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Graphical Abstract

Triple-quadrupole ICP-MS using O₂ mass-shift technology is superior for removing gadolinium interference on selenium in serum.
Determinantion of selenium in serum in the presence of gadolinium with ICP-QQQ-MS

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Gadolinium (Gd)-based magnetic resonance imaging (MRI) contrasting agents interfere with the determination of Se when analysed by single quadrupole inductively coupled plasma-mass spectrometry (ICP-MS). This paper demonstrates that an ICP-triple quadrupole-MS (ICP-QQQ-MS) with oxygen mass shift overcomes Gd⁺⁺ interference on Se⁻ and mitigates typically encountered matrix and spectral based interferences. Normal human serum was diluted in a solution containing isopropanol, EDTA, NH₄OH and Triton X-100. Samples were unspiked (control) serum; serum spiked with 0.127 µmol L⁻¹ Se or 127 µmol L⁻¹ Gd; and serum spiked with both 0.127 µmol L⁻¹ Se and 127 µmol L⁻¹ Gd. Consideration of collision/reaction gases and conditions for interference mitigation included helium (He); a ‘low’ and ‘high’ hydrogen (H₂) flow, and oxygen (O₂). The instrument tune for O₂ was optimised for effective elimination of interferences via a mass shift reaction of Se⁺ to SeO⁻. The ICP-QQQ-MS was capable of detecting trace (> 9.34 nmol L⁻¹) levels of Se in serum in the presence of Gd in our simulated post-MRI serum sample. The multi-tune capabilities of the ICP-QQQ-MS may be adapted to eliminate other specific isobaric interferences that cause false positive results in other analyses where the analyte is confounded by doubly charged and/or polyatomic species.

1. Introduction

Selenium (Se) is an essential trace element that is incorporated into the amino acid backbone of proteins as selenocysteine. Se has structural and enzymatic roles in 25 known human selenoproteins.¹-³ These proteins are antioxidants; involved in the production of active thyroid hormone; roles in immune function; control of viruses such as HIV; prevention of cancer;⁴ and are important for reproductive health.⁵-⁶ Selenium deficiency has been associated with diseases affecting nutrient absorption including coeliac disease, some types of cancer, restricted diets and Keshan disease.⁷-¹² Additionally, a significant decline in blood Se levels with age has been reported.¹³ Stated serum levels vary geographically. For example, in healthy European populations levels ranged from 0.5 to 1.2 µmol L⁻¹ of Se.¹⁵-¹⁷ while levels as low as 0.34 µmol L⁻¹ have been reported in populations where Keshan disease occurs.¹⁸

Single quadrupole inductively coupled plasma-mass spectrometry (SQ-ICP-MS) is the preferred standard analytical method for analysis of most elements in biological fluids, and represents 95% of all commercial ICP-MS instruments.¹⁹ The two most abundant isotopes are ⁸⁰Se and ⁷⁸Se, with ⁷⁸Se the preferred isotope pre-collision cell technology along with interference equation correction, due to less isobaric interferences from the argon plasma and the sample matrix (Table 1), whilst ⁷⁸Se is the preferred isotope when using collision cells. Gadolinium (Gd) is widely used in contrasting agents for magnetic resonance imaging (MRI) of the vasculature and tumours of the central nervous system,²⁰ and interferes with the major isotopes of Se due to doubly charged Gd species.²¹-²³

Consequently, incorrect diagnosis of Se toxicity may arise from the presence of high levels of circulating Gd. Total elimination of circulating Gd may be in the order of days or longer for those with impaired renal function.²⁴ For example, in 2008, a clinical urine sample measured by ICP-MS returned a Se concentration of 16.8 µmol L⁻¹, a level associated with acute Se poisoning. This measurement was an artefact resulting from an MRI procedure performed on the day of testing using a Gd contrasting agent.²⁵

Interference on Se is typically managed by a collision/reaction cell which removes polyatomic species with kinetic energy discrimination or by chemically induced dissociation, providing reduced backgrounds and improved limits of detection and quantification.²¹,²² The higher mass resolving power of double-focusing sector field mass-spectrometers (ICP-SF-MS) is an alternative to reducing the impact of polyatomic and isobaric interferences on mass measurements.²⁶-²⁸ However, ICP-SF-MS are costly, complex and high resolution settings compromise the sensitivity of the analysis when resolving Ar dimers from Se. Comparisons of SQ-ICP-MS with a collision/reaction cell against a SF-ICP-MS demonstrated that ⁸⁰Se⁺ could not be resolved from ⁴⁰Ar⁺⁺ in the...
high-resolution SF-ICP-MS and the SQ-ICP-MS had superior
isotope ratio precision and a lower LOD.30 Mathematical
corrections may also be used to remove the signal from doubly
charged and polyatomic species, but are less accurate than
removing interfering species by physical methods.

The triple quadruple ICP-MS (ICP-QQQ-MS) reduces
interferences by operating in either standard single quadrupole
(SQ) mode or tandem MS/MS. In the MS/MS mode a
quadrupole (Q1) filters the mass-to-charge ratio (m/z) of
interest prior to introduction into an ion-guide (Q2), which has
the option to be filled with a collision and/or reaction gas.
The final quadrupole (Q3) again filters the desired analyte, either
on its original mass or the known reaction product.30 O2 may be
used to react with kinetically favoured analytes to an MO∗ mass
filtered by Q3, removing all other interfering species. This
approach was applied to the measurement of selenoproteins in
rat serum by liquid chromatography (LC)-ICP-MS/MS31 and
arsenic (As) and Se in food.32 This MO∗ mass shift improved
detection limits for phosphorus (P), sulfur (S) and silicon (Si)
over those obtained from isotope dilution (ID)-ICP-SF-MS in
both aqueous33 and organic matrices.34,35 O2 reaction might
produce unwanted polyatomic species, then alternative reaction
/ collision gases may be used, e.g. NH3/He for the ultra-trace
detection of titanium (Ti; as Ti(NH3)6+) in biological fluids.36

This paper describes the analysis of clinically relevant
concentrations of Se in serum samples in the presence of Gd via
an O2 mass shift approach.

2. Experimental

2.1 Instrumentation

All analyses were performed using an Agilent Technologies
8800 Series ICP-QQQ-MS (Mulgrave, Victoria, Australia).
Four tune modes were evaluated; (tune mode 1) high energy
helium (HE-He), 10 mL min-1 He flow; (tune mode 2) H2 at a
low cell flow (low H2), 5 mL min-1 H2 flow; (tune mode 3) high
flow H2 (high H2), 9 mL min-1 H2 flow; and (tune mode 4) O2,
0.28 mL min-1 O2 flow. Gd (m/z = 156, 157) was measured on
mass for tune modes 1–3. Se m/z 77 and 78 were analysed for
the HE-He tune, and Se m/z 77, 78 and 82 for the two H2 tune
modes. The elements were measured with a 40O mass shift with
the O2 tune (m/z 77→93, 78→94, 80→96, 82→98, 156→172,
157→173). The octopole bias (OctP Bias) and the energy
discrimination were optimised for each tune mode (see Table
2).

| Table 1 | Target Se isotopes and potential interferences. Adapted from
| | May and Wiedenmeyer.39 |
| Isotope | Natural abundance (%) | Potential molecular interferences |
| 34Se | 0.89 | 32Cl, 34Ar, 34ArH, 34ArH2, 36ArH2 |
| 36Se | 9.37 | 32Cl, 36Ar, 36ArH, 36ArH2 |
| 77Se | 7.63 | 32Cl, 36Ar, 36ArH, 36ArH2 |
| 79Se | 23.8 | 32Cl, 36Ar, 36ArH, 36ArH2 |
| 80Se | 49.6 | 32Cl, 36Ar, 36ArH, 36ArH2 |
| 82Se | 7.35 | 32Cl, 36Ar, 36ArH, 36ArH2 |

2.2 Reagents

Human serum, A.C.S.-grade EDTA-acid, Triton X-100 and
NH4OH were obtained from Sigma Aldrich (Castle Hill, New
South Wales, Australia). HPLC-grade isopropanol was
purchased from Chem-Supply (Gilman, South Australia,
Australia), 99.999% purity O2, He, and H2 from BOC (North
Ryde, New South Wales, Australia), and high purity Se, Gd and
tellurium (Te) standards from Choice Analytical ( Thornleigh,
New South Wales, Australia).

2.3 Sample preparation

The sample preparation was modified from that described by
Burri and Haldimann.35 Briefly, a diluent containing 4% isopropanol, 0.1% EDTA, 0.1% Triton X-100 and 2% NH4OH
was prepared. Te was added to this solution as the internal
standard to a final concentration of 0.130 μmol L-1 in the
sample. 0.25 mL of serum was mixed with 2.5 mL of diluent
with the volume made to 5.0 mL with MilliQ water and element
of interest prior to introduction into an ion-guide (Q2), which has
the option to be filled with a collision and/or reaction gas.
The final quadrupole (Q3) again filters the desired analyte, either
on its original mass or the known reaction product.30 O2 may be
used to react with kinetically favoured analytes to an MO∗ mass
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facilitate desirable in-cell reactions, a low negative voltage OCP bias accelerated ions into the cell, and a slight positive bias limited in-cell products from exiting the cell, providing more time for reaction. Tune mode 2 used an \( \text{H}_2 \) flow rate of 5.0 mL min\(^{-1} \) (standard conditions for Se determination, to reduce the \( ^{40}\text{Ar}^{16}\text{Ar}^+ \) dimer at \( m/z \ 78 \)), and tune mode 3 used a higher flow rate (9.0 mL min\(^{-1} \)), determined experimentally as the optimal rate to reduce \(^{156}\text{Gd}^{++} \) interference on \( m/z \ 78 \), whilst maintaining adequate sensitivity for \(^{78}\text{Se} \) (Figure 1). In the case of tune mode 4 (\( \text{O}_2 \) mass shift), the energy and cell gas flow was optimised to maximise the low yield endothermic \( \text{Se}^+ + \text{O} \rightarrow \text{SeO}^+ \) reaction (\( \delta \ 0.69 \) eV), and to minimise the exothermic reaction of \( \text{Gd}^+ + \text{O} \rightarrow \text{GdO}^+ \), \( \delta \ 2.39 \) eV). Energy discrimination of -5 V was experimentally determined to minimise \(^{156}\text{Gd}^{3+} \text{O}^+ \) and maximise \(^{78}\text{Se}^{16}O^+ \) passage through the collision/reaction cell and into Q3. The first mass filter (Q1) limited the transmission to the cell of plasma generated ions to the selected mass. The results are shown in Table 3.

### Table 3: Concentration of Se (± 1 standard deviation) in samples per tune mode (\( \mu \text{mol L}^{-1} \)).

| Serum blank | \(^{77}\text{Se} \) HE-HE | \(^{78}\text{Se} \) HE-HE | \(^{78}\text{Se} \) Low \( \text{H}_2 \) | \(^{78}\text{Se} \) Low \( \text{H}_2 \) | \(^{78}\text{Se} \) High \( \text{H}_2 \) | \(^{78}\text{Se} \) High \( \text{H}_2 \) | \(^{82}\text{Se} \) O\(_2\) | \(^{80}\text{Se} \) O\(_2\) | \(^{42}\text{Se} \) O\(_2\) |
|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 0.0524±0.00065 | 0.0528±0.00061 | 0.0405±0.00051 | 0.0445±0.0046 | 0.0506±0.0050 | 0.0478±0.0046 | 0.0478±0.0046 | 0.0534±0.0050 | 0.0532±0.0047 | 0.0520±0.0045 |

### Table 4: Limits of analysis (\( \mu \text{mol L}^{-1} \)) for each tune mode. \( \text{NA} \) = not acquired.

| \(^{77}\text{Se} \) | \(^{78}\text{Se} \) Low \( \text{H}_2 \) | \(^{78}\text{Se} \) High \( \text{H}_2 \) | \(^{82}\text{Se} \) O\(_2\) | \(^{80}\text{Se} \) O\(_2\) | \(^{42}\text{Se} \) O\(_2\) |
|----------------|----------------|----------------|----------------|----------------|----------------|
| LOD | 0.00945 | 0.00982 | 0.00944 | 0.0102 |
| LOQ | 0.0315 | 0.0327 | 0.0314 | 0.0341 |

The isotopes of Se that may be determined are shown in Table 1. The most commonly measured isotope is \(^{78}\text{Se} \) (23.8% NA), graphically represented in Figure 2a-d for each tune mode. The presence of the \( \text{Gd}^{++} \) interference was not mitigated by the HE-
He tune (tune mode 1), the low H₂ tune (tune mode 2), or the high flow H₂ tune (tune mode 3), with Se concentrations
overestimated by up to 20 times. The O₂-induced mass shift using tune mode 4 sufficiently removed doubly-charged Gd interference, which also allowed measurement of the more highly-abundant 78Se isotope by shifting the measured m/z away from the 40Ar²⁺ interference (Figure 2e). The O₂ mass shift (tune mode 4) was clearly the most accurate and precise method with recoveries of 99.7-101.8% for 79Se, 80Se, 81Se and 82Se from Se + Gd spiked serum samples.

Helium is often used as a collision gas to reduce interferences on the majority of elements. Tune mode 1 (HE-He) removed the ArAr interference on 77,78Se⁺, however it did not eliminate the 154,156Gd²⁺ signal. This tune did not remove the 40Ar²⁺Ar⁺ interference on 80Se⁺, whilst 82Se⁺ was not examined due to the high number of polyatomic interferences arising from the biological matrix (Table 1).

Tune mode 2 (low H₂) effectively removed the polyatomic interferences, but was unable to sufficiently reduce the interference caused by the Gd⁷⁺ ions. Tune mode 3 (high H₂) mitigated the polyatomic interferences on 77,78Se⁺ and eliminated the 154Gd²⁺ (NA 2.18%) interference on 80Se⁺ (p < 0.05). Tune mode 3 failed to eliminate the 156Gd²⁺ (NA 20.5%) interference on 78Se⁺. This contrasts with Harrington et al. who found that H₂ in the collision cell with a flow rate of 3.26 mL min⁻¹ removed Gd²⁺ interference on the Se signal in serum due to the concentration of Se in the form of a mixture of elements higher than this study (0.127 μmol L⁻¹ vs 1.01-3.56 μmol L⁻¹). Similarly, Jackson et al. 35 removed the interference of 156Gd²⁺ on the measurement of 78Se⁺ with a H₂ cell gas flow of 6 mL min⁻¹ in food samples, also due to high to relatively high concentrations of Se; and a Gd spike 200 times lower than our simulation of post-MRI serum Gd concentration.

Other concerns with H₂ as a reaction gas include patients with high levels of circulating bromine, arising from bromhexine hydrochloride, a common ingredient in expectorants. H₂ reacts with 79Br⁻ and 81Br⁻ to form isobaric interferences on 79Se⁺ and 81Se⁺, respectively. Deuterium has been used to overcome BrH⁺ interferences, 42 though high expense limits its practical usage.

Tune mode 4 maximised the formation of Se₁⁰O²⁺ adducts and minimised the influence of doubly-charged Gd species on Se detection across all isotopes and was the superior method for Se determination, irrespective of isotope. Others have increased the yield of SeO²⁻ with mixed gases of H₂ and O₂ in the collision cell 35. We also trialled mixed cell gases and did not observe any benefit.

Minimising unwanted masses entering, and preventing undesirable interactions, in the collision cell, is a significant feature of the ICP-QQQ-MS. For example Se⁺ may be removed from the ion path before it reaches the collision cell, where it could potentially form kinetically favourable species such as 3²S₂O³⁻. The ICP-QQQ-MS may also find application in method validation strategies by ensuring isotopically pure signals particularly for analytes known to be confounded by polyatomic or isobaric interferences.

5. Conclusions

Gd-based MRI contrasting agents interfere with Se analyses by ICP-MS. Reaction with O₂ using the ICP-QQQ-MS allowed a mass shift reaction of Se, which enabled detection of all major isotopes of Se with adequate sensitivity in the presence of Gd. Additionally, this approach overcame interferences from Ar dimers, further improving the sensitivity of the analysis. ICP-QQQ-MS has the unique capability to selectively isolate ions of interest from interferences or confounding signals at low concentrations, as demonstrated in this simulated scenario of Gd interference on serum Se levels.

Notes and references

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1. G. V. Krylov, Science, 2003, 300, 1439-1443.
2. S. J. Fairweather-Tait, Y. Bao, M. R. Broadley, R. Collings, D. Ford, J. E. Hesketh and R. Hurst, Antioxid. Redox. Signal., 2011, 14, 1337-1383.
3. K. M. Brown and J. R. Arthur, Public Health Nutr., 2007, 10, 635-645.
4. D. L. Hatfield, P. A. Tsuji, B. A. Carlson and V. N. Gladyshnev, Trends Biochem. Sci., 2014, 39, 112-120.
5. I. L. Heras, M. Palomo and Y. Madrid, Analyst, 2011, 14B, 1717-1727.
6. M. Roman, P. Jitaru, M. Agostini, G. Cozzi, S. Pucciarelli, D. Nitti, C. Bedin and C. Barbante, Microchem. J., 2012, 105, 124-132.
7. J. Salonen, G. Alfthan, J. Huttunen, J. Pikkarainen and P. Puska, Lancet, 1982, 320, 175-179.
8. G. N. Schrauzer, D. A. White and C. J. Schneider, Bioinorg. Chem., 1977, 7, 23-34.
9. G. N. Schrauzer, D. A. White and C. J. Schneider, Bioinorg. Chem., 1978, 8, 387-396.
10. T. D. Shultz and J. E. Leklem, J. Clin. Chem., 1983, 37, 114-118.
11. P. Collin, K. Kaukinen, M. Välimäki and J. Salmi, Endocr. Rev., 2002, 23, 464-483.
12. I. Hafström, B. Ringertz, A. Spängberg, L. von Zweigbergk, S. Brannemark, I. Nylander, J. Rönnelid, L. Laasonen and L. Klareskog, Rheumatology, 2001, 40, 1175-1179.
13. M. A. Reeves and P. R. Hoffmann, Cell. Mol. Life Sci., 2009, 66, 2457.
14. J. L. Llosalzo, N. Engl. J. Med., 2014, 370, 1756-1760.
15. S. Letsiou, T. Nomikos, D. Panagiotakos, S. Pergantos, E. Fragopoulou, S. Antonopoulou, C. Pitsavos and C. Stefanadis, Biol. Trace Elem. Res., 2009, 12B, 8-17.
16. M. P. Rayman, Lancet, 2000, 356, 233-241.
17. M. Rökgauer, J. Klein and J. D. Kruse-Jarres, J. Trace Elem. Med. Biol., 1997, 11, 92-98.
18. A. T. Diplock, Am. J. Clin. Nutr., 1993, 57, 256S-258S.
19. D. Potter, J. Anal. At. Spectrom., 2008, 23, 690-693.
20. J. M. Idee, M. Port, I. Raynal, M. Schaefer, S. Le Greneur and C. Corot, Fundam. Clin. Pharmacol., 2006, 20, 563-576.
21. C. F. Harrington, A. Walter, S. Nelms and A. Taylor, Ann. Clin. Biochem., 2014, 51, 386-391.
22. A. Walter, S. Nelms, C. F. Harrington and A. Taylor, Ann. Clin. Biochem., 2011, 48, 176-177.
23. A. J. Steuerwald, P. J. Parsons, J. G. Arnason, Z. Chen, C. M. Peterson and G. M. B. Louis, J. Anal. At. Spectrom., 2013, 28, 821-830.
24. S. Aime and P. Caravan, J. Magn. Reson. Imaging, 2009, 30, 1259-1267.
25. L. Hinojosa Reyes, J. M. Marchante-Gayon, J. I. Garcia Alonso and A. Sanz-Medel, J. Anal. At. Spectrom., 2003, 18, 31-16.
26. A. M. Featherstone, A. T. Townsend, G. A. Jacobson and G. M. Peterson, Anal. Chim. Acta, 2004, 512, 319-327.
27. C. S. Muniz, J. M. Larchante-Gayon, J. I. G. Alonso and A. Sanz-Medel, J. Anal. At. Spectrom., 1999, 14, 193-198.
28. G. A. Jacobson, Y. C. Tong, A. T. Townsend, A. M. Featherstone, M. Ball, I. K. Robertson and G. M. Peterson, Eur. J. Clin. Nutr., 2007, 61, 1057-1063.
29. T. W. May and R. H. Wiedmeyer, At. Spectrosc., 1998, 19, 150-155.
30. N. Elwaer and H. Hintelmann, Talanta, 2008, 75, 205-214.
31. A. L. Gray and J. G. Williams, J. Anal. At. Spectrom., 1987, 2, 81-82.
32. J. Goossens, L. Moens and R. Dams, Talanta, 1994, 41, 187-193.
33. L. Balcaen, G. Woods, M. Resano and F. Vanhaecke, J. Anal. At. Spectrom., 2013, 28, 33.
34. Y. Amin, Y. Hatakeyama, M. Tokumoto and Y. Ogra, Anal. Sci., 2013, 29, 787-792.