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Quantification of selenium-tagged proteins in human plasma using species-unspecific isotope dilution ICP-DRC-*q*MS coupled on-line with anion exchange chromatography†

Ming Xu,[a] Limin Yang[a] and Qiuquan Wang*[ab]

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Inductively coupled plasma dynamic reaction cell-quadrupole mass spectrometry (ICP-DRC-*q*MS) coupled on-line with anion exchange chromatography (AEC) has been developed for the quantification of selenium-tagged proteins in human plasma. Methane was employed as a reaction gas in the dynamic reaction cell to achieve the determination of ^{80}Se free of spectroscopic interference from $^{40}Ar_2^+$ and $^{79}BrH^+$. Five selenium species including selenoprotein P (SelP), glutathione peroxidase (GPx), selenoalbumin (SeAlb), and two unknown selenospecies (U1 and U2) in a pooled plasma sample from five healthy people were separated using AEC, and the distribution of selenium in SelP, GPx, SeAlb, U1 and U2 (about 45.5%, 19.1%, 15.1%, 2.9% and 8.1%) was determined by ICP-DRC-*q*MS using species-unspecific isotope dilution ($^{80}Se/^{77}Se$). Based on the detection limit (DL) of selenium (0.54 ng mL^{-1}), we estimated that the DLs for SelP and GPx were 0.59 pmol mL^{-1} and 1.7 pmol mL^{-1}, respectively. Through the stoichiometry of the selenium atom in the selenium-tagged proteins, SelP (2.7 ± 0.1 μg mL^{-1}) and GPx (5.4 ± 0.2 μg mL^{-1}) were successfully quantified.

Introduction

Selenium, an essential trace element with important biochemical functions when it is in a reasonable concentration range, plays a key role in human health, while its deficiency or overabundance causes disease.[1,2] In the past few decades, about 25 selenoproteins have been identified which are distributed in different tissues in the human body.[3,4] Among these, three selenium-tagged proteins, namely selenoprotein P (SelP), glutathione peroxidase (GPx) and selenoalbumin (SeAlb), have been identified in human plasma so far.[5] SelP and GPx belong to selenoproteins in which the selenium is incorporated as selenocysteine while SeAlb is a nonspecific selenium-containing protein in which selenium is incorporated as selenomethionine in place of methionine. Compared with using the total selenium concentration or GPx activity in human blood plasma and/or serum to evaluate the selenium status in the body, the quantitative concentrations of specific selenium-tagged proteins provide more significant information concerning physiological changes and the relationship between the level of specific selenium-tagged proteins and diseases.[6,7] However, quantification of selenium-tagged proteins in human plasma is sometimes difficult and a time-consuming process and, therefore, still remains a challenge in physiological research and clinical diagnosis.

Although ICPMS has been widely used in the analysis of selenoprotiens, it suffers spectroscopic interference from polyatomic ions formed by the presence of argon and the sample matrix.[8] For example, determination of the major selenium isotope (^{80}Se) is hampered by the spectroscopic interference originating from the $^{40}Ar_2^+$ dimmer and $^{79}BrH^+$. To eliminate this spectroscopic interference, methane was employed in the dynamic reaction cell (DRC) for realizing accurate quantification of selenium.[8–10]

In the articles published about analysis of selenoproteins in human plasma and/or serum, size exclusion chromatography (SEC), affinity chromatography (AC) and anion exchange chromatography (AEC) are frequently used.[11–16] However, SEC cannot provide precise quantitative results for selenoproteins because its poor resolving power results in overlap between high abundance selenium-containing proteins (SeAlb) and selenoproteins.[15] In AC, the non-retained target selenoprotein (GPx) is eluted together with the unwanted matrix, causing difficulties for the accurate quantification of selenium in the GPx.[14] Compared with the former two chromatographic methods, AEC is more suitable for separating selenoproteins because the surface charge of different selenoprotein molecules is discrepant under a certain pH condition. Based on the principle of ion exchange in AEC, different kinds of selenoprotein could be separated.[14] Unfortunately, previous attempts of separating selenoproteins by AEC were not fully satisfied. Recently, the principle of anion exchange was applied in the analysis of selenoproteins combined with AC to alleviate the spectral interferences of Cl and Br, making it possible to use a conventional ICPMS without DRC and/or a collision cell (CC).[17,18] Relative to the somewhat complicated on-line and off-line procedures which need sophisticated design and skillful technicians, a simpler and easier method is needed for a broad and routine application.

[a]*Department of Chemistry & the MOE Key Laboratory of Analytical Sciences, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China. E-mail: qqwang@xmu.edu.cn; Fax: +86 5922181796; Tel: +86 5922181796*
[b]*State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, 361005, China*

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In this study, a novel AEC/ICP-DRC-*q*MS was developed so as to allow effective separation of SelP, GPx and SeAlb in a single run, and determination of the most abundant selenium isotope ^{80}Se without the possible polyatomic interference. Post-column species-unspecific isotope dilution analysis was employed for quantifying the amount of selenium in seleno- and selenium-containing proteins. Finally, the newly established method was applied to quantification of selenoproteins in a pooled human plasma sample of five healthy people.

Experimental

Apparatus

Chromatographic separations were carried out on a SERIES 200 HPLC system (PerkinElmer, SCIEX, Canada). An anion exchange column (ProPac SAX-10, 2.0 I.D. × 250 mm in length, 10 μm particle size, Dionex, Sunnyvale, USA) was used to separate the selenium species in human plasma. The effluent from the column was mixed with the enriched ^{77}Se spike solution continuously pumped by a syringe pump (Cole-Parmer, East Bunker Court Vernon Hills, USA) through a three-way connector for on-line determination using an ELAN DRC II ICPMS (PerkinElmer, SCIEX, Canada) equipped with a concentric pneumatic nebulizer and a Cyclonic spray chamber. Size exclusion chromatography was performed with a ZORBAX Bio Series GF-250 column (Agilent Technologies, UK). To obtain the best sensitivity, parameters such as lens voltage and nebulizer gas flow were optimized daily. Deadtime of the detector caused by high count rates was corrected using a Deadtime Correction function in the software. The CH_4 (99.999%) and O_2 (99.999%) used as reaction gases were purchased from the Beijing AP Beifen Gases Industry Co. (Beijing, China). To analysis the total selenium content in human plasma, steel liner PTFE pressure digestion vessels were used to digest the human plasma samples.

Reagents and materials

All reagents used were at least of analytical-reagent or high-purity grade in this study. A stock standard solution of 1007 μg mL^{-1} of Se stabilized in 2.2% (v/v) nitric acid for ICPMS was purchased from Sigma–Aldrich (Saint Louis, Missouri, USA). Seleno-DL-methionine, seleno-DL-cystine, GPx from bovine erythrocytes, and albumin from human serum were also purchased from Sigma–Aldrich. Sodium selenite and sodium selenate were purchased from Beijing Chemical Reagent Co. Ltd. (Beijing, China). For the purification of SelP, heparin-sepharose CL-6B (Pharmacia, Uppsala, Sweden) was used. Tris-(hydroxymethyl) aminomethane and ammonium acetate (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were used to prepare the mobile phase. Mobile phase and other solutions were all prepared with ultra-pure water (18.2 MΩ cm, Pen-Tung Sah Micro-Electro-Mechanical Systems Research Center of Xiamen University, China).

The enriched isotopic spike solution of ^{77}Se was prepared by dissolving approximately 2 mg enriched ^{77}Se powder (99.66%, Cambridge Isotope Laboratories, Inc., USA) in high-purity grade nitric acid. Then the solution was diluted to 100 mL with ultra-pure water as a stock solution. The exact concentration of the spike solution (2.66 μg mL^{-1}) was determined by reverse isotope dilution analysis.

Human plasma samples from healthy persons were purchased from Xiamen Blood Center as fresh frozen plasma. All plasma samples were stored at −20 °C until analysis.

Procedures

Purification of selenoprotein P by affinity chromatography. Because SelP was not commercially available, it was purified from human plasma using affinity chromatography as described previously.[11,19] Briefly, 10 mL plasma was diluted with an equal volume of 10 mM Na_2HPO_4 + 150 mM NaCl buffer (pH = 7.4) and applied to a glass column (1.0 I.D. × 20 cm in length) packed with 3 g of heparin-sepharose CL-6B. After being equilibrated with 30 mL 10 mM Na_2HPO_4 + 150 mM NaCl buffer on a Shimadzu LC-10A_{DVP} HPLC (Kyoto, Japan) equipped with a SPD-M10A detector, SelP on the column was eluted at a flow rate of 1.0 mL min^{-1} by 20 mM Tris + 800 mM NH_4Ac buffer (pH = 7.4) and collected under 280 nm monitoring. It was confirmed using SDS-PAGE and ICP-DRC-*q*MS (Fig. S1 and S2†) and stored at −20 °C before use.

Anion exchange chromatography of selenium species. The chromatographic conditions were optimized for separating seven standard selenium species, namely SelP, GPx, albumin, $(SeCys)_2$, SeMet, SeO_3^{2-} and SeO_4^{2-}, and these are summarized in Table 1. Before sample loading, the column was first equilibrated for 30 min with mobile phase A and then a 35 min gradient elution program (Table 1) was used for separating these species. For real sample analysis, plasma samples were just filtered using a 0.45 μm syringe filter, avoiding the loss of selenium species.

Isotope dilution analysis of selenium in human plasma. To quantify the selenium-tagged proteins in human plasma using post-column species-unspecific isotope dilution, an enriched ^{77}Se spike solution was mixed continuously into the chromatographic effluent to change the isotope ratio of $^{80}Se/^{77}Se$. In order to keep the flow stable and mixed sufficiently, a flow rate of 20 μL h^{-1} was adjusted using an accurate syringe pump. The signals of ^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se were monitored when CH_4 was used as a reaction gas and the signal of $^{32}S^{16}O$ was monitored for sulfur determination when O_2 was used. In order to compare the quantity of different selenium species with the total selenium content, a 500 μL aliquot of human plasma and another aliquot spiked with 15 μL enriched ^{77}Se solution were added, respectively, into pressure digestion vessel systems together with 2 mL high-purity grade nitric acid. The digestion vessels were heated at 170 °C for 10 h. After cooling, the digests were each diluted to 10 mL with ultra-pure water, and the isotope ratio of $^{80}Se/^{77}Se$ was measured using ICP-DRC-*q*MS to obtain the total selenium content.

Results and discussion

Optimization of DRC operating conditions

So far, many reaction gases have been tried in the DRC for reducing spectroscopic interference. In terms of selenium quantification, CH_4 is more effective in eliminating the interference

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Table 1 Operating conditions for AEC/ICP-DRC-*q*MS

AEC parameters		
Anion exchange column	ProPac SAX-10 (250 × 2 mm I.D., 10 μm)	
Sample volume	200 μL	
Mobile phase	Eluent A: 10 mM Tris-HAc buffer, pH = 8.0	
	Eluent B: A + 500 mM ammonium acetate, pH = 8.0	
Gradient program	0–3 min	100% eluent A
	3–10 min	100–60% eluent A 0–40% eluent B
	10–16 min	60–0% eluent A 40–100% eluent B
	16–35 min	100% eluent B
Flow rate	0.25 mL min^{-1}	
ICP-DRC-*q*MS parameters		
Isotopes monitored	^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se, $^{32}S^{16}O$	
Reaction gas	CH_4,[a] O_2[b]	
Reaction gas flow rate	0.7 mL min^{-1},[a] 0.4 mL min^{-1}[b]	
Rejection parameter *q*	0.45,[a] 0.35[b]	

[a] CH_4 used as a reaction gas for ^{80}Se determination. [b] O_2 used as a reaction gas for $^{32}S^{16}O$ determination.

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than NH_3, H_2 and O_2 as described in a previous work.[9] A standard selenium solution of 10 ng mL^{-1} was used here for optimizing the DRC conditions. The main optimized parameters of the DRC were the flow rate of CH_4 and the value of the rejection parameter *q* (Rpq). Results indicated that the ratio of signal to noise for ^{80}Se is at a maximum when Rpq = 0.45 and V_{CH_4} = 0.7 mL min^{-1} (Fig. S3).† Sulfur determination was achieved by introducing O_2 into the DRC to form $^{32}S^{16}O^+$ instead of monitoring $^{32}S^+$, thus eliminating the interference of $^{16}O^{16}O^+$, and Rpq = 0.35 and V_{O_2} = 0.4 mL min^{-1} were chosen for sulfur determination in this study.

Separation of selenium species by anion exchange chromatography

For an effective separation of selenium species including SelP, GPx, albumin and $(SeCys)_2$, SeMet, as well as SeO_3^{2-} and SeO_4^{2-} (5 ng mL^{-1} as Se each), pH and ionic strength gradient elution should be considered and applied as well as a suitable selection of column. Among these selenium species, only SelP interacts weakly with the stationary phase of the column. In order to strengthen SelP to retain in the column, the pH of the mobile phase was adjusted to 8.0 to ensure its elution (retention time, 2.4 min) after the deadtime (<2.0 min) as shown in Fig. 1. Because a high pH value makes a protein molecule's surface more negatively charged, it enhances its interaction with the stationary phase.

During the process of chromatographic separation, the isotopes of ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se were monitored simultaneously on-line with ICP-DRC-*q*MS to ensure no isobaric interference existed. All the standard selenium species were detected with ^{80}Se except albumin, because the content of selenium in this economic product is too little (Fig. S4).† The albumin used was probably produced by recombinant technology in a low-selenium environment. Thus, $^{32}S^{16}O^+$ was monitored when albumin was analyzed, and a shoulder peak was observed probably caused by the co-existence of different molecular configurations in the standard.

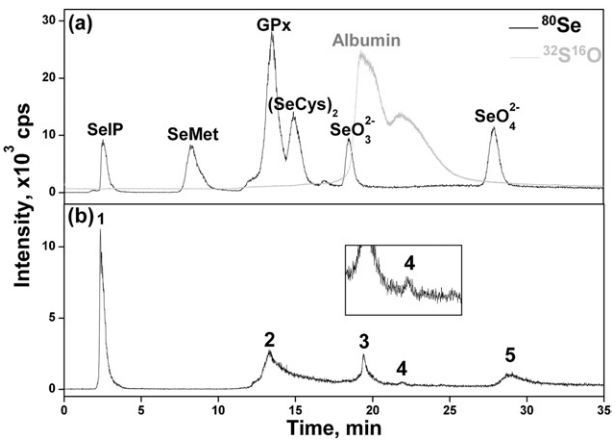

Fig. 1 Comparison of chromatogram (a) standard selenium species with (b) selenium species in healthy human plasma. Peak in chromatogram (b): (1) SelP, (2) GPx, (3) SeAlb, (4) U1, (5) U2. All the selenium species were monitored by ^{80}Se except albumin monitored by $^{32}S^{16}O$. Detailed conditions were listed in Table 1.

Quantification of selenium-tagged proteins in human plasma

Although species-specific isotope dilution (SSID) can provide the most credible data,[20] species-unspecific isotope dilution (SUID) is more universal for analysis of all species known and unknown because the limited availability of isotopically labeled compounds impedes the application of SSID. For SUID, the compound used as the spike solution does not need to be the same as the target analyte in the sample based on the knowledge that all species are broken and ionized into atoms and ions in ICP. ^{80}Se and ^{77}Se were detected during the quantification of selenium species by AEC/ICP-DRC-*q*MS, and the detailed quantitative calculation process for the amount of selenium was described previously.[21–23]

Comparing with using H_2 as a collision gas in CC or anion exchange solid phase extraction unit before AC and/or AEC for eliminating Cl and Br to reduce spectroscopic interferences from

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$^{40}Ar^{40}Ar$ and $^{1}H^{79}Br$ to ^{80}Se as well as $^{40}Ar^{37}Cl$ to ^{77}Se to achieve accurate quantification of selenoproteins,[14,17,18] separation of all Se species in a single AEC run coupled on-line with ICP-DRC-MS detection using CH_4 as a reaction gas is more attractive. In this case, no obvious interference was observed by monitoring multi-isotopes of selenium, suggesting that the spectral interferences from $^{40}Ar^{37}Cl$, $^{1}H^{79}Br$ and $^{40}Ar^{40}Ar$ could be eliminated by both AEC separation and CH_4-based DRC.

Plasma has been reported to give higher background signals owing to its more complex matrix than serum in AAS, but they produced similar distribution patterns of selenium-tagged proteins.[11] A pooled fresh plasma sample of five healthy people was analyzed for quantification of selenium-tagged proteins in this study. Fig. 2 shows the quantitative chromatogram of selenium species in the plasma by SUID AEC/ICP-DRC-*q*MS using a modified chromatographic condition to obtain better peak shape and ensure the elution of SelP beyond the void of the column. One can see that the signal of ^{77}Se in Fig. 2a increased along with the gradient and its intensity was enhanced about 3 times, suggesting that increasing the concentration of NH_4Ac in the mobile phase resulted in the enhancement of selenium sensitivity. However, the exact principle is not yet clear. It has been suggested that, during ICP, the addition of volatile carbon compounds would enhance the efficiency of element ionization, which have lower ionization potential than carbon.[24,25] An unexpected benefit of this phenomenon makes it possible to detect trace selenium species such as U1 and U2 as shown in Fig. 2. Moreover, the change of the isotopic signal intensity would not affect accurate quantification because the value of R_m would be independent of the efficiency of ionization. Following, we collected the fraction of U1 and U2 to analyze them by SEC/ICP-DRC-*q*MS (Fig. S5).† The result shows their molecule weights are much less than 18 kDa.

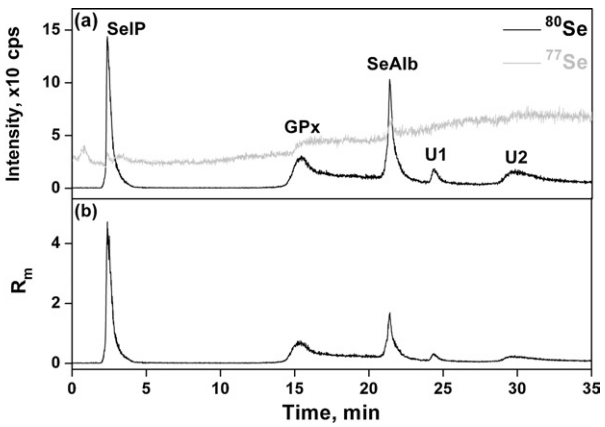

Fig. 2 (a) Chromatogram of selenium species in healthy human plasma obtained using species-unspecific isotope dilution AEC/ICP-DRC-*q*MS. (b) Isotope ratio (R_m) chromatogram of $^{80}Se/^{77}Se$ after being corrected for mass bias. Detailed conditions are the same as those in Fig. 1 except that the pH of mobile phase A was modified to be 8.6.

The concentrations of selenium in SelP, GPx and SeAlb in the pooled plasma sample were 42.85 ± 2.11, 18.01 ± 0.70 and 14.26 ± 0.13 ng mL^{-1}, respectively, which are basically coincident with previously reported results.[11,14–16] Selenium distribution was determined to be 45.5% in SelP, 19.1% in GPx, 15.1% in SeAlb, 2.9% in U1 and 8.1% in U2 based on the amount of total selenium in the plasma (94.24 ± 1.48 ng mL^{-1}). The sum of the selenium quantified for each selenium species amounts for 91% of the total Se in plasma. We guess the loss is mainly caused by the reservation of the column under high pH of the mobile phase. From the selenium concentration determined in the selenium-tagged proteins, it is possible to obtain the content of SelP and GPx in the plasma through stoichiometry of the selenium in the molecules, since there are ten selenium atoms in each SelP molecule and four in GPx (structured by 4 subunits).[3,27] The results obtained indicate that most of the selenium in the plasma is stored in SelP, although the abundance of this protein (2.7 ± 0.1 μg mL^{-1}) is less than GPx (5.4 ± 0.2 μg mL^{-1}) and SeAlb (a high-abundance protein in plasma[15]) (Table 2), suggesting that SelP might become a biomarker indicating the status of selenium nutrition. Based on the DL of selenium (0.54 ng mL^{-1}), we estimated that the DLs for SelP and GPx should be 0.59 pmol mL^{-1} and 1.7 pmol mL^{-1}, respectively. The concentration of SeAlb was estimated to be less than 12.0 ± 0.1 μg mL^{-1} based on the fact that there is at least one selenium atom in each SeAlb molecule. It is not possible to provide the exact value here due to the fact that selenomethionine was incorporated nonspecifically into albumin in place of methionine forming SeAlb.

Conclusions

The SUID AEC/ICP-DRC-*q*MS method developed here demonstrated the feasibility of selenium-tagged protein quantification in human plasma through the determination of selenium in the protein molecules, and offered a simple alternative for selenium speciation analysis. This heteroatom (such as selenium in this study)-tagged strategy is expected to be useful, after suitable modification, in moving qualitative proteomics towards quantitative proteomics, as long as the information of the heteroatom stoichiometry in the peptide and/or protein molecule is known and quantitative separation of the peptides and proteins can be achieved.

Table 2 Quantitative analysis of selenium-tagged proteins in human plasma

Selenium species[a]	SelP	GPx	SeAlb	U1	U2
Retention time/min	2.38	15.48	21.32	24.40	29.81
Molecule weight/kDa	50.5[26]	95.0[27]	66.4[b]	—	—
Se concentration/ng mL^{-1}	42.85 ± 2.11	18.01 ± 0.70	14.26 ± 0.13	2.77 ± 0.06	7.60 ± 0.23
Se percentage (%)	45.5	19.1	15.1	2.9	8.1
Protein concentration/μg mL^{-1}	2.7 ± 0.1	5.4 ± 0.2	<12.0 ± 0.1	—	—

[a] Mean ± SD (n = 3). [b] Producer's information.

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References

1 G. F. Combs and W. P. Gray, *Pharmacol. Therapeut.*, 1998, **79**, 179–192.
2 M. P. Rayman, *Lancet*, 2000, **356**, 233–241.
3 D. Behne and A. Kyriakopoulos, *Annu. Rev. Nutr.*, 2001, **21**, 453–473.
4 G. V. Kryukov, S. Castellano, S. V. Novoselov, A. V. Lobanov, O. Zehtab, R. Guigó and V. N. Gladyshev, *Science*, 2003, **300**, 1439–1443.
5 C. D. Thomson, *Analyst*, 1998, **123**, 827–831.
6 Y. J. Hu and A. M. Diamond, *Cancer Res.*, 2003, **63**, 3347–3351.
7 H. Steinbrenner, L. Alili, E. Bilgic, H. Sies and P. Brenneisen, *Free Radical. Biol. Med*, 2006, **40**, 1513–1523.
8 J. J. Sloth and E. H. Larsen, *J. Anal. At. Spectrom.*, 2000, **15**, 669–672.
9 K. S. K. Danadurai, Y. L. Hsu and S. J. Jiang, *J. Anal. At. Spectrom.*, 2002, **17**, 552–555.
10 R. Y. Wang, Y. L. Hsu, L. F. Chang and S. J. Jiang, *Anal. Chim. Acta*, 2007, **590**, 239–244.
11 I. Harrison, D. Littlejohn and G. S. Fell, *Analyst*, 1996, **121**, 189–194.
12 K. T. Suzuki and M. Itoh, *J. Chromatogr., B*, 1997, **692**, 15–22.
13 H. Koyama, K. Omura, A. Ejima, Y. Kasanuma, C. Watanabe and H. Satoh, *Anal. Biochem*, 1999, **267**, 84–91.
14 L. H. Reyes, J. M. Marchante-Gayón, J. I. G. Alonso and A. Sanz-Medel, *J. Anal. At. Spectrom.*, 2003, **18**, 1210–1216.
15 Ò. Palacios, J. R. Encinar, D. Schaumlöffel and R. Łobinski, *Anal. Bioanal. Chem.*, 2006, **384**, 1276–1283.
16 K. Shigeta, K. Sato and N. Furuta, *J. Anal. At. Spectrom.*, 2007, **22**, 911–916.
17 P. Jitaru, M. Prete, G. Cozzi, C. Turetta, W. Cairns, R. Seraglia, P. Traldi, P. Cescon and C. Barbante, *J. Anal. At. Spectrom.*, 2008, **23**, 402–406.
18 P. Jitaru, G. Cozzi, A. Gambaro, P. Cescon and C. Barbante, *Anal. Bioanal. Chem.*, 2008, **391**, 661–669.
19 B. Akesson and B. J. Martensson, *Inorg. Biochem.*, 1988, **33**, 257–261.
20 A. Polatajko, J. R. Encinar, D. Schaumlöffel and J. Szpunar, *Chem. Anal. (Warsaw)*, 2005, **50**, 265–278.
21 K. G. Heumann, S. M. Gallus, G. Rädlinger and J. Vogl, *Spectrochim. Acta, Part B*, 1998, **53**, 273–287.
22 C. S. Muñiz, J. M. Marchante-Gayón, J. I. G. Alonso and A. Sanz-Medel, *J. Anal. At. Spectrom.*, 2001, **16**, 587–592.
23 P. Rodríguez-González, J. M. Marchante-Gayón, J. I. G. Alonso and A. Sanz-Medel, *Spectrochim. Acta, Part B*, 2005, **60**, 151–207.
24 P. Allain, L. Jaunault, Y. Mauras, J.-M. Mermet and T. Delaporte, *Anal. Chem.*, 1991, **63**, 1497–1498.
25 E. H. Larsen and S. Stürup, *J. Anal. At. Spectrom.*, 1994, **9**, 1099–1105.
26 S. G. Ma, K. E. Hill, R. M. Caprioli and R. F. Burk, *J. Biol. Chem.*, 2002, **277**, 12749–12754.
27 G. A. Jacobson, C. Narkowicz, Y. C. Tong and G. M. Peterson, *Clin. Chim. Acta*, 2006, **369**, 100–103.

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