsinothio(y)l metabolite, Angewandte Chemie (Int. Ed.) 43, 337-340 (2004) A. Raab, H. Schat, A.A. Meharg, J. Feldmann, Uptake, translocation and transformation of arsenate and arsenite in sunflower (Helianthus annuus) Part 1: formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations, New Phytologist 168, 551-558 (2005)

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