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International Journal of Mass Spectrometry 213 (2002) 217–224

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Isotopes and concentrations of Zn in human blood and serum by ICP-MS

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Received 14 March 2001; accepted 6 October 2001

Abstract

The double focusing-Jeol Inductively Coupled Plasma Mass Spectrometer PLASMAX2 is used at different resolving powers, for the separation between zinc isotopes and their interfering ions. NIST standard solution samples of zinc as single and multielements are used in the analysis. Variation of different Zn isotopic abundances and their interfering ions are studied as a function of concentrations at low and high resolutions. The precision for different isotopic ratios in multielement solutions, after taking the experimental considerations, at high resolution are calculated and found to have a value between 0.19 and 0.42. While the accuracy for the isotopes can reach a value between 0.27 and 1.17, blood and serum samples in human are used as an application for this method in the biological samples. The obtained results are compared with that obtained by using flame atomic absorption spectrometer and it is found in the same range of concentration. (Int J Mass Spectrom 213 (2002) 217–224)

Keywords: ICPMS; Zn isotopes; Interfering ions

1. Introduction

Zinc has five stable isotopes at mass numbers 64, 66, 67, 68, and 70 and their abundances are 48.6%, 27.9%, 4.1%, 18.8%, and 0.6%, respectively. The possibility of interferences of zinc with polyatomic ions in the inductively coupled plasma mass spectrometer (ICP-MS) and the required resolution for their separation are shown in Table 1. In the used ICP-MS the sampler and the skimmer cones are made from Cu, therefore it is expected to have an influence from Cu in the interfering peaks as shown in Table 1. Moreover Si, which comes from the material of the torch, can interfere with mass 68 as shown in Table 1. S is found in blood and serum and therefore causes interferences as in Table 1.

2. Experimental

All measurements are performed using Jeol high-resolution inductively coupled plasma mass spectrometer HR-ICPMS (plasmax2). Fig. 1 is a schematic overview of the machine and the operating conditions are summarized in Table 2. Peaks are swept by the magnetic field in 5 replicates, where each replicate contains 20 peaks of the isotope under consideration.

3. Results and Discussion

Zinc isotopes are measured in a single element standard solution (NIST). At low resolution (500)

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1387-3806/02/$22.00
PII S1387-3806(01)00541-3

Table 1
Possible main interferences with zinc isotopes and the exact masses

Isotopes and their exact masses	Possible main interferences and their exact masses	Δm	Resolution
^{64}Zn (63.929 2)	1H63Cu (63.937 4)	0.0083	7722
	32S16O16O (63.961 9)	0.0327	1953
^{66}Zn (65.926 0)	1H65Cu (65.937 4)	0.0114	5789
^{67}Zn (66.927 1)	2H65Cu (66.941 9)	0.0148	4533
^{68}Zn (67.924 9)	28Si40Ar (67.939 3)	0.0144	4694
	$14N_2$40Ar (67.961 4)	0.0365	1862
	$18O_2$32S (67.970)	0.045	1510
^{70}Zn (69.925 3)	14N16O40Ar (69.960 4)	0.0351	1995

Table 2
The experimental conditions of the ICPMS

Parameters	Condition
Forward rf power	1 300 W
Reflected rf power	<2 W
Coolant gas flow rate	14 L/min
Auxiliary gas flow rate	0.3 L/min
Nebulizer gas flow rate	0.77 L/min
Scan type	Magnetic scan
Mass resolution(m/Δm)	500 and 5 000
Detector mode	analogue
Switching	1 000 ms
Optimization	Maximum ion intensity of 1ppb of $^{89}Y^+$, then, maximum M^+ intensity

zinc isotopes are observed as single peaks as shown in Fig. 2(a). These peaks are not only for zinc isotopes but they include other ions with the same masses, which result from the molecular ions formation (Table 1). Fig. 2(b) shows the observed peaks at high resolution (5000), where a possibility of resolution of some peaks.

At low resolution (500) zinc isotope abundances in a single element solution are studied as a function of their concentration. Fig. 3 shows that for the isotopes, by changing to higher concentrations, an increase in the ion intensity is recorded. The lines, which represent these data, do not go by extension to zero concentration. This is not clear in Fig. 3 for masses 67

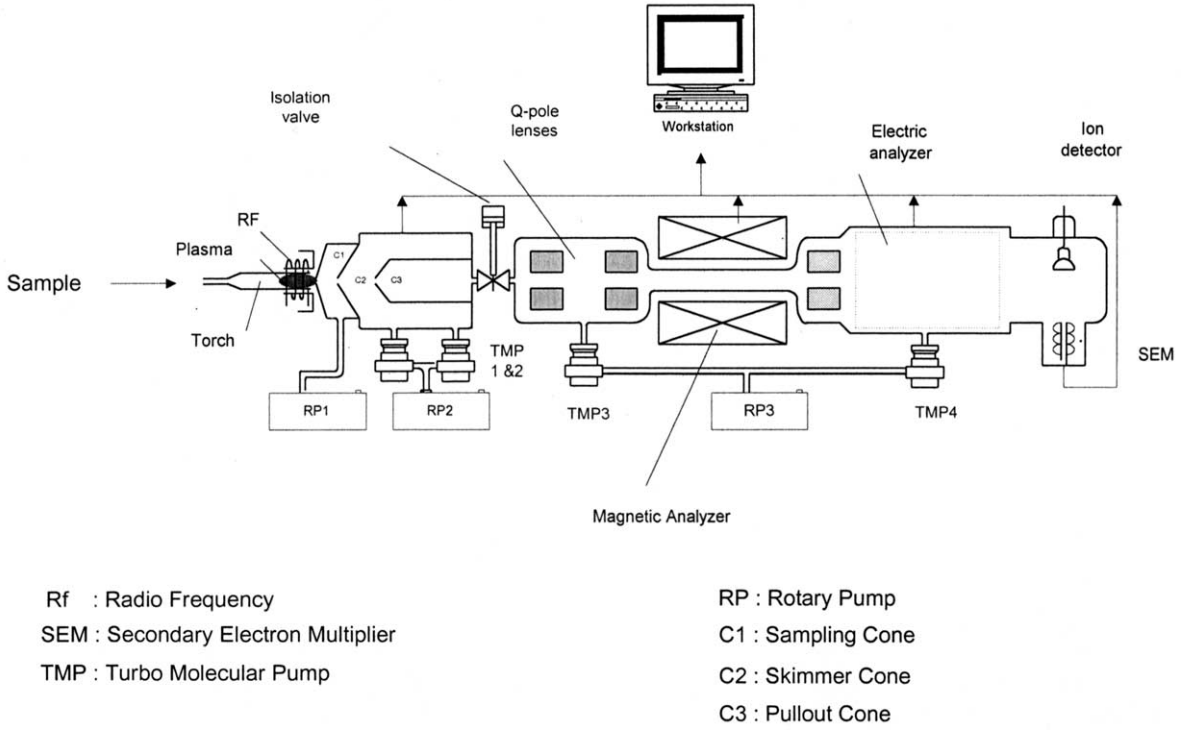

Fig. 1. Schematic diagram of plasmax2 ICPMS.

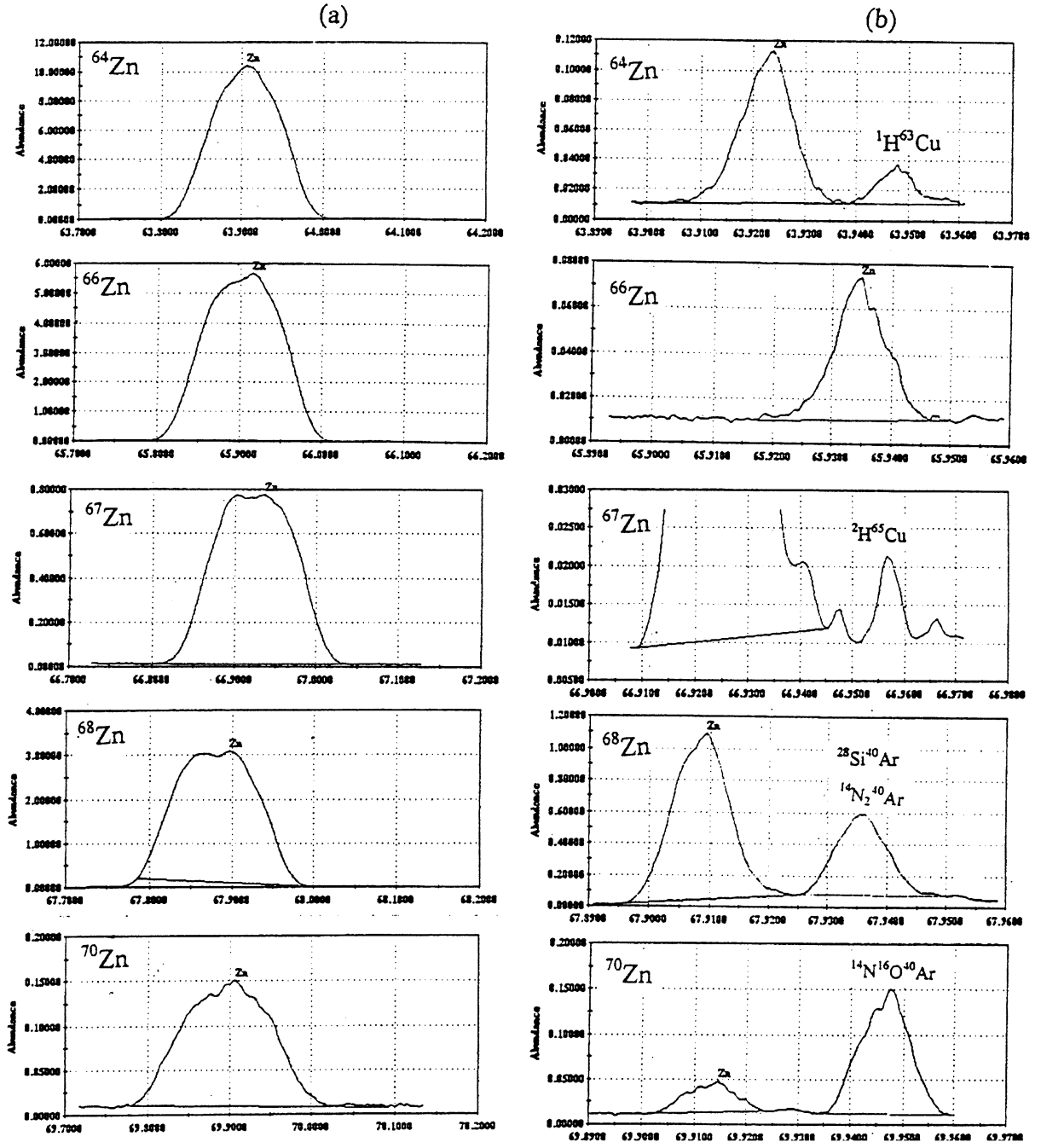

Fig. 2. Peak shapes for zinc isotopes (a) at low resolution (500), (b) at high resolution (5000).

and 68. However, enlarged scales show these clearly. This means that Zn suffers from the influence of an overlapping. At a higher resolution (5000) there is a separation between two components. The host Zn isotopes in Fig. 4 shows linear increments by increasing the concentration. The molecular ions formation

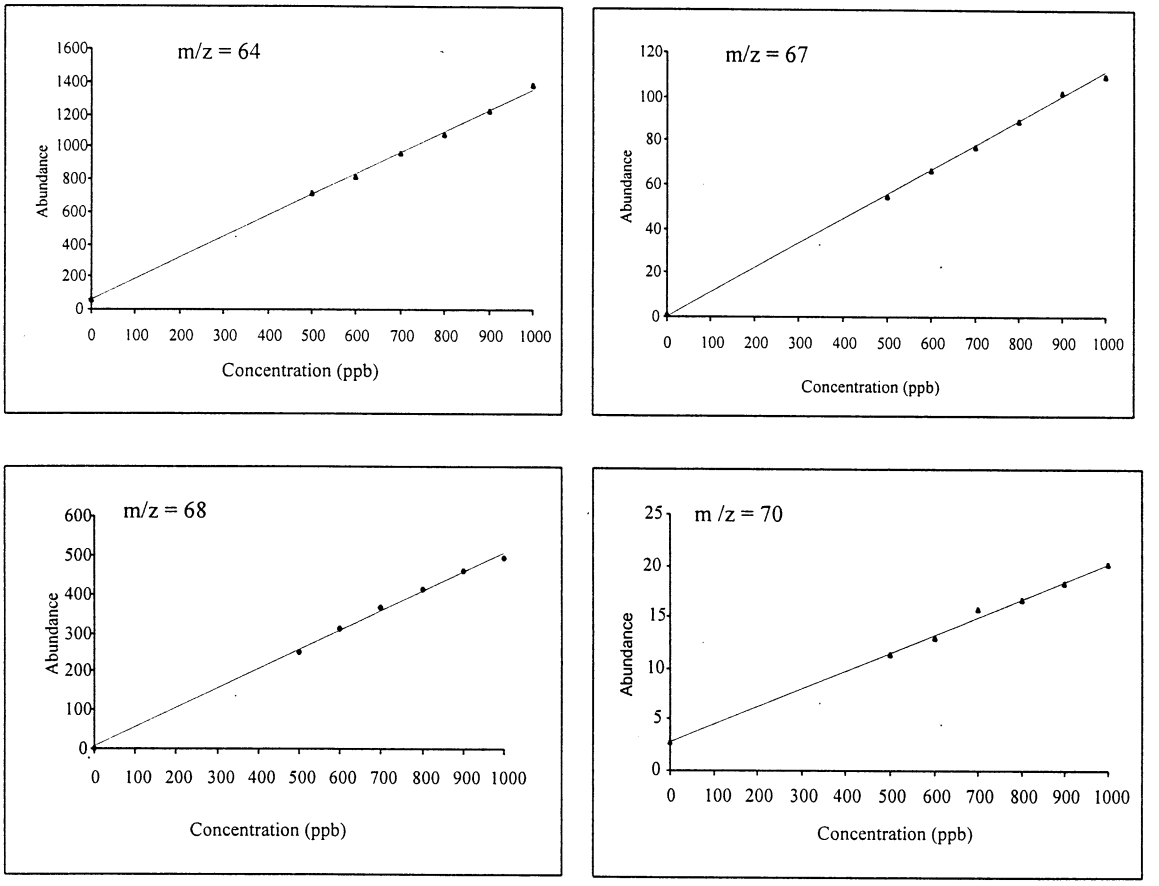

Fig. 3. Variation of ion intensity of zinc in single element standard solution as a function of concentration at low resolution (500).

are studied as a function of concentration and it is found that there is no dependence on the concentration. This means that the origin of these peaks is from (Ar, Cu, Si, N, O, and H) which exist in the gas phase of the plasma media, the cones of the interface and the material of the torch.

The isotope ratios for zinc isotopes $^{64}Zn/^{66}Zn$, $^{67}Zn/^{66}Zn$, $^{68}Zn/^{66}Zn$ and $^{70}Zn/^{66}Zn$ are calculated with their precision and accuracy in the case of single element solution at low resolution (500) and high resolution (5000) (Table 3). In the high-resolution mode, the average accuracy is improved after reduction of the molecular ion formation, since it is changed from (2.198, 0.615, 1.066, and 55.965) at low resolution to (0.528, 0.429, 0.334, and 0.648) at high resolution, for the previous isotope ratios, respectively.

To be more close to the actual cases of the existence of Zn in biological samples, the mass spectra of multielements solution, which contains 26 elements namely Ba, Be, Bi, Cd, Cs, Cr, Co, Cu, Ca, In, Fe, Pb, Li, Mg, Mn, Ni, K, Rb, Se, Ag, Na, Sr, Tl, U, V, and Zn at concentration 10 ppm are studied. This matrix is not characteristic of biological samples. An organic matrix with higher concentrations of Na, K, Mg, and Ca could be more typical. However, the present study with known matrix is a guide for further studies with different organic matrices. Fig. 5 shows the variation in the ion intensity for zinc isotopes which are obtained from the multisolution as a func-

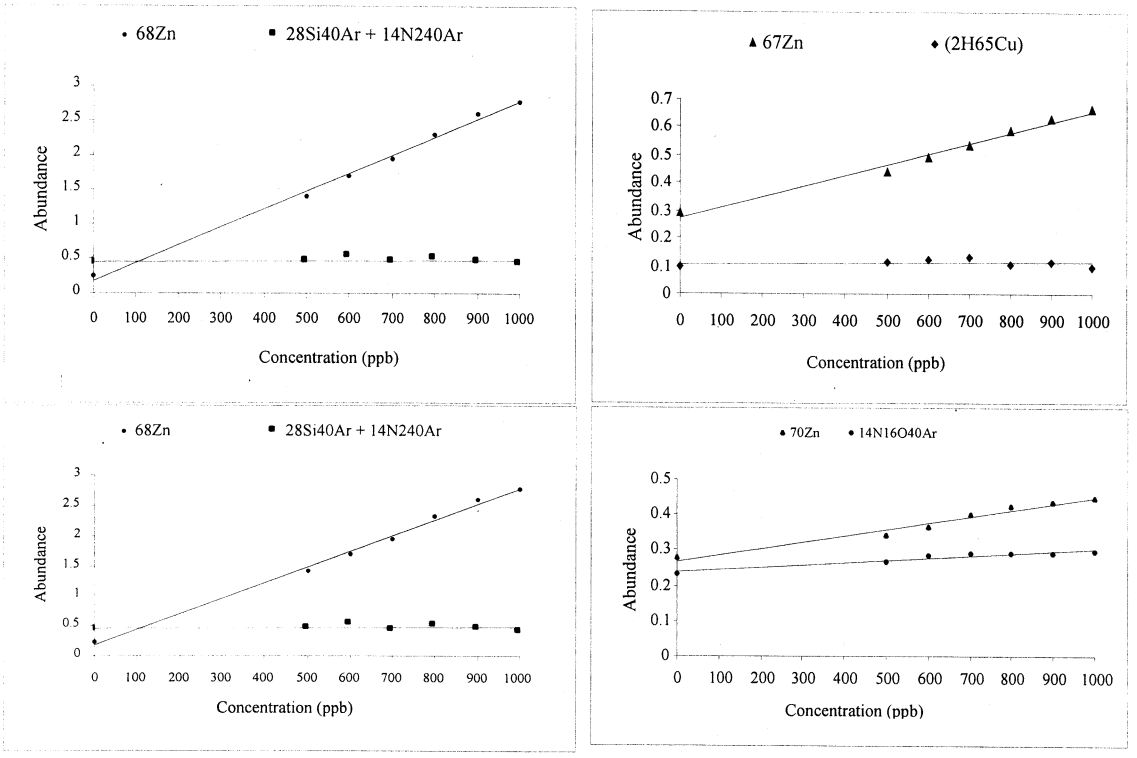

Fig. 4. Variation of ion intensity for zinc in single element standard solution as a function of concentration at high resolution (5000).

tion of their concentrations at high resolution (5000). Since the standard multisolution contains Ba, an interference occurs for ^{68}Zn with the doubly charged ion $^{136}Ba^{++}$ at $m/z = 67.9523$. Zinc isotopes can be separated from their interfering ions except the isobaric interferences in masses 64 and 70, which interfere with ^{64}Ni and ^{70}Ge, respectively. This needs very high resolution for separation. In our measurements isobaric interference is expected for ^{64}Zn alone because the multielement standard solution contains Ni and free from Ge. However, at any case, the present measurements cannot separate the isobaric interferences.

Table 4 shows the isotope ratios ^{64}Zn/^{66}Zn, ^{67}Zn/

Table 3
Zinc isotope ratios in single element standard solution

At low resolution (500)	^{64}Zn/^{66}Zn	^{67}Zn/^{66}Zn	^{68}Zn/^{66}Zn	^{70}Zn/^{66}Zn
Actual values	1.742	0.147	0.674	0.022
Average values	1.749	0.146	0.671	0.034
Average precision (%)	0.609	0.511	0.515	0.414
Average accuracy (%)	2.198	0.615	1.066	55.965
At high resolution (5000)	^{64}Zn/^{66}Zn	^{67}Zn/^{66}Zn	^{68}Zn/^{66}Zn	^{70}Zn/^{66}Zn
Average values	1.733	0.147	0.672	0.022
Average precision (%)	0.272	0.213	0.166	0.397
Average accuracy (%)	0.528	0.429	0.334	0.648

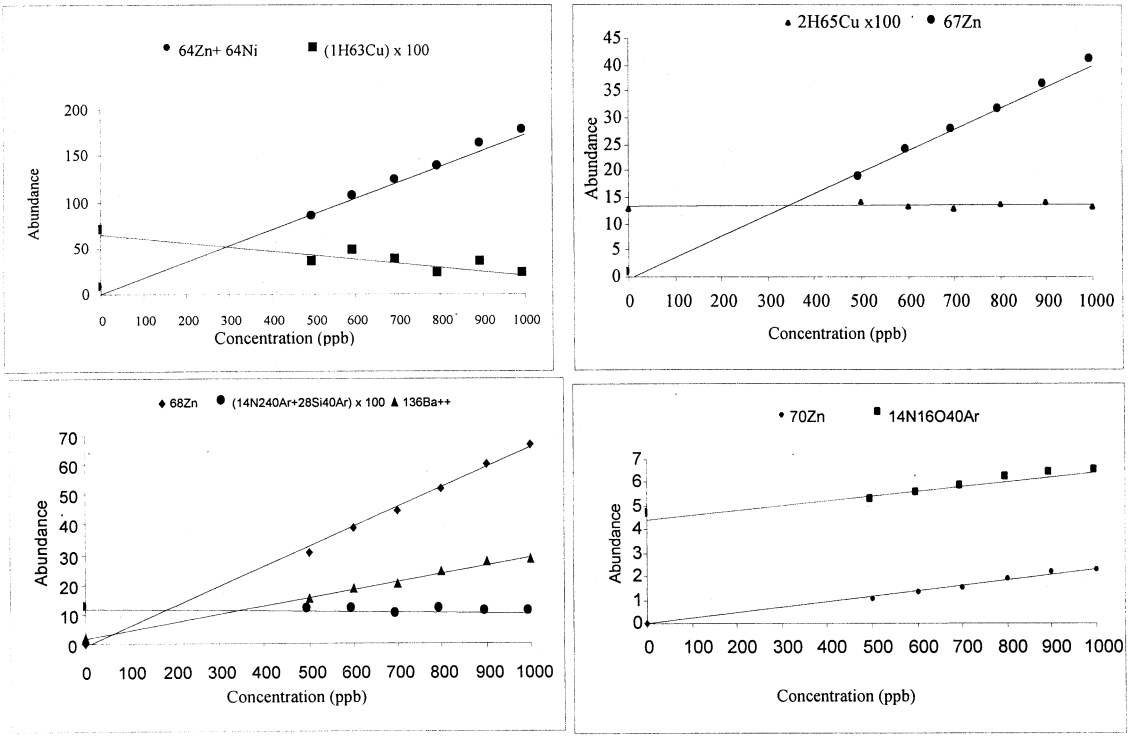

Fig. 5. Variation of ion intensity for zinc in multielement standard solution as a function of concentration at high resolution (5000).

^{66}Zn, ^{68}Zn/^{66}Zn, and ^{70}Zn/^{66}Zn in multielement solution at low and high resolutions. At low resolution a bad accuracy is obtained and this refers to the molecular ion formation in addition to the isobaric interferences. At high resolution the accuracy is improved for the separation of poly ions (Fig. 2) although the isobar 64Ni is not separated. The obtained accuracies at low resolution are (1.273, 154.394, 59.476, and 235.910) and at high resolution they become (1.171, 0.364, 0.267, and 0.472) for the previous isotope ratios, respectively.

Inspection of precisions of the isotopic ratios in Tables 3 and 4 reveals an improvement in the precisions at high resolution than that at low resolution, although the intensities of different isotopes are much reduced (70 times reduction). This could be attributed

Table 4
Zinc isotope ratios in multi elements standard solution

At low resolution (500)	^{64}Zn/^{66}Zn	^{67}Zn/^{66}Zn	^{68}Zn/^{66}Zn	^{70}Zn/^{66}Zn
Actual values	1.742	0.147	0.674	0.022
Average values	1.7 56	0.374	1.075	0.072
Average precision (%)	0.396	0.572	0.628	0.531
Average accuracy (%)	1.273	154.394	59.476	235.910
At high resolution (5 000)	^{64}Zn/^{66}Zn	^{67}Zn/^{66}Zn	^{68}Zn/^{66}Zn	^{70}Zn/^{66}Zn
Average values	1.755	0.147	0.676	0.022
Average precision (%)	0.322	0.287	0.192	0.424
Average accuracy (%)	1.171	0.364	0.267	0.472

Table 5
Microwave program for the digestion of blood and serum samples

Time (min)	3	3	5	3	3
Power (Watt)	200	0	400	0	800

to the separation of polyions, which are formed from different sources in the gas phase. This reflects the instability of these polyions, which depends mainly on the instantaneous experimental conditions of the plasma.

Blood and serum samples are collected and digested, by using the microwave digestion system. 5 ml of nitric acid are added with 2 ml of hydrogen peroxide to 0.2 ml of the sample. The mixtures are left about 30 min before starting the digestion program, to leave the reagent to react with the sample, and to prevent the explosion of H_2O_2 during the digestion. The program can be started in a way as shown in (Table 5).

Inductively coupled plasma mass spectrometer and flame atomic absorption spectrometer is used for measuring zinc concentrations in blood and serum samples for humans. ICPMS measured the concentrations and the isotopic ratios for zinc at high resolution (5000) except the isotopes, which include isobars because they need very high resolution. Comparisons between the measured concentrations in blood and serum samples are tabulated in (Table 6).

A calibration curve is prepared by using NIST

Table 6
A comparison between measured zinc concentration by two techniques

	Concentration (ppm)			
Sample name	ICP-MS	RSD%	F-AAS	RSD%
Blood 1	5.24	0.57	5.30	7.73
Blood 2	6.51	0.42	6.90	8.4
Blood 3	5.43	0.46	5.50	7.09
Blood 4	6.21	0.52	6.70	7.9
Blood 5	6.71	0.43	6.50	5.8
Serum1	0.87	0.57	0.80	10.0
Serum2	1.22	0.66	1.13	10.6
Serum3	0.94	0.64	0.92	11.9
Serum4	1.47	0.48	1.53	8.49
Serum5	0.81	0.49	0.83	10.8

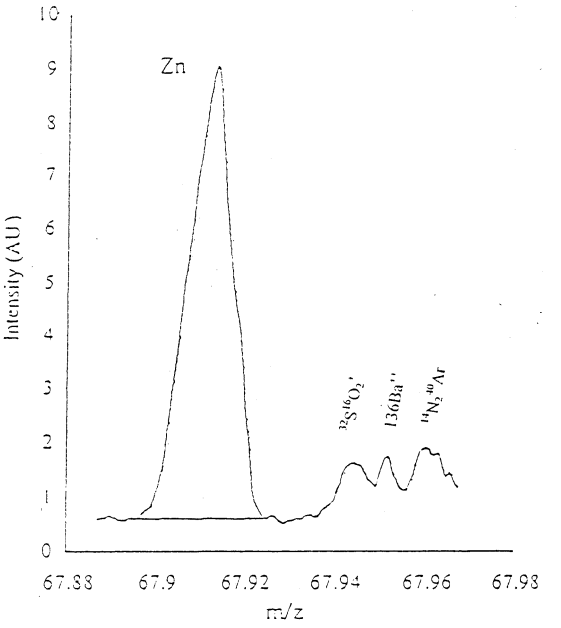

Fig. 6. Separation between ^{68}Zn, $^{32}S^{16}O_2^+$, $^{136}Ba^{++}$, and $^{40}Ar^{14}N_2^+$ in human serum samples at high resolution (5000).

(multielements standard solution) at a concentration of 10 ppm and diluted to 300, 500, and 700 ppb. Five samples are used for measuring zinc concentrations in human serum and blood at high resolution. A complete separation is obtained for ^{68}Zn in serum and blood samples from $^{36}S^{16}O_2^+$, $^{136}Ba^{++}$ and $^{14}N_2^{40}Ar^+$ as shown in Fig. 6.

The obtained results show the capability of ICP-MS for measuring concentrations and isotope ratios with good precision. Since precision values are the controlling factor in nutrition research. Therefore, it is important to compare these values in both techniques of ICP-MS and F-AAS from this point. In ICP-MS the experimental data of the isotope ratios in the analysis of serum and blood and the concentrations show values of RSD % from 0.42 up to 0.66%. In F-AAS, there is no possibility for measuring the isotopic ratios. However, the range of the precision in measuring the concentrations is from 5.8% up to 11.9%. This may lead to the possibility of measuring traces and ultra traces in biological materials, which leads to the development of biological researches for example the nutrition researches.

Table 7
Average $^{67}Zn/^{68}Zn$ and $^{70}Zn/^{68}Zn$ isotope ratios in blood and serum for the five samples

Samples	$^{67}Zn/^{68}Zn$	RSD (%)	$^{70}Zn/^{68}Zn$	RSD (%)
Blood	0.219	0.71	0.033	0.75
Serum	0.220	0.72	0.034	0.76

Concentrations of zinc in the collected data about zinc concentrations in blood and serum samples as measured by different techniques [1–5] have a wide range from 5.3 up to 9.3 ppm for blood and from 0.67 to 1.87 ppm for serum. The obtained experimental results are close to these values, since it ranges from 5.24 up to 6.7 ppm for blood and it ranges from 0.81 up to 1.53 ppm for serum. The obtained results in the present work are found to be within the same concentration range.

The average isotope ratios of $^{67}Zn/^{68}Zn$ and $^{70}Zn/^{68}Zn$ in blood and serum of the five samples at high resolution and their precisions, after taking the previous experimental conditions under consideration, are represented in the Table 7. ^{67}Zn and ^{67}Zn are selected in these measurements because they are used as tracers in metabolic studies.

Friel at al. [6] showed that a precision better than 1% RSD on the measurement of zinc isotope ratios is considered sufficient for the use in human metabolic studies. Following this argument, the measured isotope ratios (at high resolution) as in Table 7 are in the range of 0.7%, which means that the present measurements with these precisions could be used in nutrition researches.

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