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Selenium Speciation Analysis by LC-ICP-MS with Mass Balance Calculations for Se-Enriched Yeasts

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Introduction
Alltech manufactures nutritional supplements such as organic trace minerals (Bioplex) and selenium-enriched yeast (Sel-Plex) for the animal feed industry. The level of analytical detail now required by regulatory bodies in the EU and elsewhere resulted in our decision to purchase an LC-ICP-MS instrument. We had previously used single element techniques such as atomic absorption spectrometry for mineral analysis. These methods were valid and fit for purpose but in general they were time consuming and not easily automated. Sensitivity was an issue with some types of samples and additional modules, e.g. hydride generation, were required for low level selenium analysis.

Our new Agilent 7700x system was installed early in 2011 and within weeks we had transferred our methods of analysis for total mineral content over to the new instrument. The MassHunter ICP-MS software is quite different to anything that we had used before but the excellent training provided during installation meant that this was not a problem area. We are mainly interested in elements of nutritional importance although we also screen some samples for heavy metals as well. Sample preparation steps are the same as before but the simultaneous determination of several elements on the ICP-MS has greatly reduced our analysis time. In our experience so far, the additional costs associated with ICP-MS (especially liquid argon consumption) are more than offset by the improvements and savings in other areas.

Se speciation analysis, while well established in several laboratories, is a more difficult prospect than the relatively straightforward measurement of total mineral content. It was here that we relied on help from Agilent and colleagues from other Research groups. We received specialized training on speciation analysis from Agilent and visited a couple of laboratories where Se speciation analysis is performed routinely. These experiences significantly accelerated our progress. The remarkable sensitivity of the 7700 instrument is a welcome feature when analyzing for compounds present at very low concentrations e.g. in tissue extracts.

Application of LC-ICP-MS
Speciation analysis of nutritional supplements is a basic requirement of many regulatory authorities. It is no longer sufficient to determine only the total concentration, as it is well-known that the chemical form of a trace element determines its physiological function [1]. This applies to supplementation of animal and human diets, and the onus is on the manufacturers of such products to demonstrate product efficacy.

Selenium is an important component of several antioxidant enzymes and Se-enriched yeast is routinely added to animal feed to overcome problems associated with Se deficiency e.g. impeded development, low fertility and myopathy. As a result, the Se status of the animal is increased. This, in turn, is beneficial in the case of edible products such as meat, milk and eggs, whereby this essential element may be transferred up the food chain and contribute to improved Se status in humans. Selenomethionine (SeMet) is the main species in Se-enriched yeast; yet, several other compounds are known to be responsible for important physiological effects such as cancer prevention [2].

Experimental
Samples of Se-enriched yeast (0.2 g) were extracted three times (18 hours @ 37 °C) with 5 mL volumes of 30 mM, pH 7.5 Tris buffer containing protease (20 mg) and lipase (10 mg). Extracts were centrifuged at 6,000 rpm for 30 minutes to yield soluble and insoluble fractions. The soluble fractions were pooled. All samples for total Se analysis were microwave digested in nitric acid (6 mL) and hydrogen peroxide (0.5 mL) using an Anton Paar Multiwave 3000.

Analysis for total selenium was performed by ICP-MS in helium mode (5 mL/min). Speciation analysis was performed using an LC coupled to an ICP-MS operating in hydrogen mode on the soluble fraction only using the conditions in Table 1.

<table>
<thead>
<tr>
<th>LC system</th>
<th>Agilent 1260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Zorbax C8, 4.6 x 250 mm (5µm)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>0.1% TFA/2% MeOH @ 1 mL/min</td>
</tr>
<tr>
<td>Inj. volume</td>
<td>50 µL</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Agilent 7700x</td>
</tr>
<tr>
<td>Forward RF power (W)</td>
<td>1550</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>Glass Concentric</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>1.05 L/min</td>
</tr>
<tr>
<td>KED (V)</td>
<td>3</td>
</tr>
<tr>
<td>Cell gas</td>
<td>H₂ @ 6.5 mL/min</td>
</tr>
<tr>
<td>Isotopes monitored</td>
<td>77, 78, 82</td>
</tr>
<tr>
<td>Integration time</td>
<td>0.1 s</td>
</tr>
</tbody>
</table>

Table 1. LC-ICP-MS operating parameters for selenium speciation analysis
A sample of certified reference material (SELM-1, Total Se: 2,059 mg/kg, SeMet Se: 1,389 mg/kg) was included to check method accuracy.

**Results and Discussion**

Total selenium concentrations were determined in the soluble and insoluble fractions after microwave digestion (Table 2). In all cases, the bulk of the selenium (70-90%) was extracted into the soluble fraction, following enzymatic treatment. This is important because it means that the subsequent speciation analysis, which is performed on the soluble fraction only, concerns most of the selenium in the sample.

The chromatogram in Figure 1 and the data in Table 3 indicate that SeMet is the main selenium containing compound in the soluble fraction. This concurs with earlier work published by many different research groups. In the case of SELM-1, we found that SeMet accounted for 66.9% of the total selenium. This agrees well with the NRC-certified value of 67.5%.

To perform mass balance calculations on the speciation data, we used “compound independent calibration” (CIC) to quantify the total selenium recovery from each chromatogram. This feature of the MassHunter software permits the use of a “known” calibrant, in our case selenomethionine, to estimate the Se content of other “unknown” peaks in the chromatogram. The last column in Table 3 demonstrates the usefulness of this approach, especially when working with samples that contain compounds not yet identified or characterized.

**Conclusions**

We found LC-ICP-MS well suited for speciation analysis of Se compounds extracted from selenium-enriched yeast. The CIC function within the MassHunter software allowed us to obtain useful information relating to the selenium content of unknown peaks. This enabled us to perform more detailed analysis of the results, including a full mass balance calculation. We plan to use LC-ICP-MS for the continuing work on characterization of nutritional supplements as well as for routine analytical work.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Se (µg)</th>
<th>Soluble Se (µg)</th>
<th>Insoluble Se (µg)</th>
<th>Se recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Yeast 1</td>
<td>424.4</td>
<td>387.0</td>
<td>25.6</td>
<td>97.2</td>
</tr>
<tr>
<td>Se Yeast 2</td>
<td>205.0</td>
<td>176.0</td>
<td>13.8</td>
<td>92.6</td>
</tr>
<tr>
<td>Se Yeast 3</td>
<td>236.3</td>
<td>178.6</td>
<td>32.2</td>
<td>89.2</td>
</tr>
<tr>
<td>Se Yeast 4</td>
<td>395.9</td>
<td>301.8</td>
<td>74.1</td>
<td>93.2</td>
</tr>
<tr>
<td>SELM-1</td>
<td>374.3</td>
<td>341.6</td>
<td>22.6</td>
<td>97.3</td>
</tr>
</tbody>
</table>

Table 2. Recovery of selenium from soluble and insoluble fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Se (µg)</th>
<th>*SeMet Se (µg)</th>
<th>CIC, Se recovery from LC (µg)</th>
<th>Mass balance Se recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Yeast 1</td>
<td>387.0</td>
<td>256.2 (66.2)</td>
<td>392.4</td>
<td>101.4</td>
</tr>
<tr>
<td>Se Yeast 2</td>
<td>176.0</td>
<td>115.8 (65.8)</td>
<td>169.5</td>
<td>96.3</td>
</tr>
<tr>
<td>Se Yeast 3</td>
<td>178.6</td>
<td>120.0 (67.2)</td>
<td>172.7</td>
<td>96.7</td>
</tr>
<tr>
<td>Se Yeast 4</td>
<td>301.8</td>
<td>194.2 (64.3)</td>
<td>311.2</td>
<td>103.1</td>
</tr>
<tr>
<td>SELM-1</td>
<td>341.6</td>
<td>225.8 (66.9)</td>
<td>354.9</td>
<td>103.9</td>
</tr>
</tbody>
</table>

Table 3. Recovery of selenium following speciation analysis of the soluble fraction

*Numbers in brackets represent the % of total Se recovered as SeMet

**References**


**Acknowledgements**

The authors are grateful for the assistance of Heidi Goenaga-Infante and Christian Deitrich at LGC, Teddington, UK, Katarzyna Bierla at LCABIE-IPREM, Pau, France and Richard Rigg and Raimund Wahlen of Agilent Technologies.
ICP-MS Analysis of Metal Impurities in Pharma Ingredients in Advance of New USP Methods

Amir Liba, 1Samina Hussain and Ed McCurdy
Agilent Technologies Inc., 1Exova, CA, USA

Introduction
The pharmaceutical industry is currently using a 100 year old “heavy metals limit test” (USP<231>) to determine metal impurities in pharmaceutical products, such as active pharmaceutical ingredients (API) and excipients. USP<231> measures the sum of 10 sulfide-forming elements (Ag, As, Bi, Cd, Cu, Hg, Mo, Pb, Sb and Sn), using a precipitation reaction with a reagent such as thioacetamide. The colored precipitate is compared to a 10 ppm Pb standard to determine compliance with the heavy metals limit. Besides the obvious potential bias associated with a subjective visual comparison, the use of thioacetamide and H2S is not allowed in many parts of the world due to safety and environmental concerns. Moreover, USP<231> does not give individual concentrations for each element and, as the method may require ignition of the sample in a furnace at up to 600°C, loss of volatile analytes during sample preparation is inevitable. These issues have led to a program to replace USP<231> with a new method that is more reliable, specific, performance-based, and uses modern instrumental analysis.

Three proposed new USP General Chapters for elemental impurities are being developed, USP <232>/<233> and <2232>. USP <232> is limited to dietary supplements, while USP <2232> and <233> deal with drug products and drug substances. USP<232> defines Permitted Daily Exposure (PDE) limits for a new list of analytes, based on potential toxicity, rather than method capability, and for the first time metal catalysts are included. USP<233> defines the analytical and validation procedures that labs must use to ensure that the analysis is “specific, accurate, and precise.” USP<232>/<233> permits the use of any “suitable” analytical technique, as long as it can be shown to give equivalent data to the reference ICP-OES and ICP-MS methods. In practice, most labs are likely to opt for one of the ICP techniques, due to their ability to measure all required elements in a single rapid analysis.

While the required PDE limits can be measured easily with either ICP-OES or ICP-MS, the limits must be corrected for any dilution factor during sample preparation, and are also adjusted depending on the type of pharmaceutical product and the route of administration. For example drug products administered by a parenteral or inhalational route must meet a modified PDE that is 10x lower than the limit for oral administration. In a digest of a solid sample material, a dilution factor of 250x would give a measurement limit (the “J” value) of 2 ppb for Cd. Accurate recovery must be demonstrated at 0.5J, suggesting a required detection limit at least 10x lower than this – 0.1 ppb – a limit easily achieved using ICP-MS.

In the most recent revision of USP<232>, the previous Group I and Group II analytes have been combined into a single table, but the more toxic elements (As, Cd, Hg and Pb, sometimes referred to as the “Big Four”) are controlled at much lower levels than the other analytes, and must be measured in all samples. In this study, we performed method validation for several pharmaceutical products and ingredients, according to the May 2011 revision of USP <232>/<233>, using an Agilent 7700 Series ICP-MS.

Experimental
An Agilent 7700x ICP-MS was used to measure the 16 regulated elements in calibration standards, digested samples, and samples spiked at the levels specified in USP<233>.

The 7700 Series ICP-MS utilizes a 3rd generation Octopole Reaction System (ORS3) for removal of polyatomic interferences using helium (He) mode with kinetic energy discrimination (KED). He mode has the major benefit that it can deliver reliable results for all analytes regardless of the sample matrix, which simplifies method development and routine analysis.

The 7700x ICP-MS also benefits from very robust plasma (indicated by the CeO/Ce ratio of around 1%), which ensures that good sensitivity is obtained for the poorly ionized elements As, Cd, Hg and the PGEs Os, Ir, Pt and Au. The 7700x is also equipped with High Matrix Introduction (HMI) capability which further improves plasma robustness and enables analysis of samples with very high (% level) total dissolved solids (TDS), as may be the case in pharmaceutical samples.

Figure 1. Calibration curves for 75As, 111Cd, 201Hg, 208Pb
Results and Discussion

Calibration standards were prepared in 1% HNO₃ and 0.5% HCl, while the samples were prepared in 2% HNO₃ and 0.5% HCl. HCl is not specifically required in USP<233>, which refers to the use of “strong acids” for sample preparation, but a complexing agent such as Cl⁻ (HCl) is in fact essential for sample preparation, but a complexing agent required in USP<233>, which refers to the use of “strong acids” for sample preparation, but a complexing agent. However, He mode on the 7700x effectively reduces all these Cl-based matrix elements. A further benefit of He mode is that it reduces the polyatomic interferences on all isotopes under a single set of cell conditions, which allows secondary isotopes to be measured for many analytes. The data shown in Table 1 also demonstrate this secondary isotope capability, as consistent results were obtained for the conformational isotopes of Cr, Ni, Cu, Mo, Cd, Os, Ir, Pt, Hg and Pb.

USP<233> also requires that the analysis provides the ability to "unequivocally" assess each analyte in the presence of other analytes and matrix elements. A further benefit of He mode is that it reduces the polyatomic interferences on all isotopes under a single set of cell conditions, which allows secondary isotopes to be measured for many analytes. The data shown in Table 1 also demonstrate this secondary isotope capability, as consistent results were obtained for the conformational isotopes of Cr, Ni, Cu, Mo, Cd, Os, Ir, Pt, Hg and Pb.

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The analysis used He mode for all analytes, and example calibration curves for the “Big 4” analytes ⁷⁵As, ¹¹¹Cd, ²⁰¹Hg and ²⁰⁸Pb are shown in Figure 1. Method validation for quantitative (as opposed to limit) methods under USP<233> requires assessment of spike recovery (between 70% and 150%) and precision (<20% RSD) at concentrations between 0.5J and 1.5J. Table 1 shows the mean recoveries and external repeatability for spikes at 0.5J, 1.0J and 1.5J into Gelatin Capsule samples (10 separate samples for each spike level – USP<233> requires 6 replicates). The recovery and %RSD data in Table 1 demonstrate accurate and precise determination of all required analytes. While the results were well within the 70% to 130% recovery limits, it is noticeable that the recovery of Os was consistently slightly low, probably because of the relatively low concentration of HCl used for these samples (up to 5% HCl may be needed to ensure long-term stability of the PGEs in some sample types).

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Monitoring Radioactive $^{129}$Iodine using ICP-MS

Kazumi Nakano, Yasuyuki Shikamori, Naoki Sugiyama and Shinichiro Kakuta Agilent Technologies, Japan

Introduction

Iodine $^{129}$ ($^{129}$I) is a long-lived radionuclide (half-life of 15.7 million years), which is present in the environment as a result of nuclear weapons testing and accidental release from nuclear power stations and nuclear fuel reprocessing plants. The commonly-used techniques for the measurement of $^{129}$I, neutron activation analysis (NAA) and accelerator mass spectrometry, or liquid scintillation counting and gamma spectrometry, are all slow (from several hours to several weeks) and may require radiochemical separation prior to analysis.

Inductively coupled plasma mass spectrometry (ICP-MS) can quickly screen for $^{129}$I at low levels in food and environmental samples, and requires minimal sample preparation. But $^{129}$I is affected by a spectral overlap from the $^{129}$I isotope of Xe, which is present as an impurity in the argon plasma gas. The Agilent 7700 Series ICP-MS can resolve this interference using oxygen reaction mode with the optional reaction gas line of the Octopole Reaction System (ORS®). O$_2$ cell gas removes the Xe$^+$ ion by the reaction $\text{Xe}^+ + \text{O}_2 \rightarrow \text{Xe} + \text{O}_2^+$. O$_2$ reaction gas also removes the $^{127}$I$^+$ interference from high levels of natural I in samples. The effectiveness of O$_2$ reaction mode on the 7700 ICP-MS is illustrated in Figure 1.

Measurement of trace level $^{129}$I and $^{129}$I/$^{127}$I isotope ratios

The removal of the spectral overlaps at mass 129 allows the determination of $^{129}$I at low levels in natural samples. The detection of $^{129}$I using O$_2$ reaction mode on the Agilent 7700x ICP-MS was evaluated using the reference materials NIST 3231 Level I and Level II. These isotope ratio standards have certified values for the $^{129}$I/$^{127}$I ratio of 0.981$x10^{-6}$ ± 0.012$x10^{-6}$ in Level I, and 0.982$x10^{-8}$ ± 0.012$x10^{-8}$ in Level II. The $^{129}$I/$^{127}$I isotope ratio was measured with a precision of less than 2% RSD in a 10x dilution of the Level I standard (total I concentration 88.9 mg/L (ppm)), and less than 10% RSD in a 100x dilution ($^{129}$I present at single ppt). This is remarkable performance for an isotope ratio with such a large difference in isotopic abundance.

Conclusions

The measurement of low-level radioactive iodine ($^{129}$I) is now possible using O$_2$ reaction mode on the Agilent 7700x ICP-MS. The new method:

• Provides a detection limit of 1 ng/L (ppt) for $^{129}$I
• Is fast (a few minutes per sample)
• Requires minimal sample preparation (simple dilution or digestion).

The method can therefore be used for fast routine screening or monitoring of $^{129}$I in food and environmental samples.
ICP-MS Manufacturing Returns to Full Capacity Following Japanese Earthquake

Ken Suzuki
ICP-MS Marketing Manager, Agilent Technologies

On 11 March 2011, Japan’s eastern coast was devastated when a massive tsunami triggered by the most powerful earthquake in the country’s recorded history smashed ashore.

Direct impact to Agilent’s ICP-MS manufacturing facility, Tokyo Analytical Division (TAD), based in Hachioji, Tokyo was minor and the site remained operational. However, a few of our suppliers’ sites were seriously damaged in the disaster. Furthermore, the rolling power outages by Tokyo Electric Power Company throughout March occurred almost daily for 3-4 hours at a time, seriously impacting on manufacturing and product development. With every blackout, it took 1.5 hours to shut down and reboot the R&D computer servers, leaving R&D engineers without access to their server for 6-7 hours.

Manufacturing Challenges
Despite the adversity, all staff at TAD worked hard to minimize the impact to customers by continuing production. Some started work earlier in the mornings and others stayed on later in the evenings. In parallel with our improvised manufacturing operation we had daily updates from all direct suppliers on their recovery status. While some suppliers’ operations were severely interrupted, most of our key suppliers were either unaffected or had suffered only limited damage and were quick to recover. Where there were problems with supply, we selected and evaluated alternative parts with our R&D engineering team or selected alternative vendors with our partner and direct suppliers. We were also fortunate that none of our suppliers was located within the restricted area around the damaged Fukushima nuclear power plant.

According to Mitsuki Goto, ICP-MS Manufacturing Manager, “Teamwork of our suppliers, manufacturing partners and all functions within TAD helped ICP-MS manufacturing to recover quickly.”

The fact that there were no delays to customer shipments is a testament to TAD manufacturing personnel and the Agilent supply chain management. Toshifumi Matsuzaki, General Manager for ICP-MS commented: “I’m very proud of my team in Hachioji for their dedication, commitment, flexibility and hard work. I was also impressed by our suppliers’ recovery speed.” This resilience and speed to recovery is commendable and is a clear example of why manufacturing is a key factor to Agilent’s ICP-MS success.

In relation to R&D Matsuzaki-san added: “There has been almost no schedule impact to new product development projects. According to my software and solution project manager, one key project has been delayed by three days because of the power outage – that’s it!”

Return to Full Production
In just two months following the natural disaster, ICP-MS production had fully recovered and was back to pre-earthquake production capacity (Figure 1). We also took the opportunity to address any potential risk areas in our supply chain and are confident that we can continue to fulfill forward-looking ICP-MS demand.

Help for the Devastated Areas
Since the earthquake on March 11, Agilent and the Agilent Technologies Foundation have contributed over $850,000 in donations and equipment to help with the disaster response.
Agilent Donates a 7700 to Measure Radioactive Elements

Together with the methodology summarized on page 6, Agilent has donated a 7700 ICP-MS to Gakushuin University in Japan so that they can identify radioactive iodine quickly and accurately.

Prof. Yasuyuki Muramatsu's lab measures ultra-trace levels of radioactive elements, including iodine isotopes 129, 131 and 127, as well as trace elements such as Cs and Sr, which are relevant to radioisotopes released from the reactor into the environment.

"Radioactive iodine-129 is of particular interest because of its long half-life," said Prof. Muramatsu. "Monitoring its levels will be important for the safety assessment of radioiodine deposited in the environment."

Agilent India Hosts First ICP-MS Training Event in Center of Excellence

The first Agilent Academy ICP-MS customer education event to be held at the recently extended Bangalore Center of Excellence took place over 2 days beginning on July 14, 2011.

Response to the "ICP-MS: Fundamentals and Applications" training course was overwhelming, with all 10 places being snapped up quickly. Two additional places were created to ease demand and another training course is planned for September 20-21, 2011.

Participant feedback was positive with praise being given to the knowledge base of the Agilent trainers, Arun Kumar Raju & Dharmendra Vummiti. Attendees especially appreciated the practical format of the training that included live demonstrations, discussions and hands-on operation of the 7700 Series ICP-MS for Method setup, Data Analysis, Reporting etc.

Conferences. Meetings. Seminars.

Agilent ICP-MS Publications

To view and download the latest ICP-MS literature, go to www.agilent.com/chem/icpms and look under “Literature Library” and search using the title or publication number.

• **Application Note:** EPA approved standard operating procedure for the analysis of flue gas desulfurization wastewater, 5990-8114EN

• **Application Note:** Trace elemental analysis of trichlorosilane by Agilent 7700s ICP-MS, 5990-8175EN

• **Application Note:** The ultratrace determination of iodine 129 in aqueous samples by ORS-ICP-MS using oxygen reaction mode, 5990-8171EN

• **Advertorial:** Plasma Robustness in ICP-MS: Benefits of a Low CeO/Ce Ratio, 5990-8060EN

Agilent ICP-MS Journal Editor
Karen Morton for Agilent Technologies

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