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September 2020

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Norbert Jakubowski, Sarah Borrmann, Sebastian Recknagel, Janina Roik, and Friedhelm Rickert
Inductively coupled plasma–atomic emission spectroscopy (ICP-AES) relies on the use of a peristaltic pump for sample introduction. Here, two conventional peristaltic pumps are compared with a new pump based on the “easy click” principle for the analytical figures of merit.

Daniel Kutscher, Dhinesh Asogan, Ian Mudway, Patricia Brekke, Charlie Beales, Xiaohan Wang, Matthew W. Perkins, Wolfgang Maret, and Theodora J. Stewart
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Comparison of Peristaltic Pumps Used for Sample Introduction in Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES)

In this investigation, two conventional peristaltic pumps are compared with a new pump based on the “easy click” principle using a simultaneous ICP-AES instrument with standard operating conditions. It is found that the figures of merit achieved are quite comparable for all three pumps. Relative standard deviations (RSDs) range between 0.2% and 1.8%, and limits of detection as low as 0.1 µg/L have been achieved, demonstrating that the easy click principle of the new pump does not compromise the analytical figures of merit.

Norbert Jakubowski, Sarah Borrmann, Sebastian Recknagel, Janina Roik, and Friedhelm Rickert

A
tomic spectroscopy has a long history that starts with Sir Isaac Newton (1643–1727) when he was theorizing about color in 1704 (1). He introduced the idea of the “spectrum,” which he believed was made up of different components of light.

Many years later, in 1814, German physicist Joseph von Fraunhofer paved the way for modern absorption spectroscopy when he interpreted the black lines in the sun’s spectrum (2). In the later nineteenth century, German chemist Robert Bunsen, together with physicist Gustav Kirchhoff, examined the colors of the spectrum of a flame, and laid the foundation for today’s atomic spectroscopy (3).

In 1955, Sir Alan Walsh published his work on atomic absorption spectroscopy (AAS) (4). Since then, this analytical method has been in daily use in many thousands of analysis systems to determine the concentration of various elements, primarily in liquids. Considerable efforts were made to develop AAS from a single-element method into a multielement method (5), but other techniques, based on plasmas instead of a flame, namely inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (6) and inductively coupled plasma–mass spectroscopy (ICP-MS) (7), were used to utilize element emissions or ionization of the analytes for today’s multi-element analysis.

All of these methods mentioned are quite similar in their construction. Each method is made up of a system for introducing the sample, comprising of a pump and an atomizer (such as an aerosol generator) to transfer the sample into the hot zone (flame or plasma), a spectrometer (spectrograph, monochromator, or mass spectrometer), a detector, and a control computer complete with software for operating the machine and analyzing the results. Although analysis systems from different vendors differ radically, some components are nevertheless very similar. This is true of the pump systems used to feed the
**TABLE I**: Calibration data measured with the original pump of the Arcos system. Data is shown for all selected elements and wavelengths. The line finally selected for the comparison of the different pumps is shown in the last column with the abbreviation. For some lines the calibration did not show a satisfying correlation. They are marked with -.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>BEC (μg/L)</th>
<th>DL (μg/L)</th>
<th>Correlation Coefficient</th>
<th>Selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al 167.078</td>
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<td>0.6</td>
<td>0.99784</td>
<td>Al 167</td>
</tr>
<tr>
<td>Al 176.641</td>
<td>538</td>
<td>11</td>
<td>0.98907</td>
<td></td>
</tr>
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<td>Al 309.271</td>
<td>7,540</td>
<td>97.6</td>
<td>0.09168</td>
<td></td>
</tr>
<tr>
<td>Al 394.401</td>
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<td>120</td>
<td>0.98656</td>
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</tr>
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<td>Al 396.152</td>
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<td>12.7</td>
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<td>Bi 190</td>
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<td>Bi 222.825</td>
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<tr>
<td>Cr 205.618</td>
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<td>0.53</td>
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<td>Cr 205</td>
</tr>
<tr>
<td>Cr 267.716</td>
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<td>0.99818</td>
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</tr>
<tr>
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<tr>
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<td>Cu 219.958</td>
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<td>7.46</td>
<td>0.98868</td>
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<tr>
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<tr>
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<td>294</td>
<td>7.65</td>
<td>1.00000</td>
<td></td>
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<tr>
<td>Fe 275.573</td>
<td>235</td>
<td>5.97</td>
<td>0.99942</td>
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</tr>
<tr>
<td>Fe 373.486</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Continued on Page 8*
As a rule, users take little notice of these components, and regularly fail to maintain and monitor them properly, even though they can. Despite the small size of the nebulizer and atomizer, these small components have a significant impact on the results of the analysis.

Peristaltic pumps are based on the principle of peristalsis, which often occurs in biological processes or organs such as the esophagus, where it transports liquids. In instrumental analysis, peristaltic pumps can be used to transport liquid samples. The basic principle by which a sample is transported is based on the alternate compression and relaxation of elastic pump tubing. The pump tubing is passed around a roller head, and pressed against the roller head with a curved pressure plate. The tubing is pressed so tightly against the roller head that it is completely sealed. When the tubing returns to its original shape behind the roller as the pump head rotates, a vacuum is created, drawing the liquid sample into the tube. Further rollers on the pump head seal the pump tubing again to form a closed segment of liquid. The rotation of the pump head drives the fluid forward in the tubing, thus causing it to flow.

The use of peristaltic pumps offers the advantage that there is no direct contact with the liquid sample at any time. Furthermore, as already described, peristaltic pumps allow constant delivery rates. As a result, these pumps help alleviate viscosity transport variations of different samples. Peristaltic pumps are available in a wide variety

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**TABLE I (CONTINUED):** Calibration data measured with the original pump of the Arcos system. Data is shown for all selected elements and wavelengths. The line finally selected for the comparison of the different pumps is shown in the last column with the abbreviation. For some lines the calibration did not show a satisfying correlation. They are marked with -.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>BEC (μg/L)</th>
<th>DL (μg/L)</th>
<th>Correlation Coefficient</th>
<th>Selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg 202.647</td>
<td>449</td>
<td>7.51</td>
<td>0.98347</td>
<td></td>
</tr>
<tr>
<td>Mg 279.079</td>
<td>959</td>
<td>17.1</td>
<td>0.97431</td>
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</tr>
<tr>
<td>Mg 279.553</td>
<td>2.44</td>
<td>0.39</td>
<td>0.99701</td>
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</tr>
<tr>
<td>Mg 280.270</td>
<td>3.71</td>
<td>0.36</td>
<td>0.99687</td>
<td>Mg 280</td>
</tr>
<tr>
<td>Mg 285.213</td>
<td>26.2</td>
<td>0.55</td>
<td>0.99702</td>
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</tr>
<tr>
<td>Mn 257.611</td>
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<td>0.16</td>
<td>0.99861</td>
<td>Mn 257</td>
</tr>
<tr>
<td>Mn 259.373</td>
<td>9.29</td>
<td>0.18</td>
<td>0.99868</td>
<td></td>
</tr>
<tr>
<td>Mn 260.569</td>
<td>13.3</td>
<td>0.25</td>
<td>0.99861</td>
<td></td>
</tr>
<tr>
<td>Mn 294.921</td>
<td>50.6</td>
<td>1.02</td>
<td>0.99823</td>
<td></td>
</tr>
<tr>
<td>Mn 403.076</td>
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<td>86.4</td>
<td>0.99630</td>
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</tr>
<tr>
<td>Ni 221.648</td>
<td>53.6</td>
<td>3.61</td>
<td>0.99986</td>
<td>Ni 221</td>
</tr>
<tr>
<td>Ni 231.604</td>
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<td>2.61</td>
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<td>5.12</td>
<td>0.99872</td>
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<tr>
<td>Ni 300.249</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Se 196.090</td>
<td>715</td>
<td>12.5</td>
<td>0.96577</td>
<td>Se 196</td>
</tr>
</tbody>
</table>

Continued from Page 7

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of designs, with significant differences in the size of the pump head and the number of rollers. The rotation speed of the pump head is usually infinitely variable, so that, depending on the inner diameter of the pump’s tubing and the rotation speed of the pump head, samples can be delivered at precise rates from a few microliters up to several milliliters per minute.

One problem that arises when using single-channel peristaltic pumps is the pulsation of the sample liquid, which can affect the aerosol generation process in the nebulizer used, and thus will have an impact on the stability of the signal intensity measured. When a roller contacts the pump tubing, the sample liquid is displaced on both sides. The excess pressure in the tubing accelerates the sample liquid briefly, resulting in pulsating delivery to the downstream atomizer. Fluctuating delivery rates can thus potentially have a negative impact on the precision as well as on the detection threshold of the method used. When the tubing relaxes again, the volume available to the sample liquid in the tubing increases, intensifying pulsations.

A pulsation-damped pump can be used to reduce pulsation. Pulsation-damped pumps consist of at least two half-channels, with the rollers of each half-channel arranged in such a way that they are phase-shifted. This arrangement means that each peak of one half-channel is canceled out by a valley of the other half-channel. Peaks arise from acceleration of the sample liquid when the pump tubing is compressed, whereas troughs result when the pump tubing relaxes. Ideally, the pulsations of the individual half-channels cancel each other out when they are merged, so that a constant sample flow is delivered to the atomizer.

In a previous investigation, the noise spectra of different peristaltic pumps were measured, and it was shown that the noise frequency and amplitude strongly depend on the rotation speed, the number of rolls in the pumping head, and the pressure by which the elastic tubing is pressed against the roller head. For many commercial peristaltic pumps, this tubing pressure, which is used to press the pressure plate against the elastic tubing, will usually be increased while the tubings are altered or optimized to reduce pulsations. The rolls, meanwhile, can be adjusted manually. A new alternative is pumps with preselected settings, which do not need adjustment at all, and the required pressure is achieved by use of a spring, allowing faster changing of tubing and no need for optimization or control of the pressure.

This investigation compares three different peristaltic pumps to see how the analytical figures of merit, such as detection limit (DL), background equivalent concentration (BEC), and the relative standard deviation (RSD) of wavelength intensity measurements, depend on peristaltic pump conditions. In this study, multielement standards are applied for measuring 10 selected elements simultaneously using standard operating conditions of an ICP-AES instrument. The elements are selected to cover a broad range of excitation energies, and should cover the whole mass range from lowest to highest.

**Materials and Methods**

**Standards**

Stock solutions of two multielement standards with 24 elements each (Merck VIII and XVI Certipur multielement standards, Merck Millipore) with a concentration of 100 mg/L plus 2 mL concentrated HNO₃ (stock solution) were diluted in bi-distilled water acidified with HNO₃. The stock solutions were diluted in bi-distilled water, and two different concentrations were diluted: concentration I: 2.5 µg/L; concentration II: 25 µg/L were prepared for each element. These concentrations are selected to be 10 times or 100 times above the instrumental DL provided by the manufacturer.
### TABLE II: Comparison of analytical figures of merit for three different peristaltic pumps: 1) Peristaltic pump of the Arcos device, 2) “Easy Click” peristaltic pump, 3) Perimax peristaltic pump.

<table>
<thead>
<tr>
<th>Element</th>
<th>Spectro</th>
<th></th>
<th>Easy Click</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wavelength (nm)</td>
<td>DL (µg/L)</td>
<td>BEC (µg/L)</td>
<td>RSD (N = 10)</td>
</tr>
<tr>
<td>Al</td>
<td>167</td>
<td>0.6</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Bi</td>
<td>190</td>
<td>13</td>
<td>747</td>
<td>0.8</td>
</tr>
<tr>
<td>Co</td>
<td>238</td>
<td>0.6</td>
<td>49</td>
<td>0.75</td>
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<tr>
<td>Cr</td>
<td>205</td>
<td>0.5</td>
<td>26</td>
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<tr>
<td>Cu</td>
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<td>2.7</td>
<td>116</td>
<td>0.85</td>
</tr>
<tr>
<td>Fe</td>
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<td>2.2</td>
<td>43</td>
<td>0.9</td>
</tr>
<tr>
<td>Mg</td>
<td>280</td>
<td>0.4</td>
<td>3.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Mn</td>
<td>257</td>
<td>0.2</td>
<td>6.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Ni</td>
<td>221</td>
<td>3.6</td>
<td>54</td>
<td>0.9</td>
</tr>
<tr>
<td>Se</td>
<td>196</td>
<td>13</td>
<td>715</td>
<td>0.85</td>
</tr>
</tbody>
</table>

**Instrumentation**

For all measurements, a Spectro Arcos (12) (Spectro Analytical Instruments GmbH) simultaneous measuring optical emission spectrometer with inductively coupled plasma excitation (ICP-AES) was used (13). The instrument includes a Paschen-Runge mount optical system, where all elemental lines are focused onto a curve, called a Rowland circle, on which a linear array of electronic detectors is installed covering the wavelength range from 130 to 770 nm. The instrument provides two different orientations of the plasma source, and thus can be easily operated in radial or axial observation mode. All measurements presented here were performed in axial observation mode.

The following plasma and operational conditions were selected: a plasma forward power: 1400 W, a plasma gas flow rate at 13 L/min, a Nebulizer flow rate at 0.85 L/min; a single measurement time at 28 s. A crossflow neb-
TABLE II (CONTINUED): Comparison of analytical figures of merit for three different peristaltic pumps: 1) Peristaltic pump of the Arcos device, 2) “Easy Click” peristaltic pump, 3) Perimax peristaltic pump.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>DL (µg/L)</th>
<th>BEC (µg/L)</th>
<th>RSD (N = 3)</th>
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<td>10</td>
<td>377</td>
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All pumps were connected to an auto-sampler (Asx-260 Cetac).

All pumps were operated using a sample up-take rate of 0.8 mL/min, controlled by a volumetric measurement. For all experiments new tubing (ID color code: orange-orange, Spetec GmbH) was used, except in the Spectro device; here the tubing was in use for more than a fortnight, and this “matured” condition served as a reference value used to see if old tubing compromised the figures of merit.

Measurement Details

For each pump the following measurement procedure was applied: First, a blank measurement was performed, and then two multielement solutions with concentrations of 2.5 µg/L and 25 µg/L were measured.

Results

Wavelength Selection and Data Evaluation

In a first test run, 10 (n = 10) repeated intensity measurements for at least 5 preselected lines per
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element were performed. The calibration data for all lines are compiled in Table I, and the number of wavelengths is reduced to a single one (indicated in Table I) to compare all three pumps. However, the total measurement time, and time needed for calculations exceeded more than 60 min, so that the number of repetitions was decreased for all further measurements to $n = 4$. As a result, the final measurement and calculation time of 25 min was achieved. Standard conditions were chosen as the integration time for each element.

Because the peristaltic pump of the Arcos system was operated with a higher pumping speed in between intensity measurement cycles, the washout phase was doubled for the Perimax and “Easy Click” pump, which were operated manually. However, the first run of a new measurement series showed a higher concentration for these two pumps, with much higher RSD, DL, and BEC values, an indication that the sample introduction system was not in a stable equilibrium. Therefore, the first run of each series of new intensity measurement has been deleted, and thus data is given for three ($n = 3$) repeated measurement cycles only.

Except for Se, several lines were proposed by the software of the Arcos system for most elements, and were confirmed after a blank measurement to evaluate the background structure. For all lines selected, calibration data are available, but for the comparison of the three different pumping systems, a single wavelength with the lowest DL was selected, provided the correlation coefficient was satisfactory. Selected lines, used further on, are shown in the last column of Table I, with their abbreviations.

The stability of the plasma was controlled manually using the Ar wavelength at 430.010 nm, and the resulting RSD measured was always equal or better than 1% for all pumps and all concentrations measured.

**Comparison of Analytical Figures of Merit for Three Different Peristaltic Pumps**

Results for the analytical figures of merit for of all three pumps are compiled in Table II. Data provided includes the DL, BEC, and the RSD. The latter is calculated as mean value from the RSDs of both concentrations used for calibration.

**Discussion**

As stated before, the original peristaltic pump of the Arcos system was operated with matured peristaltic tubing. Nevertheless, all RSD values (shown in Table II) calculated are better than 1% (except Cr: 1.4%). Blank values (not shown) are slightly higher by 10–20% compared to new tubing (likely because the tubing was extensively used for analysis of highly concentrated digestions of steel and alloys of Al, Cu, and Ni). However, DL and BEC do not significantly deviate from the values measured for the two other pumps. The Perimax pump, for which the pressure plate was manually adjusted to the lowest pulsation setting and for which new pumping tubing were used, showed the best RSD values for all elements, being better than 1%. Even the Easy Click pump with manufacturer settings showed relatively good RSD values for some elements very close to the values achieved for the Perimax pump. This result demonstrates that the manual alignment of the pressure block does not significantly improve the figures of merit, and thus is not needed if a pump with the lowest maintenance is needed. For the lines selected, all DL values are lower than 5 µg/L (except Bi and Se). For Bi, the DL could be improved by more than a factor of two mainly using new tubing, which also demonstrates that memory effects did not significantly worsen the analytical figures of merit. Finally, it should be mentioned that all three pumps tested are very comparable concerning their analytical figures of merit. In particular, low RSD values were achieved in this
experiment for all pumps investigated, and can be attributed to the simultaneous detection capability of the instrument used in this investigation. Such low precision is required for traceability studies. Applying a precision measurement method for element contents, developed using ICP-AES, combined measurement uncertainties between 0.05% and 0.1% can be achieved (14).

**Conclusion**
In this investigation, three different peristaltic pumps were compared using an ICP-AES instrument with simultaneous detection for all elemental wavelengths of interest to demonstrate that a peristaltic pump based on an “easy click” adjustment will not compromise the analytical figures of merit. It was found that comparable analytical figures of merit can be achieved for all three pumps using standard operating conditions and very low standard deviations (below 1%) can be achieved for all pumps, if simultaneous detection of the intensity measurements is applied. For multielement analysis, such very low standard deviations and LOD values can only be achieved with ICP-AES operated with peristaltic pumps, and are essential for the development of new standard reference materials (15).

**References**

Norbert Jakubowski, Sarah Borrmann, and Friedhelm Rickert are with Spetec GmbH, in Erding, Germany. Sebastian Recknagel and Janina Roik are with Federal Institute for Materials Research and Testing (BAM), in Berlin, Germany. Direct correspondence to: Sarah.Borrmann@spetec.de •

The qualitative and quantitative determination of trace elements is a stronghold of inductively coupled plasma–mass spectrometry (ICP-MS). Whereas in most cases the concentrations of a series of elements are determined in the bulk sample (often requiring a digestion upfront), the use of laser ablation (LA) in combination with ICP-MS allows further insight into an isotope of a given element within a sample, with the added benefit of avoiding labor intensive and contamination-prone digestion processes. LA-ICP-MS also allows the analysis of small samples with highly localized differences in trace elemental concentrations. Higher sensitivity and better interference removal using triple quadrupole instrumentation, as well as faster laser systems with specialized ablation cells enabling fast washout of material from individual laser pulses, has paved the way for new applications for LA-ICP-MS. The software supported recombination of data generated in a series of line scans covering the entire area of a sample. This was in conjunction with information on the laser position over time (using ablation rate, spot size, and stage movement velocity), allowing for the creation of a full image of elemental distributions in a sample. This is especially useful for biological samples, where the role of trace elements involved in biological processes is often unknown. This article will outline the principle of LA-ICP-MS for imaging applications, highlighting its potential to gain additional insights into the field of bioanalysis and metallomics. More specifically, the technique will be used to track the effect of environmental pollution on the distribution of heavy metals in the brain of a sentinel species in a highly urban area.

Daniel Kutscher, Dhinesh Asogan, Ian Mudway, Patricia Brekke, Charlie Beales, Xiaohan Wang, Matthew W. Perkins, Wolfgang Maret, and Theodora J. Stewart

Metallomics takes an integrated approach to understanding the metallobiochemistry of cells and organisms in health and disease, utilizing analytical tools to elucidate the biological roles of metals in both space and time (1). In humans, every tenth protein depends on zinc for structure, catalysis, or regulation, and, together with other metal ions, every second protein contains a metal ion essential for its function. Most importantly, both the metal composition and the distribution in cells and tissues change during physiological and pathophysiological processes, with consequences in disease development, progression and treatment (2–4). Every cell utilizes a wide range of metal ions (such as sodium, potassium, magnesium, calcium,
iron, molybdenum, manganese, zinc, and copper) and nonmetals (such as chloride, bromide, iodide, and selenium) in conjunction with the elemental building blocks of biological molecules (such as carbon, oxygen, nitrogen, hydrogen, sulfur, and phosphorus). Thus, depending on their specialized functions and activities, each type of cell has a unique metal ion composition or “metalome.” These attributes can be exploited to distinguish cells with altered characteristics (such as highly proliferative or malignant, diseased, or transitioning toward cell death through regulated or unregulated pathways), or senescent in a range of disease settings (such as dementia, diabetes, and cancer).

Prior to the development of elemental bioimaging achieved through coupling instrumentation, such as laser ablation to inductively coupled plasma–mass spectrometry (ICP-MS), metal analysis of biological samples largely consisted of bulk quantification and speciation approaches. However, it became clear that this bulk tissue concentration level information lacked the spatial resolution necessary to fully explore the roles of biological metals at the cellular and subcellular levels. Advancing

**FIGURE 1:** Procedure for image generation using LA-ICP-MS to avoid potential artefacts. Steps 1 through 4 are illustrated.
ICP-MS Cones: Why, When and How to Maintain

Authors: Ryan Brennan and Justin Masone

Introduction
In this article we highlight why, when and how to properly maintain your ICP-MS interface cones, in addition to the importance of cone conditioning. We also discuss the benefits of Platinum (Pt) tipped cones. These are suggested maintenance guidelines, many of which are borrowed from the ICP-MS manufacturers. Always refer to your instrument manufacturer’s recommended maintenance guidelines.

Why
The most common type of problems associated with ICP-MS cones is related to blockage of the orifice or corrosion. The interface cones are also very fragile, particularly the tip. Damage to the tip changes the diameter of the orifice and performance of the cone. Operating your ICP-MS with cones that are in poor condition can lead to increased background signal, memory effects, loss of sensitivity and poor precision — all of which can lead to interrupted analysis and re-run sample batches, resulting in lost time and lower profits.

When
It is important that the interface cones are regularly inspected. The frequency at which the cones are cleaned depends very much on the application and the workload of the instrument. You want to avoid unnecessary cleaning, as overcleaning the interface cones could lead to shortened lifetime.

If the samples are clean and the usage is low, the cones may only need cleaning monthly. But if the instrument is in continuous use and/or the samples contain high levels of dissolved solids or are highly corrosive, the cones may need daily cleaning.

If analyzing different sample types, and in one sample matrix an element of interest is commonly a higher concentration, but in another matrix that same element of interest is at trace levels, you would want to clean the cones in between analyses, to ensure no cross-contamination.

Cones should be cleaned if there are visible deposits near the orifice or if the orifice is blocked or distorted. Deterioration in the performance of the ICP-MS can also indicate that the cones may need cleaning. Watch for increased background signal, memory effects, loss of sensitivity or distorted peak shapes. A change in the instrument vacuum reading can also indicate cone problems. If the orifice is blocked, the vacuum will increase (pressure decrease), although there will usually be a deterioration in performance before this point. If the vacuum decreases (pressure increases), this could indicate that the orifice is worn and has increased in size. If this happens the cone needs to be replaced.

As the sampler cone is more exposed to the plasma, it will usually need cleaning more frequently than the skimmer cone. If the performance of the instrument does not recover when the cones are cleaned, they may need to be replaced or refurbished (Pt tipped).

How
The method of cleaning also depends on the application. If the samples are relatively clean, a gentle cleaning process is sufficient. But, if the samples contain high levels of dissolved solids or are highly corrosive, a more aggressive cleaning procedure is required.
A dilute Citranox solution is a gentle and effective cleaning agent, and we recommend that it be tried first. If Citranox is not effective, it may be necessary to use a more aggressive cleaning agent such as nitric acid. However, we recommend that nitric acid not be used unless it is necessary. Nitric acid is more corrosive than Citranox, and prolonged use will reduce the lifetime of the cones. Note that even Citranox will attack copper cones so the cones should not be exposed to high concentrations of Citranox or exposed for long periods.

When cleaning cones that have a screw thread, it is important that the thread is not contacted by any corrosive solution. If the thread becomes corroded, the cone may not seal correctly or it may bond to the base and be difficult to remove. And with Pt tipped cones, the thread is likely to wear out before the Pt insert. It is also important to keep the thread in good condition to prevent the possibility of cross-threading and potential damage to the instrument housing.

Glass Expansion developed the ConeGuard Thread Protector to seal the threads and protect them from corrosion during the cleaning process. The ConeGuard simply screws onto the threaded part of the cone, and O’ring seals prevent any solution from coming in contact with the thread. The ConeGuard (P/N 70-803-1024) is shown with a PerkinElmer NexION Pt sampler cone (P/N PE3013-PT) in Figure 1.

![Figure 1. Glass Expansion ConeGuard with NexION Pt Sampler Cone.](image1)

There are three common methods of cleaning cones, visit [www.geicp.com](http://www.geicp.com) for these detailed procedures.

We recommend that you do not use nitric acid any more than is necessary since it will attack the cone materials. If nitric acid is used excessively, the size of the cone orifice may be increased. If this happens, or if the tip is damaged or deformed, then the cone needs to be replaced.

After cleaning your sampler and skimmer cones, it is also important to check the condition of the cone consumable parts. Many sampler and skimmer cones rely on a gasket or O’ring to make a proper seal. Installing a new or recently cleaned cone with a worn gasket or O’ring can prevent a proper vacuum and result in rapid overheating of the cone. Some cones require additional screws for installation within the interface. Heavily corroded screws can result in the same issues and, in some cases, result in elevated iron background since many are made from steel. You do not want to ruin a cone for a $5 O’ring or gasket. We recommend that the sampler gasket be changed each time you change the sampler cone to ensure a proper seal. Regularly check the conditions of the O’rings and replace if they show any signs of cracking.

Pre-soaking the cones in a detergent such as Fluka RBS-25 prior to cleaning with Citranox or nitric acid helps the cleaning process. Fluka is available for purchase through Glass Expansion (P/N FLUKA25).

Always use safety glasses and protective gloves. Be careful when handling the cone – the tip is very easily damaged. Hold the cone by its edge and only use light pressure with your hand when cleaning the tip. Never use tools for cleaning cones. The cleaning process does not necessarily need to reproduce the original as-new polished appearance. Sample deposits need to be removed, but it is not usually a problem if the cone is discolored. This may actually result in a more stable signal.

**Cone Conditioning**

To ensure the lowest background levels of copper and nickel, conditioning cones before use is recommended. A conditioned cone with a uniform coating will also lead to improved long-term stability. Brand-new cones and cones that have gone through cleaning should be conditioned prior to use.

To condition your cones, prepare the following conditioning solutions:

- 1.0 % nitric acid blank
- 50 ppm calcium in 1.0 % nitric acid

![Figure 2. Nickel Plated Nickel Sampler Cone and Nickel Skimmer Cone to suit the Agilent 7900 ICP-MS.](image2)
Install the new cones or recently cleaned cones into the instrument. Turn on the plasma, and establish robust plasma conditions:

1. Aspirate the 50 ppm calcium solution for 10 minutes.
2. Change to 1.0% nitric acid blank solution and aspirate for a further 10 minutes.

Another recommended cone conditioning procedure is to aspirate your highest matrix sample for at least 30 minutes, followed by 10 minutes of aspirating your blank or rinse solution. This method is helpful if your laboratory is analyzing a common matrix each day.

**Platinum Tipped Cones**

When dealing with a high TDS matrix analyzed by ICP-MS, Pt cones are often preferred in place of Ni. The Pt insert runs hotter than Ni, which slows the buildup of salts at the orifice. This allows the ICP-MS analyst to run for longer periods of time before cone maintenance is required. Pt tipped cones are also recommended for analysis of sample matrices with aggressive acids like hydrofluoric (HF), sulfuric (H₂SO₄) and phosphoric (H₃PO₄). For the lowest level detection limits, Pt tipped cones are often used in the semiconductor industry.

For more aggressive samples – e.g., a combination of high TDS and high acid concentration – a sampler cone with a larger diameter Pt insert can be used. For some ICP-MS models a sampler is available with a 10, 15 or 18 mm Pt insert. The larger insert provides a much greater life due to the larger surface area.

One Glass Expansion customer found that the 15 mm and 18 mm inserts would last for 18-24 months compared to 6-8 months with the 10 mm insert, or as long as the orifice was good.

Agilent ICP-MS instruments have a variety of different cone and skimmer insert combinations to help the laboratory choose which cone option is the best suited for their application. As an example, Glass Expansion’s sampler and skimmer cone options for the Agilent 7900 ICP-MS are listed in Table 1 and Table 2, respectively. It is recommended that the Pt skimmer cones be used with a brass skimmer base and Ni skimmer cones be used with a stainless-steel skimmer base.

Selecting the proper skimmer base helps to prevent overheating and prolong the life of the skimmer cone.

<table>
<thead>
<tr>
<th>P/N</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT7701-Ni</td>
<td>Nickel sampler cone</td>
</tr>
<tr>
<td>AT7701-Ni/Ni</td>
<td>Nickel plated Nickel sample cone</td>
</tr>
<tr>
<td>AT7701-Al</td>
<td>Aluminum sampler cone</td>
</tr>
<tr>
<td>AT7706-Pt</td>
<td>Platinum sampler cone</td>
</tr>
<tr>
<td>AT7706A-Pt</td>
<td>Platinum sampler cone (18 mm insert)</td>
</tr>
<tr>
<td>AT7706-Pt-BF</td>
<td>Platinum sampler cone Boron Free</td>
</tr>
<tr>
<td>AT7706-Pt/NiP</td>
<td>Nickel plated Platinum sample cone</td>
</tr>
<tr>
<td>AT7706-Pt/Ni</td>
<td>Platinum sampler cone with Nickel base</td>
</tr>
<tr>
<td>AT7706B-Pt/Ni</td>
<td>Platinum sampler cone (15 mm insert) with Nickel base</td>
</tr>
</tbody>
</table>

**Table 2. Glass Expansion Agilent® 7900 Skimmer Cone Options**

<table>
<thead>
<tr>
<th>P/N</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT7902X-Ni</td>
<td>Nickel skimmer cone for x-lens</td>
</tr>
<tr>
<td>AT7902X-Al</td>
<td>Aluminum skimmer cone for x-lens</td>
</tr>
<tr>
<td>AT7908X-Pt</td>
<td>Platinum skimmer cone Copper base for x-lens</td>
</tr>
<tr>
<td>AT7908X-Pt/Ni</td>
<td>Platinum skimmer cone Nickel base for x-lens</td>
</tr>
<tr>
<td>AT7702S-Ni</td>
<td>Nickel skimmer cone for s-lens</td>
</tr>
<tr>
<td>AT7702S-Cu</td>
<td>Copper skimmer cone for s-lens</td>
</tr>
<tr>
<td>AT7708S-Pt</td>
<td>Platinum skimmer cone Copper base for s-lens</td>
</tr>
<tr>
<td>AT7708S-Pt/Ni</td>
<td>Platinum skimmer cone Nickel base for s-lens</td>
</tr>
</tbody>
</table>
Glass Expansion ICP-MS Cones

Glass Expansion has over 30 years of experience in manufacturing ICP-MS sample introduction components and has earned a worldwide reputation for quality and reliability. We are proud to be the first and only ICP-MS cone manufacturer with the required technology and equipment to produce the full range of ICP-MS cones entirely in-house. Glass Expansion’s manufacturing capabilities for this include: CNC machining, precision grinding, electron beam welding and electroless nickel plating. This results in our company being able to maintain the highest level of precision, quality and reliability standards.

We manufacture ICP-MS cones to the same exacting standards as all of our products, and they are guaranteed to perform to your satisfaction. We supply cones for all of the popular ICP-MS instruments, including models from Agilent®, Nu Instruments, PerkinElmer®, Shimadzu and Thermo Fisher Scientific™. We recently added the Fluidigm® TOF-ICP-MS to our ICP-MS cone catalog. These options, along with the corresponding cone raw material, are listed in Table 3. To make sure you get all the support you need, we have a staff of technical experts and a fully equipped ICP-MS laboratory for testing and evaluation.

Table 3. Glass Expansion Cone Availability

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Ni</th>
<th>Pt</th>
<th>Al</th>
<th>Cu</th>
<th>Pt-Boron Free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent®</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Fluidigm®</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nu Instruments</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PerkinElmer®</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Shimadzu</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Thermo™</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

The next time you are in need of replacement ICP-MS cones, we invite you to take advantage of our No-Risk Guarantee:

- Meet or exceed OEM specifications
- Available for all common ICP-MS models
- Satisfaction guaranteed

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**Head office**
6 Central Boulevard,
Port Melbourne VIC 3207,
Australia
+61 3 9320 1111
enquiries@geicp.com

**Americas**
31 Jonathan Bourne Drive,
Unit 7 Pocasset, MA 02559,
USA
508 563 1800
geusa@geicp.com

**Europe**
Friedenbachstrasse 9,
35781 Weilburg,
Germany
+49 6471 3778517
gembh@geicp.com

Visit www.geicp.com for more information
our understanding of the role of biometals in fundamental biology and disease, therefore, required increased information on the spatial localization of metals in tissues, and this need has been met, to a large extent, through a number of elemental bioimaging techniques including, but not limited to, synchrotron X-ray spectroscopy, secondary ion mass spectrometry (SIMS), and LA-ICP-MS (5).

Materials and Methods
Elemental images or maps refer to an LA-ICP-MS analysis that provides information on the elemental distribution across a sample’s two-dimensional area. An example would be the surface of a biological tissue section. As the laser is fired at the sample surface, the sample is moved at a defined and constant rate. This process allows the time profile of a line scan to be translated into a distance profile. Gathering multiple profiles across the sample generates a 2D image of the elemental distribution in the sample (3D after the vertical reconstruction of sequential 2D image maps), where signal intensity is directly proportional to concentration. To avoid artifacts, the duty cycle of the ICP-MS instrument and the translation speed of the laser must be synchronized. Figure 1 highlights this process of image generation.

To image a given sample, a suitable area or region must be chosen through complementary imaging techniques such as classical histology or unstained microscopy images. Samples are typically provided for analysis as thin sections on microscopy slides. Depending on the sample type and level of structural preservation required, samples are either formalin-fixed paraffin-embedded (FFPE), or cryopreserved with or without embedding material. If an embedding medium is used, a material of suitable purity for trace elements must be selected to avoid unnecessarily high backgrounds.

To balance the analysis time versus image size, the spot size of the laser must be optimized. This parameter affects the spatial resolution of the image and the sensitivity and the speed of the analysis. Larger spot sizes ablate more material, and therefore allow a greater quantity of sample to reach the plasma while the image generation is achieved in a shorter time. Spot sizes of 10–25 µm can provide reasonable spatial resolution depending on tissue type and the biological questions posed. Smaller spot sizes (below 5 µm) provide superior cellular resolution, but require high sensitivity to obtain a signal-to-noise ratio (S/N) of 10, often used as a minimum for quantitative work. Subcellular resolution (below 1 µm) can be obtained through an approach called oversampling (6).

The correct choice of the laser wavelength and carrier gas is also important. A combination of a laser system in the deep ultraviolet (UV) range (such as 193 nm), in combination with helium as a carrier gas, often provides the best results. Several key comparative studies highlight the performance characteristics between 193, 213, and 266 nm laser systems (7–9).

To obtain high-quality images with enough contrast to differentiate detailed structures, it is important that the ICP-MS system used for analysis provide high signal-to-noise ratios for the elements under study. This includes not only detection sensitivity, but also the complete suppression of interferences. Particularly important elements in metallomics studies, such as iron, manganese, copper, or zinc, may be significantly biased by polyatomic and other in-
terferences, such as $^{40}$Ar$^{16}$O$^+$ (on $^{56}$Fe), $^{54}$Fe$^+$ and $^{32}$Fe$^+$ (on $^{55}$Mn), $^{40}$Ar$^{23}$Na$^+$ (on $^{63}$Cu) or $^{32}$S$^2$ on $^{64}$Zn. There is also growing interest in understanding nutrient metabolism, and imaging additional elements such as Se, P, and S, is again biased by significant polyatomic interferences. Complete removal of these interferences in combination with high detection sensitivity is often only achieved using triple quadrupole–based ICP-MS instruments. Typical conditions for analysis often are similar to those summarized in Table I.

**Results**

An increasing body of evidence has linked air pollution exposure to impacts on the brain, ranging from cognitive impairments in childhood (10) to increased incidence of dementia in the aged population (11).

**TABLE I: Typical analysis conditions for a LA-ICP-MS system used for imaging applications**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analysis Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injector</td>
<td>2.5 mm i.d., quartz</td>
</tr>
<tr>
<td>Interface</td>
<td>High sensitivity (2.8 mm) skimmer insert; Ni cones</td>
</tr>
<tr>
<td>RF power</td>
<td>1100 – 1550 W</td>
</tr>
<tr>
<td>CRC flow</td>
<td>TQ-O2 modes: 0.4 mL/min</td>
</tr>
<tr>
<td>LA, Teledyne CETAC Iridia LA System</td>
<td></td>
</tr>
<tr>
<td>Ablation cell gas flow</td>
<td>0.3 – 0.8 L/min He (cell and transport tubing dependent)</td>
</tr>
<tr>
<td>Spot size</td>
<td>5–25 μm</td>
</tr>
<tr>
<td>Scan speed</td>
<td>Depends on laser spot size and either ICP-MS dwell time (traditional imaging) or laser repetition rate (single dosage mode)</td>
</tr>
<tr>
<td>Laser energy</td>
<td>0.5 – 1.0 J/cm²</td>
</tr>
<tr>
<td>Repetition rate</td>
<td>10 Hz, up to 500 Hz possible with fast-washout systems</td>
</tr>
</tbody>
</table>
Metal dyshomeostasis has been implicated in a range of neurodegenerative disorders (12), and there is preliminary evidence linking these disorders to the accumulation of metals in the brains of dogs (13) and humans in highly polluted areas (14). Uncertainties remain, however, about how exogenous metals accumulate in the brain, with some evidence supporting the uptake of metal-rich nanoparticles by the olfactory neurons in the nose to the olfactory bulb region of the brain, and some speculation about particles that have reached the systemic circulation crossing the blood–brain barrier (13–14). Allowing for either uptake pathway, there is even less knowledge about how particles and particle-associated metals move through the parenchyma of the brain, or whether they accumulate. Studying these questions is challenging due to the requirement to address long-term accumulative processes and in the limited access to relevant material in the clinical setting.

To overcome this, the option of using animal sentinel species to determine metal concentrations and distributions within their brains was explored, focusing on urban populations with clear exposure contrasts to traffic-related air pollutants. This study focused on the use of the Eastern gray squirrel (Sciurus carolinensis), because these are routinely culled as part of population control efforts in London and throughout the United Kingdom. Therefore, they provide a unique resource to explore the impact of environmental pollution on long-term metal accumulation in the brain. In this article, preliminary data exploiting LA-ICP-MS to explore elemental patterns within the brains of squirrels collected from inner London (high pollution) and outer London (low pollution) are presented. Following culling, whole brains were removed by pathology technicians at the Zoological Society of London before being snap frozen. The frozen samples were then cryosectioned at 20 μm thickness before air drying on glass microscopy slides prior to LA-ICP-MS at 40 μm resolution for $^{63}$Cu, $^{57}$Fe, and $^{208}$Pb, using $^{31}$P as a morphology indicator.

The representative images are presented in Figure 2. This data are preliminary, but clear contrasts are apparent in the spatial distribution of the selected elements between the low- and high-pollution sites (clearest in panel C). The data show that Cu (often associated with brake dust) is elevated in the olfactory bulb region of animals living in inner London whereas Pb appeared elevated in this brain region in the animals from outer London. Additionally, Cu concentrations appeared to be higher throughout the brains of the squirrels collected from inner London. Overall, Fe appeared higher in the outer London sample with evidence of high concentrations in the mid-brain region and clear high-intensity foci scattered throughout the parenchyma.

As stated, these images at this time are simply indicative of the power of this technique to provide semiquantitative spatial data on metal concentrations within the brain, and it is planned to expand this work to a more comprehensive set of samples to complement our work using traditional ICP-MS analysis of digested samples from discrete brain regions. Ultimately, the power of these techniques requires that elemental maps be related to classical pathohistological examination of the tissues so that metal intensity can be correlated to markers of overt neurological disease, or, through the application of other multi-model analytical techniques, correlated to mechanistic
pathways linking air pollutant exposure to disease initiation and progression.

Conclusions
LA-ICP-MS allows isotopic mapping of a range of elements in biological samples to be performed with high sensitivity and high resolution. One major caveat of LA-ICP-MS is its destructive nature, particularly when applied to biological samples. In contrast to its application in geology, where nanometer-thick layers can be removed from surfaces of hard materials, when applied to elemental bioimaging, it typically completely ablates and removes cells and tissues from their support substrates during the analysis.

The resolution that can now be achieved using LA-ICP-MS, particularly with oversampling approaches, is beginning to open up new applications in subcellular imaging (15), which is still highly dependent on cell size and the concentration of elements of

FIGURE 2: Laser Ablation ICP-MS images of an axial section of representative squirrel brains from inner London (IL) and outer London (OL) at a resolution of 40 μm. Panel (a) shows 31P concentrations from OL, used as a general morphological indicator, with the anatomical brain regions indicated. ‘*’ indicates an area that was used to optimize the ablation method. Panel (b) shows Cu, Fe and Pb concentrations. Panel (c) illustrates the combined elemental distribution in the samples.
interest. In the field of elemental bioimaging, rapid developments in laser hardware, affording smaller spot sizes and therefore higher-resolution mapping, now require the sensitivity to measure such small amounts of material. Depending on the type of ICP-MS (single quadrupole, triple quadrupole, sector field, time-of-flight), sensitivity may be a limiting factor to image endogenous metals at high resolution (such as 1 µm), especially when found in lower concentrations. However, particularly in metallomics, the power of this analytical technique lies in its incorporation into correlative multimodel imaging and molecular mass spectrometry workflows so that information relating to the chemical environment associated with specific elements is characterized and can be placed within the context of specific biological processes and their underlying mechanisms.

Acknowledgments
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References

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Accurate, Low-Level Sulfur Analysis by ICP-MS Using MS/MS with Oxygen Reaction Cell Gas

Sulfur (S) is one of a small group of elements that have historically been difficult to analyze at low levels using conventional inductively coupled plasma–mass spectrometry (ICP-MS). Accurate low-level analysis of sulfur is required in applications in life science, clinical research, pharmaceutical development, food safety, environmental monitoring, geochemistry, and petrochemistry. ICP-MS analysis of sulfur is problematic because of the element’s high first ionization potential, which reduces sensitivity. Also, intense polyatomic interferences affect all the isotopes of sulfur. Background levels are also often high, due to the presence of S in many components used in laboratory equipment. ICP-MS fitted with a collision/reaction cell can use oxygen (O₂) cell gas to mass shift the ³²S⁺ ions to SO⁺ product ions at mass-to-charge (m/z) 48, avoiding the on-mass interferences at m/z 32. However, mass 48 can also be affected by interference from ⁴⁰Ca⁺, ⁴⁰Ti⁺, and ³⁶Ar¹²C⁺, as well as from overlapping product ions such as ³¹P"O⁺. Also, applications such as isotope ratio measurements and isotope dilution mass spectrometry (IDMS) require the accurate determination of a minor isotope, ³⁴S or ³³S, as well as ³²S. We describe how tandem mass spectrometry (MS/MS) controls the O₂ reaction gas chemistry to resolve multiple spectral overlaps on all the sulfur isotopes. Combined with appropriate selection of instrument components to reduce the sulfur background, MS/MS can provide accurate low-level analysis of sulfur and sulfur isotope ratios in aqueous and organic matrices.

Ed McCurdy, Glenn Woods, Bastian Georg, and Naoki Sugiyama

Inductively coupled plasma–mass spectrometry (ICP-MS) is widely used for multielement analysis of trace analytes in a wide variety of sample types. A few elements, including sulfur (S), are difficult to measure using conventional (single) quadrupole ICP-MS.

Applications that require low level sulfur analysis include petrochemical analysis (1,2), the study of biomolecules (such as proteins) in life science research (3,4), detection of sulfur compounds in foods (5), and development of sulfur-based pharmaceuticals (6,7). Accurate determination of sulfur at low concentrations is affected by several factors:

1. Sulfur has a high first ionization energy (or ionization potential) of 10.36 electron volts (eV), meaning it is only around 10% ionized in a normal ICP plasma. This translates into 10x lower sensitivity than for a fully ionized element.

2. The main isotope of sulfur (³²S, 95.04% abundance) suffers intense background overlap from polyatomic ions including ¹⁶O₂⁺ and ¹⁴N¹⁸O⁺, making trace analysis...
impossible by conventional quadrupole ICP-MS.

3. The next most abundant isotope, $^{34}$S, accounts for only 4.2% of total sulfur, meaning a further reduction in sensitivity by a factor of more than 20. This isotope also suffers from intense background interferences, as does the final potentially useful isotope, $^{33}$S (0.75% abundance). The fourth naturally occurring isotope, $^{36}$S, is only 0.015% abundant, and is overlapped by a minor isotope of argon. The most significant background polyatomic ions that overlap the isotopes of sulfur are shown in Table I.

4. Sulfur is present in many laboratory reagents and components, causing background contamination.

The commercial development of collision–reaction cells (CRCs) for ICP-MS in 1997 (8) provided analysts with a novel approach to controlling interferences, solving many of the problematic spectral overlaps that had hampered ICP-MS analysis (9,10). However, a small number of analytes, including sulfur, remained difficult to determine at low concentrations.

Approaches to improve the detection of sulfur have included the use of xenon (Xe) cell gas to reduce the intensity of the $^{16}$O$_2^+$ interference by collisional dissociation and charge transfer (11), and sector field high resolution ICP-MS to mass resolve the O$_2^+$ peak (4). However, the use of xenon cell gas does not give low enough detection limits for trace sulfur analysis, and sector field ICP-MS suffers from poor abundance sensitivity (peak tailing), which compromises the separation of the trace $^{32}$S peak from the much more intense neighboring $^{16}$O$_2^+$ peak.

Oxygen (O$_2$) cell gas can be used in the CRC of a single quadrupole ICP-MS instrument to promote the formation of SO$^+$ product ions. The O$_2^+$ interfering ions at m/z 32 do not react with the O$_2$ cell gas, so S$^+$ can be mass-shifted away from the original O$_2^+$ overlap (12). But the SO$^+$ product ion masses are overlapped by existing ions, including $^{48}$Ca$^+$, $^{48}$Ti$^+$, and $^{36}$Ar$^1$C$^+$ at m/z 48 for $^{32}$S$^{16}$O$^+$. This approach is therefore not suitable for samples that might contain these other interfering elements, such as high Ca mineral waters and other environmental materials, or organic-based samples in petrochemical and life sciences applications (13).

Analysis of sulfur isotope abundance is of interest in several applications in environmental science, geochemistry, and hydrogeology. Such analysis requires interferences to be resolved on at least one of the minor isotopes of sulfur, $^{34}$S or $^{33}$S, as well as the major $^{32}$S isotope. Minor isotopes must also be measured accurately to quantify sulfur using isotope dilution. CRC methods using O$_2$ cell gas with single quadrupole ICP-MS (with or without a bandpass cell) cannot control the formation of interisotope overlaps such as $^{31}$S$^{16}$O$^+$ on $^{34}$S$^{16}$O$^+$, so accurate isotope ratio and isotope dilution analysis that requires analysis of $^{34}$S or $^{33}$S is impossible. Potential matrix-based and interisotope overlaps on the SO$^+$ product ion masses are shown in Table II.

---

**TABLE I:** Potential polyatomic interferences on the main isotopes of sulfur

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Abundance (%)</th>
<th>m/z</th>
<th>Ions Causing Spectral Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{32}$S$^+$</td>
<td>95.04</td>
<td>32</td>
<td>$^{16}$O$_2^+$, $^{14}$N$^{16}$O$^+$, $^{15}$N$^{16}$O$^{1H}$, $^{31}$P$^{1H}$</td>
</tr>
<tr>
<td>$^{33}$S$^+$</td>
<td>0.75</td>
<td>33</td>
<td>$^{32}$S$^{1H}$, $^{16}$O$_2^{1H}$, $^{16}$O$^{15}$O$^+$, $^{15}$N$^{16}$O$^+$, $^{14}$N$^{16}$O$^{1H}$</td>
</tr>
<tr>
<td>$^{34}$S$^+$</td>
<td>4.2</td>
<td>34</td>
<td>$^{12}$S$^{1H}_2$, $^{13}$S$^{1H}$, $^{32}$O$^{16}$O$^+$</td>
</tr>
</tbody>
</table>
The development of triple quadrupole ICP-MS (ICP-QQQ) in 2012 (14) offered a way to address the limitations of reaction mode methods on single quadrupole and bandpass ICP-MS. ICP-QQQ uses an additional quadrupole mass filter (Q1) before the CRC, allowing tandem mass spectrometry (MS/MS) operation. In MS/MS, Q1 functions as a 1 u mass filter to select the specific mass that is passed to the cell. This excludes any ions that might be present at the mass where analyte product ions would appear (15). As each analyte precursor mass is passed to the CRC while excluding all other masses, ICP-MS/MS also resolves interisotope overlaps so accurate isotope ratio and isotope dilution analysis is straightforward. We investigate the potential interferences on sulfur from several common matrix elements and demonstrate ICP-MS/MS conditions that resolve the interferences on the sulfur isotopes of interest. We compare these results with operating the ICP instrument without MS/MS (Q1 >1 u mass window), and show examples of applications where complete resolution of the interferences is needed.

**Experimental**

**Instrumentation and Reagents**

Measurements were performed using an Agilent 8900 ICP-QQQ (model #100, Advanced Applicata-
The instrument was fitted with the standard glass concentric nebulizer, quartz spray chamber, quartz torch with 2.5 mm injector, and nickel sampling and skimmer cones. The instrument model used includes an inert, low contamination argon gas flow path, which reduces levels of sulfur (and silicon) in the gas supplied to the plasma torch. The effectiveness of this inert gas flow path is illustrated by the low ng/L (ppt) detection limit that can be achieved for sulfur in high purity semiconductor reagents (16). Samples were introduced via a peristaltic pump with 1.02 mm i.d. pump tubing. Operating conditions are shown in Table III.

Sulfur standards and matrix element solutions were prepared from single element stock standards (Agilent Technologies). Sulfur isotope standards were prepared from sulfur isotope CRMs IAEA-S-1, S-2, and S-3 (National Institute of Standards and Technology [NIST]). Ultrapure water was prepared in the laboratory using a Milli-Q Element system, and acids used for sample stabilization and matrix preparation were Ultra pure Normatom grade (VWR Chemicals).

Results and Discussion

Comparing MS/MS and Single Quad Mode for Analysis of S as SO\(^{+}\)

The mass-shift approach to resolve interferences has been described extensively (17). To assess the ability of MS/MS to resolve matrix interferences on the sulfur isotopes, mass spectra were acquired covering the region where the \(^{32}\text{S}^{16}\text{O}^{+}\) product ions would appear (m/z 48 for \(^{34}\text{S}^{16}\text{O}^{+}\), m/z 49 for \(^{35}\text{S}^{16}\text{O}^{+}\), and m/z 50 for \(^{33}\text{S}^{16}\text{O}^{+}\)). The same acquisition was performed in MS/MS mode and “single quad” mode, where Q1 is operated as a simple ion guide (no mass filtering). A 10 \(\mu\)g/L (ppb) S standard and separate solutions containing potentially interfering matrix elements Ca, Ti, and C were measured. The overlaid spectra (sulfur standard and matrix solutions) are shown for both modes in Figure 1.

The good isotope template fit and freedom from matrix overlaps for sulfur in MS/MS mode contrasts markedly with the severe overlaps apparent in “single quad” mode. The difference is due to the inability of the single quadrupole to reject the existing Ca\(^{+}\), Ti\(^{+}\), and ArC\(^{+}\) ions at m/z 48 to 50, where the SO\(^{-}\) product ions are measured. In MS/MS, these interfering ions are all rejected by Q1, which is set to pass only the mass of the respective precursor S\(^{+}\) ions, so m/z 32 for \(^{32}\text{S}\), m/z 33 for \(^{33}\text{S}\), and m/z 34 for \(^{34}\text{S}\).

Controlling Product Ion Formation with Q1 in MS/MS Mode

With O-atom addition reactions, the large (16 u) mass difference between the precursor and product ion masses means any existing ions at the product ion masses can be rejected, even if Q1 is operated in bandpass mode (mass window > 1 u). However, existing ion overlaps are not the only potential interferences that can affect the accurate measurement of S as SO\(^{+}\). If the sample contains phosphorus (P), \(^{31}\text{P}^{+}\) ions could also react with the \(\text{O}_2\) cell gas to form \(^{31}\text{P}^{17}\text{O}^{+}\) product ions that would overlap the \(^{32}\text{S}^{16}\text{O}^{+}\) product ions at m/z 48. This is illustrated in Figure 2, where a scan of a sulfur standard (25 \(\mu\)g/L) is shown overlaid with scans of 0.5% H\(_3\)PO\(_4\) and 0.5% HCl. The solutions were measured in MS/MS mode and with Q1 set to pass a 3 u mass window (simulating bandpass operation). In bandpass mode, the P matrix gives rise to an intense interference at m/z 32, while MS/MS ensures effective resolution of the \(^{31}\text{P}^{17}\text{O}^{+}\) product ions. Rejecting \(^{31}\text{P}^{+}\) while allowing transmission of \(^{32}\text{S}^{+}\) requires Q1 to operate as a 1 u mass filter (MS/MS mode).

Sulfur Isotope Analysis in Natural Waters

The relative abundance of the stable (non-radioactive) sulfur isotopes varies a lot in natural mate-
The variation in abundances is calculated as a deviation (or delta, δ) in the abundance of the minor isotope (such as δ\textsuperscript{34}S), expressed in per mil (parts per thousand, ‰). Natural materials such as groundwaters, soil, vegetation, and rocks can contain sulfur isotopes with δ\textsuperscript{34}S values ranging from -40‰ to +40‰, and occasionally more (18). This variation enables researchers to study processes such as sedimentary rock formation and biogeochemical cycles.

High precision sulfur isotope analysis is usually performed using an isotope ratio mass spectrometer (IRMS), such as a thermal ionization mass spectrometer (TIMS), secondary ionization mass spectrometer (SIMS), or multi-collector ICP-MS. However, the large variation in natural sulfur isotope abundances means that an ICP-MS/MS method with O\textsubscript{2} cell gas can measure the differences in S ratios, albeit less precisely.

Determining accurate S isotope abundances by conventional ICP-MS can be affected by errors from interisotope overlaps. Where the mass that enters the CRC is not tightly controlled, multiple sulfur isotopes can react to form product ion overlaps. For example, \textsuperscript{32}S\textsuperscript{18}O\textsuperscript{+} appears at the same mass as \textsuperscript{34}S\textsuperscript{16}O\textsuperscript{+} (m/z 50), so accurate determination of \textsuperscript{34}S abundance is only possible if \textsuperscript{32}S\textsuperscript{+} is excluded from the CRC. As with adjacent mass overlaps (such as \textsuperscript{31}P\textsuperscript{17}O\textsuperscript{+} on \textsuperscript{32}S\textsuperscript{16}O\textsuperscript{+}), resolving

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Overlaid spectra of 10 μg/L (ppb) S standard (gray), 100 μg/L Ca matrix (blue), 100 μg/L Ti matrix (green), and 5% isopropyl alcohol (IPA, pink). (a) ICP-triple quadrupole MS in “single quad” mode (Q1 operated as an ion guide). (b) ICP-MS/MS (Q1 operated as 1 u mass filter). Existing element overlaps on the SO\textsuperscript{+} product ion masses are eliminated with MS/MS, enabling accurate sulfur isotope abundance template match (red outlines).}
\end{figure}
Inter-isotope overlaps require Q1 to operate as a 1 u mass filter (MS/MS).

Sulfur isotopes were measured in a range of mineral water, river water, spring water, and seawater samples, using ICP-MS/MS in mass-shift mode with O₂ cell gas. Sulfur isotope calibration was based on IAEA-S-1, and bracketing standards were used for mass bias correction. The results are summarized in Figure 3. Excellent δ³⁴S precision of less than 0.9‰ SD (n = 10) was obtained. The accuracy of the method was confirmed by the measured δ³⁴S value for NASS 5 seawater (21.5‰), which agreed well with the global average value for oceanic seawater of 21‰ (18).

Sulfur isotope analysis in geochemistry applications may require the measurement of the ³³S:³²S ratio as well as ³⁴S:³²S. The ³³S isotope can be measured as ³³S¹⁶O⁻ using ICP-MS, but the large difference in abundance between ³²S and ³³S isotopes can cause errors in the analysis of the minor isotope. In ICP-MS/MS analysis using O₂ mass shift, ³³S must be excluded from the CRC when ³²S is measured. This separation can

**FIGURE 2:** Overlaid spectra of 25 µg/L (ppb) S standard (gray) and 0.5% H₃PO₄ matrix (pink); 0.5% HCl matrix (green) also shown. (a): ICP-triple quadrupole MS in “bandpass” mode (Q1 set to 3 u mass window). (b): ICP-MS/MS (Q1 operated as 1 u mass filter). PO⁺ product ion overlap on SO⁻ is eliminated with MS/MS, as confirmed by accurate S isotope abundance template match (red outlines).
be degraded either by poor Q1 resolution (mass window >1 u), or by poor abundance sensitivity (peak tail of $^{32}$S overlaps $^{33}$S). In both cases, the $^{32}$S peak would contribute to the signal at the adjacent mass 33, affecting the $^{33}$S:$^{32}$S ratio.

This potential error was assessed by measuring digested sulfide minerals and calculating the mass-independent deviation in the measured abundance of the $^{33}$S isotope ($\delta^{33}$S). Mass independent fractionation of sulfur isotopes rarely occurs naturally, so the defect in $^{33}$S abundance calculated from $\Delta^{34}$S = $\delta^{33}$/15S - $\delta^{34}$/15S x 0.515 should equal zero, assuming equilibrium fractionation (19). A measured value of zero (within experimental error) therefore indicates no contribution from $^{32}$S on $^{33}$S. The results obtained with ICP-MS/MS are shown in Table IV, with a $\delta^{33}$S of -0.1 confirming no contribution from $^{32}$S on $^{33}$S measured as the $^{33}$S$^{16}$O$^+$ product ion.

**Conclusions**

ICP-MS/MS using O$_2$ cell gas to mass shift the S$^+$ ions to their respective SO$^+$ product ion masses provides the opportunity to determine sulfur accurately at lower levels than was previously possible by ICP-MS. By allowing only the target S$^+$ precursor ion into the CRC, MS/MS eliminates potential interferences from other reactive ions (such as $^{31}$P$^+$) and from existing ions at the product ion masses (such as $^{48}$Ca$^+$, $^{48}$Ti$^+$, and $^{36}$Ar$^{12}$C$^+$).

MS/MS also excludes the possibility of interisotope overlaps, enabling accurate sulfur isotope analysis in complex sample matrices. These measurements cannot be performed successfully using conventional single quadrupole or bandpass ICP-MS.

**References**

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TABLE IV: Calculated defect ($\Delta^{33}\text{S}$) in measured $^{33}\text{S}$ isotope abundance, confirming no mass-independent fractionation ($^{32}\text{S}$ did not contribute to $^{33}\text{S}$ signal using ICP-MS/MS)

<table>
<thead>
<tr>
<th></th>
<th>$\delta^{33/32}\text{S} (\text{‰})$</th>
<th>$\delta^{34/32}\text{S} (\text{‰})$</th>
<th>$\Delta^{33}\text{S} (\text{‰})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average ($n=7$)</td>
<td>0.1</td>
<td>0.4</td>
<td>-0.1</td>
</tr>
<tr>
<td>2 $\times$ SD</td>
<td>2.8</td>
<td>2.1</td>
<td>1.9</td>
</tr>
</tbody>
</table>


**Ed McCurdy** and **Glenn Woods** are with Agilent Technologies LDA (UK) Ltd, in the United Kingdom. **Bastian Georg** is with Agilent Technologies in Canada. **Naoki Sugiyama** is with Agilent Technologies International in Japan. Direct correspondence to: ed_mccurdy@agilent.com. •
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