

Application of Methane Mixed Plasma for the Determination of Ge, As, and Se in Serum and Urine by ICP/MS

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An analytical method for the simultaneous determination of trace Ge, As and Se in biological samples by inductively coupled plasma/mass spectrometry has been investigated. The effects of added organic gas into the coolant argon gas on the analyte signal were studied to improve the detection limit, accuracy and precision. The addition of a small amount of methane (10 mL/min.) into the coolant gas channel improved the ionization of Ge, As and Se. The analytical sensitivity of the proposed Ar/CH₄ system was superior by at least two-fold to that of the conventional Ar method. In the present method, the detection limits obtained for Ge, As and Se were 0.014, 0.012 and 0.064 $\mu\text{g/L}$, respectively. The analytical reliability of the proposed method was evaluated by analyzing the certified standard reference materials (SRM). Recoveries of 99.9% for Ge, 103% for As, 96.5% for Se were obtained for NIST SRM of freeze dried urine sample. The proposed method was also applied to the biological samples.

Key Words : Methane mixed plasma, ICP/MS, Ge, As, Se

Introduction

Organic compounds of germanium have been used as antitumor agents and intestinal astringents in veterinary medicine. And arsenic compounds were used for a long time as pesticides in agriculture. Such compounds are still increasingly used as wood and cotton preservatives. Selenium is an essential antioxidant mineral. We have applied a more sensitive ICP/MS Ge, As and Se determination in biological samples. The concentration of these analytes in serum and urine is important and was significantly lower than pure water.

The non-spectroscopic matrix effects in ICP/MS typically come from a biological sample matrix such as Na, K and organic compounds.¹ The use of an internal standard is not adequate with most elements because the interference effects are not in the same direction.¹ Reduction of these effects can be achieved by a careful setting of the instrumental parameters. Other methods, such as hydride generation or electrothermal vaporization, can partially overcome these interferences.

A more direct method of interference removal is the use of 1. molecular and inert gases, 2. addition into the gas, or 3. replacing one of the three gas flows of the ICP. Characteristics of mixed gas plasmas have been widely studied in inductively coupled plasma/atomic emission spectrometry (ICP/AES) and ICP/MS, though most of the literature has focused on the effect of nitrogen added to the outer argon gas.²⁻¹¹ Several researchers have also reported the use of nitrogen addition in ICP/MS. Lam and Horlick reported that

polyatomics could be reduced and analyte signals enhanced with the addition of nitrogen to the outer gas.¹³ To date, the majority of research has focused on the use of nitrogen, oxygen and air as additional gas, but recently attention has been turned to other gases. The additions of methane and propane have already been used in ICP atomic emission spectrometry (AES) and atomic fluorescence spectrometry (AFS).¹⁵⁻¹⁸

In the present paper, the conditions for the removal of signal enhancement or suppression are optimized with the addition of methane to the coolant gas. The optimum operating conditions were defined and tested by determining detection limits and recoveries of elements and the analysis of certified reference materials (CRMs) for these elements.

Experimental Section

Instrumentation. The ICP/MS instrument used was a Perkin-Elmer SCIEX Elan 5000 (Norwalk, Connecticut, USA) run at normal resolution. A cross-flow nebulizer associated with a double-pass spray chamber was used. The mass spectrometer was set to sample ion intensities (peak jumping) at the analyte masses m/z 70, 72, 73 and 74 for Ge⁺, m/z 75 As⁻ and m/z 77, 78 and 82 for Se⁺. A peristaltic pump was used for conventional sample introduction. The instrument sensitivity was optimized by varying the instrumental setting, using aqueous standard solutions of Pb, Rh and Mg. The instrument adjustments included the physical positioning of the mass spectrometer relative to the plasma, the ion lens voltages and the adjustment of R, F, power to the argon plasma. Once optimized for germanium, arsenic and selenium, these variables were kept constant throughout all experiments involving the same element. The aerosol carrier gas

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Table 1. Instrumental and analytical parameters for ICP/MS

Descriptions	Conditions
R.F. generator	Free-running type, 40 MHz
R.F. power	1000 W
Induction coil	3-turn, 1.8 in. copper, 2.6 mm i.d.
Sampling depth	7 mm from load coil, on center
Coolant gas flow rate	15.0 L/min. (Ar), 10.0 mL min. (CH ₄)
Auxiliary gas flow rate	0.85 L/min. (Ar)
Nebulizer gas flow rate	0.92 L/min. (Ar)
Mass flow controller	4-channel
Sample introduction	Peristaltic pump
Sample uptake flow	1.0 mL/min.
Nebulizer	Cross-flow type
Spray chamber	Double pass type (Scott type)
Torch	Demountable
Interface cones	Nickel
Mass analyzer	Quadrupole
Vacuum system	Turbo molecular pumps
Quadrupole chamber	5×10^{-7} torr
Quantitative mode	
Replicate time (ms)	300
Dwell time (ms)	100
Sweeps Reading	3
Reading/Replicate	1
Number of Replicates	5
Points Spectral peak	3
Ge:Mass	70, 72, 73, 74
As:Mass	75
Se:Mass	77, 78, 82
Scan mode	Peak hopping
Resolution	0.9
Total Quant Mode Mass Range	67-87

flow was adjusted for each analyte until optimum signal intensity was reached. Methane addition to the coolant gas was achieved by using a 4-channel gas mass flow controller (MFC). The ICP/MS setting is given in Table 1.

Materials and chemicals. Human serum and urine samples were obtained from 30 healthy individuals living in the Seoul region.

Five CRMs were analyzed: Freeze-Dried Urine [NIST Standard Reference Material (SRM) No. 2670, National Institute of Standards and Technology, Gaithersburg, MD, USA], Bovine-Muscle Powder [NIST SRM No. 8414], Whole Egg Powder [NIST SRM No. 8415], Whole Milk Powder [NIST SRM No. 8435], Durum Wheat Flour [NIST SRM No. 8436].

Standard solutions were prepared from 10 mg/L stock solutions of Ge, As and Se (Spex Industries Inc., Edison, New Jersey, USA). All standard solutions were made up in 1% HNO₃. High purity reagents were used, and the water was produced in a Millipore Super-Q apparatus (Millipore, Milford, MA, USA). The liquid argon used was 99.999% pure. The methane used was 99% pure.

Sample preparation. Approximately 0.5 g of each CRMs

was digested in beakers, using a hot plate. 5 mL of nitric acid were used for the digestion. After digestion, the solutions were quantitatively transferred into calibrated flasks (10 mL) and made up with distilled, de-ionized water. CRMs were spiked with Ge to a final concentration of 5 µg/L. Five replicates of each sample were prepared. The 1 mL serum and urine samples were digested with c-HNO₃.

Standard solutions and calibration solutions were prepared from the stock solutions as required.

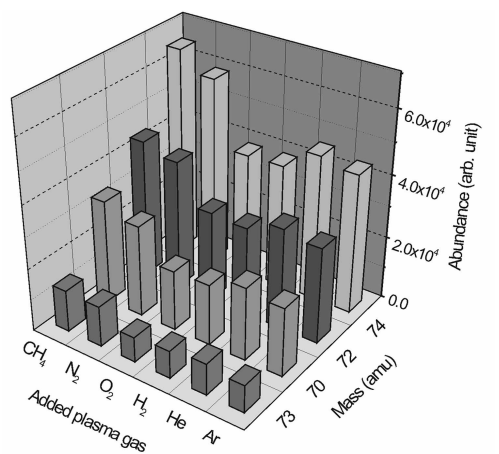
Procedure. Experiments to assess the influence of the instrumental operating parameters (species of added gas, channel for methane and gas flow rate for methane) on elements were performed. In these experiments standard solutions of 100 µg/L Ge, As and Se in 1% matrices were analyzed to find the effect of the operating parameters on analyte elements. These experiments were undertaken with and without methane addition to the coolant gas, under the following operating conditions: outer gas, 15 L/min; intermediate gas, 0.85 L/min; nebulizer gas, 0.92 L/min; power, 1000 W; and CH₄, either absent or variable up to 100 mL/min.

Optimizations of the operating parameters, with the addition of methane to the coolant gas, were performed to find the optimum conditions for the reduction of interferences. The optimal conditions defined by these simplex optimization were tested by determination of the detection limits for Ge, As and Se. Analysis of the CRMs and real samples was undertaken on typical operating parameters (Table 1).

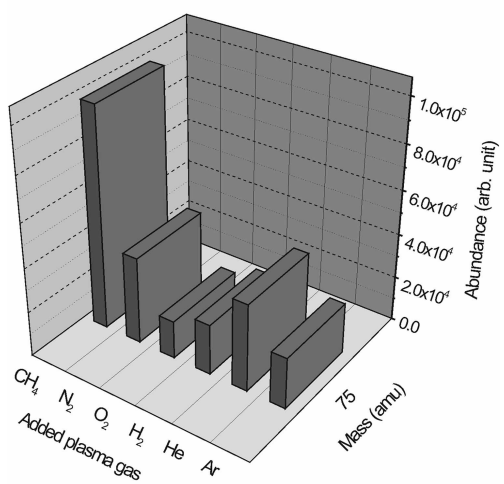
Results and Discussion

Selection of mass for analyte. Noticeable interferences were observed for Ge, As and Se, showing that below $m/z=85$ the background spectrum is not free from interfering peaks.¹⁸⁻²⁰ Moreover, at $m/z=70-82$, interfering oxides or argon dimer ion (⁴⁰Ar⁴⁰Ar) have been reported.¹⁸⁻²⁰ So the isotope $m/z=77$ and 82 was chosen instead of $m/z=80$ for Se. Because the m/z 75 (⁴⁰Ar³⁵Cl) was a possible interfering ion, it was compensated by the m/z 77 (⁴⁰Ar³⁷Cl), which was corrected for ⁷⁷Se using ⁸²Se. ⁸²Se was obtained by correcting ⁸²Kr.²¹

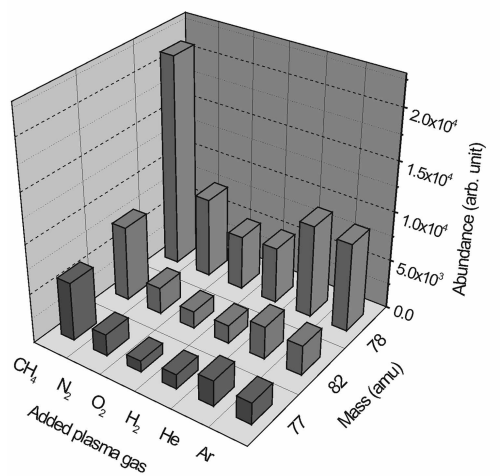
Optimization of plasma with methane. An experiment was carried out with methane in the argon coolant gas. For selection of added gas, various gases were added into the coolant gas. Figure 1 shows that the enhancement of signals caused by methane introduction is more effective than any other gases. The results of Figure 2 show that the coolant gas flow channel is the most effective for enhancement of analytes. The intensity of analytes with the addition of methane to the coolant gas is plotted in Figure 3. When a flow rate of methane up to 10 mL/min was added to the coolant gas flow, the intensity of Ge, Se was maximum. And when the flow rate of methane up to 20 mL/min was added to the coolant gas flow, the intensity of As was maximum. For simultaneous analysis, 10 mL/min methane gas flow rate was used. The optimal conditions are defined in Table 1. Detection limits are reported in Table 2. The result shows that the



(a) Ge



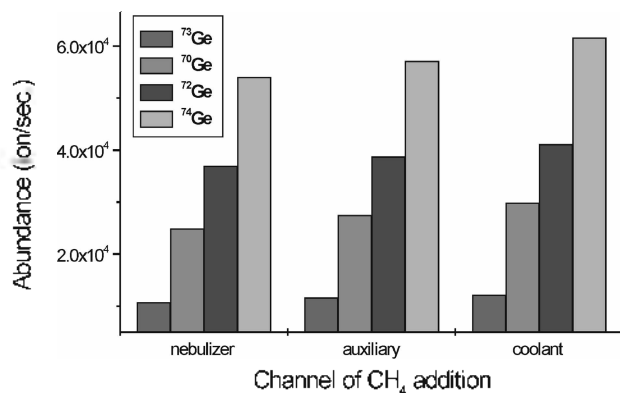
(b) As



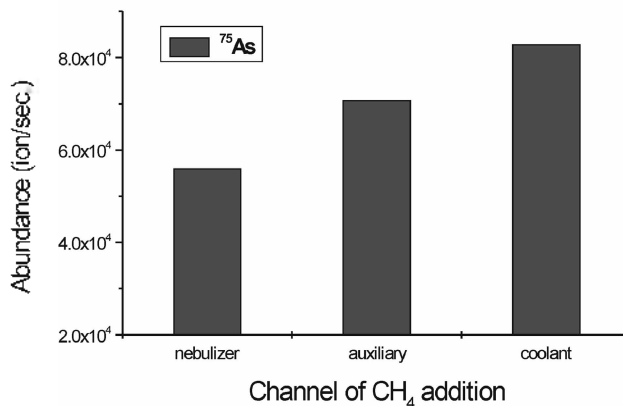
(c) Se

Figure 1. The effects of the added coolant gas on the abundance of Ge, As and Se.

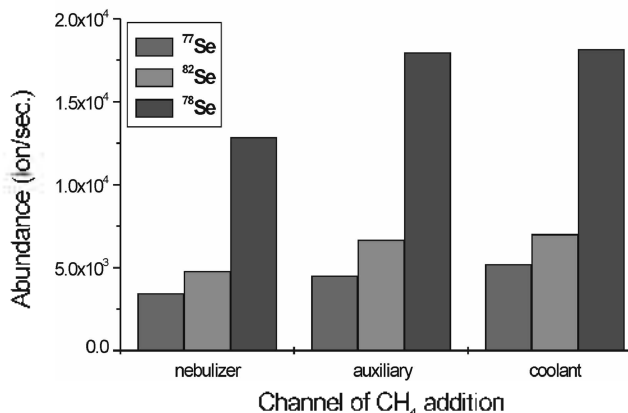
detection limit for As was effectively decreased in the Ar/CH₄ plasma. From the results of Figure 4 for the effect in some matrices, the simplex optimization of the plasma with methane added to the coolant gas reduced the enhancement or depletion of Ge, As and Se signal formed by 1% matrices.¹



(a) Ge



(b) As



(c) Se

Figure 2. The effect of the channel of CH₄ addition on the abundance of Ge, As and Se.

Determination of three metals in Korean serum and urine. The determination of Ge, As and Se in human serum and urine are useful to detect excess intake or exposure, particularly concerning pesticides, food or drugs. Before the test of human serum and urine, we checked this method by the use of CRMs (Table 3). The precision and accuracy could not be defined directly, owing to a lack of suitable certified reference material for Ge in serum. It was therefore assessed indirectly using Ge spiked NIST SRM 2670, 8414, 8415, 8435, 8436 and serum samples. The relative errors of Ge are 0.15-0.58% and C.V.s are 0.80-1.79%. For As, the

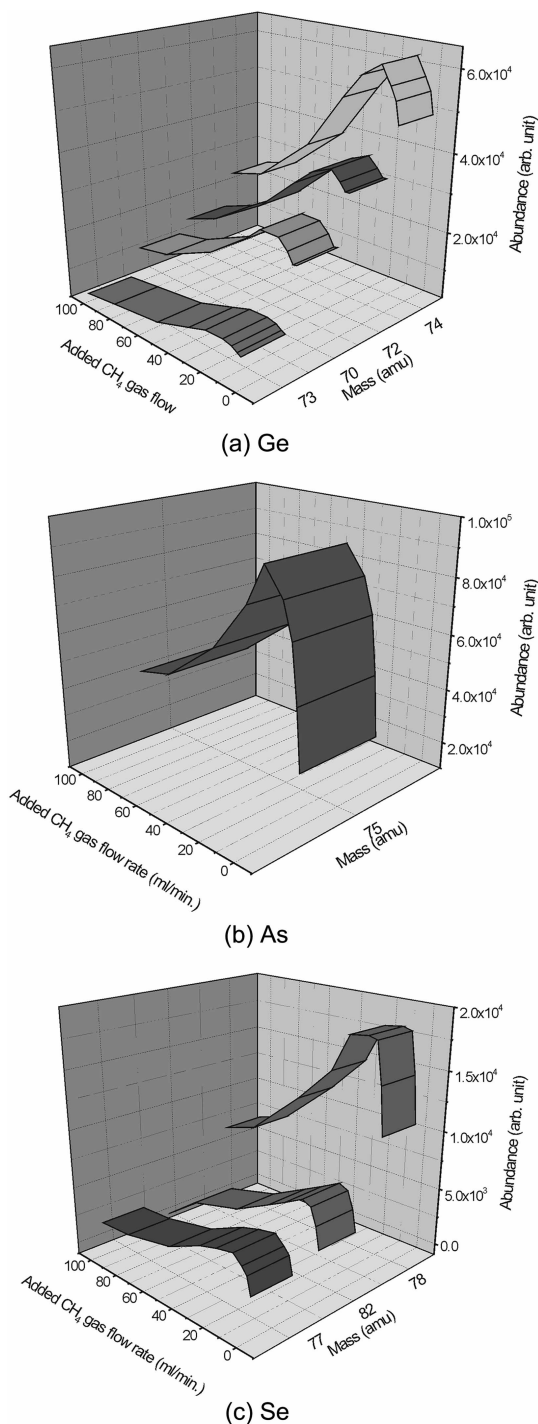


Figure 3. The effect of the CH₄ gas flow rate on the abundance of Ge, As and Se.

Table 2. Detection limits of Ge, As and Se by Ar/CH₄/ICP/MS, HG/ICP/AES and Ar/ICP/MS

Element	Detection limit ^a (μg/L), C.V. ^b (%)		
	Ar/CH ₄ /ICP/MS	Ar/ICP/MS	HG/ICP/AES
Ge	0.0138 ± 0.0002, 0.43	0.027	1.78
As	0.0120 ± 0.0004, 2.58	0.106	1.85
Se	0.0642 ± 0.0011, 7.09	0.886	2.69

^aMean values obtained from ten measurements. ^bCoefficient of variation.

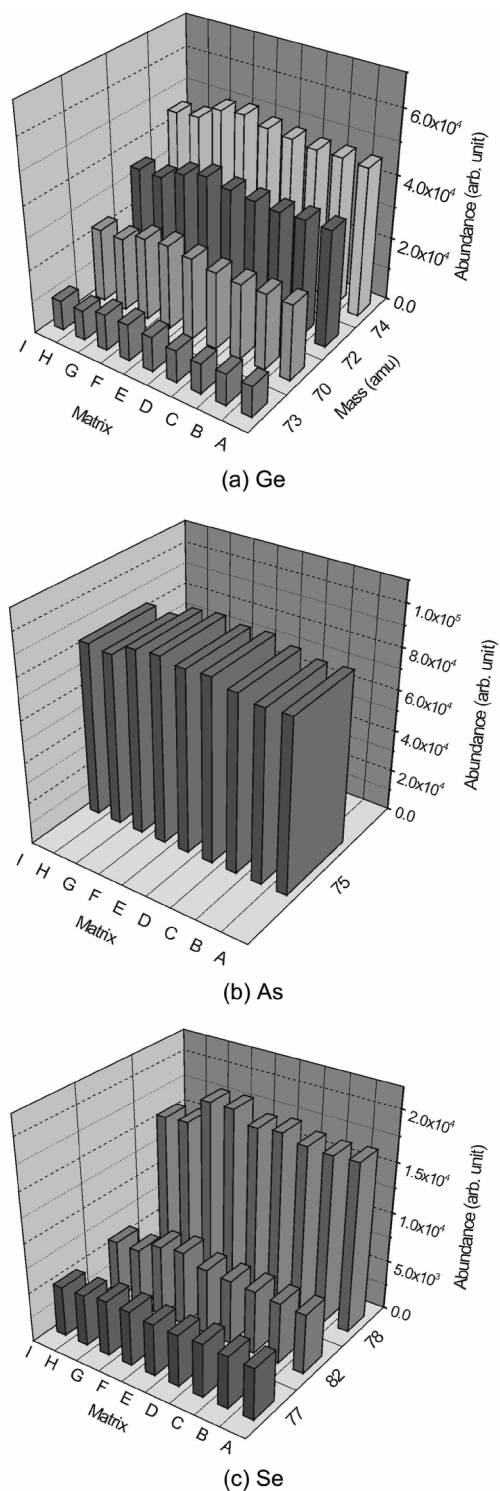


Figure 4. Effects of methane mixed plasma on Ge, As and Se signal: A: H₂O, B: NH₄NO₃, C: NH₄Cl, D: (NH₄)₂SO₄, E: NH₄H₂PO₄, F: CH₃COONH₄, G: (NH₄)₂C₂H₄O₆, H: NaNO₃, I: KNO₃.

relative errors are 2.00-20.0% and C.V.s are 1.59-8.43%. The relative errors of Se are 1.60-9.21% and C.V.s are 1.94-6.93%. The test method was established with 5 replicate measurements on the same sample. Figure 5 is the calibration curves for ⁷⁴Ge, ⁷⁵As and ⁷⁷Se. The curves show good linearity.

Table 3. Accuracy and precision test for Ge, As and Se in the certified reference materials^{a,d}

Material	Cer. Ref.	Element	Found value	Certified value	Relative error ^a (%)	C.V. ^b (%)
Freeze dried urine	SRM 2670	⁷⁴ Ge	99.9 ng	100 ng	0.15	1.06
		⁷² Ge	99.8 ng	100 ng	0.17	2.84
		⁷⁵ As	0.493 mg/L	0.48 mg/L	2.71	5.94
		⁸² Se	0.444 mg/L	0.46 mg/L	3.48	6.28
		⁷⁷ Se	0.443 mg/L	0.46 mg/L	3.71	4.75
Spiked serum sample	-	⁷⁴ Ge	99.4 ng	100 ng	0.58	1.22
		⁷² Ge	99.5 ng	100 ng	0.52	0.95
		⁷⁵ As	97.4 ng	100 ng	2.57	1.59
		⁸² Se	96.2 ng	100 ng	3.78	1.94
		⁷⁷ Se	96.4 ng	100 ng	3.65	2.37
Bovine muscle powder	SRM 8414	⁷⁴ Ge	99.7 ng	100 ng	0.26	1.33
		⁷² Ge	99.7 ng	100 ng	0.27	0.72
		⁷⁵ As	0.010 mg/kg	0.009 mg/kg	10.1	7.92
		⁸² Se	0.069 mg/kg	0.076 mg/kg	9.21	7.46
		⁷⁷ Se	0.070 mg/kg	0.076 mg/kg	7.89	6.85
Whole egg powder	SRM 8415	⁷⁴ Ge	99.6 ng	100 ng	0.41	1.79
		⁷² Ge	99.7 ng	100 ng	0.35	1.23
		⁷⁵ As	0.011 mg/kg	0.01 mg/kg	10.0	5.92
		⁸² Se	1.31 mg/kg	1.39 mg/kg	5.61	6.93
		⁷⁷ Se	1.32 mg/kg	1.39 mg/kg	5.40	3.24
Whole milk powder	SRM 8435	⁷⁴ Ge	99.7 ng	100 ng	0.28	1.13
		⁷² Ge	99.7 ng	100 ng	0.27	1.01
		⁷⁵ As	0.0012 mg/kg	0.001 mg/kg	20.0	8.43
		⁸² Se	0.127 mg/kg	0.131 mg/kg	3.05	5.80
		⁷⁷ Se	0.128 mg/kg	0.131 mg/kg	1.98	6.33
Durum wheat flour	SRM 8436	⁷⁴ Ge	99.5 ng	100 ng	0.47	0.80
		⁷² Ge	99.6 ng	100 ng	0.36	1.46
		⁷⁵ As	0.036 mg/kg	0.03 mg/kg	2.00	4.27
		⁸² Se	1.20 mg/kg	1.23 mg/kg	2.60	3.29
		⁷⁷ Se	1.20 mg/kg	1.23 mg/kg	3.50	5.81

^aMean values obtained from five measurements. ^bCoefficient of variation. ^cDigestion reagent is HNO₃. ^dCRM is digested in beaker, at 150 °C.

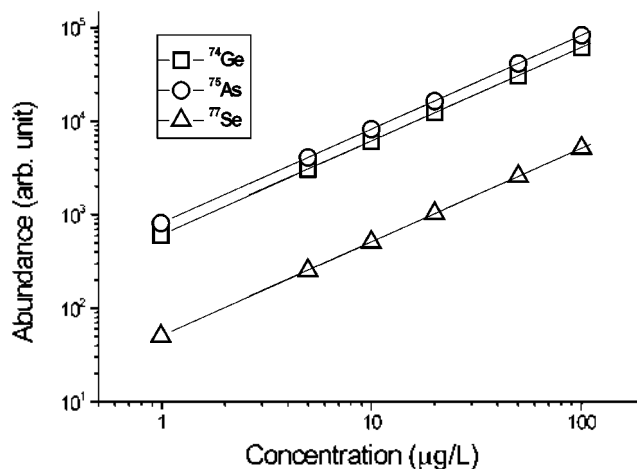
Table 4. Analytical results for Ge, As and Se in blood and urine^{a,b}

Sample	mg/L		
	Ge	As	Se
Whole blood of human	62.7	—	—
Urine of human	2.19	—	—
Urine of human ^c	—	0.042-0.64	—
Serum of human ^d	—	—	0.17-0.35

^aDigestion reagent is HNO₃. ^bSamples are digested in beaker, at 150 °C. ^cn = 30. ^dn = 30

The Ge, As and Se in the human serum and urine of 30 healthy individuals were determined. The concentration of Se in the human serum was 0.17-0.35 mg/L, the concentration of As in the urine was 0.042-0.64 mg/L.

The concentration of As and Se in the urine of 30 healthy subjects (0.042-0.64 and 0.17-0.35 mg/L, respectively) are similar to the values obtained by F. Peter and J. H. Watkinson *et al.*²²⁻²³

**Figure 5.** Calibration curves of ⁷⁴Ge, ⁷⁵As and ⁷⁷Se using ICP/MS with methane mixed plasma.

Conclusions

This work shows that signal enhancement or depletion of Ge, As and Se can be effectively reduced, by the addition of methane to the outer gas.

The success of this work is demonstrated by the analysis of CRMs, by the improved detection limits and by the enhanced recoveries obtained. Detection limits for Ge, As and Se generally were better with the addition of methane to the coolant than without the addition of methane to any of the gas flows.

We have found that the use of ICP/MS allows the rapid and easy determination of Ge, As and Se in serum and urine.

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