



SIMULTANEOUS DETERMINATION OF Cu, Mn, Bi AND Sb USING MULTIELEMENT GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETER

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ABSTRACT

Simultaneous multi-element graphite furnace atomic absorption spectrometer (SIMAA 6000) has been used for developing multi-element determinations methodology for Cu, Mn, Bi, and Sb. Firstly, the optimum experimental conditions in the case of the single-element mode have been determined (these include: pyrolysis and atomization temperature). Then, the compromised conditions for each group of upto four elements in multi-element mode have been also optimized. The setting of the compromised conditions in the case of multi-element mode have been carried out and compared with these of the single-element. The characteristic mass for each element in the simultaneous multielement determination doesn't change compared to the single mode. To study the effect of the matrix, urine standard sample (Seronorm-LOT 0511545) has been used (the urine sample has been diluted (1 : 4)). The accuracy of the methods has been confirmed by analysis of different biological reference materials. The analyzed values in the simultaneous multi-element determinations were in good agreement with the certified values. Simultaneous multi-element GF-AAS provide a rapid, low cost and sensitive method for routine analysis of trace elements.

Key words: Simultaneous multi-element graphite furnace, Copper, Manganese, Bismuth, Antimony.

INTRODUCTION

Among the instrumental techniques available for trace and ultra-trace element determinations, Graphite Furnace Atomic Absorption Spectrometry (GFAAS) occupies an outstanding position due to its high specificity, selectivity and sensitivity, low spectral interference, ease of operation, low sample volume and low cost. The major challenge facing the future of GFAAS stems from an increased competition from other modern spectroscopic techniques, e.g. ICP-MS. But in comparison with other techniques, GFAAS offers several features, especially for routine analysis. However, GFAAS suffers from the very beginning from the fact that it has developed as a single-element technique, which implies a multiplied analysis time, when more than one element has to be measured.

Among the most recent requirements in analytical atomic spectrometry is the capability for simultaneous multi-element determinations in small size samples with a variety of matrices. This capability is particularly important in such fields as clinical and biochemical fields where a large sample size is difficult to obtain. Many different multi-element instruments have been used for the simultaneous multi-

element determination of trace metals, such as Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), X-ray Fluorescence Spectrometry and Cathodic Stripping Voltammetry (CCV)¹. Among of them, the ICP-based instruments are the most commercially available. The applicability of ICP-based instruments has been somewhat limited by difficulty in dealing with high salt concentrations and the need for relatively large sample volumes. Consequently, separation or pre-concentration of analyte from matrix prior to measurement is necessary. Although ICP-MS has emerged as a method with high detection power of which is at least comparable to that of GFAAS, the MS detector is rather complex and expensive, which limited the widespread use of ICP-MS for routine analytical work in labs and hospitals².

Multi-element atomic absorption researches have aroused interest since the first AAS stage in order to conceive a spectrometer able to determine several elements simultaneously^{3,4}. At the end of the 1980s, the analytical society has been faced with the first commercial systems. Hitachi (1988), Thermo Jarrell Ash (1990), Leeman Labs (1993) and Perkin-Elmer (1994) introduced more or less successful multi-element AAS instruments on the market. At the present, the most updated commercial instrumentation is a line source simultaneous spectrometer, equipped with transversely heated graphite atomizer, THGA with integrated platform, Zeeman Effect background corrector and solid-state detector, making possible the operation under Stabilized Temperature Platform Furnace (STPF) conditions⁵⁻¹⁰. This instrument allows simultaneous determinations up to six elements, improved the analytical frequency of ETAAS, reducing costs related to instrument maintenance, sample and high purity reagent consumption. In spite of saving time and costs, multi-element determinations carried out by SIMAAS (Simultaneous Multi-element Atomic Absorption Spectrometry) require the adoption of compromised conditions, which can cause loss of sensitivity and damage the precision of analytical results^{11,12}. Therefore, the optimization of pyrolysis and atomization temperatures and chemical modifier selection are critical to evaluate a reliable method. They must be carefully chosen while taking into account all analytes to be determined simultaneously.

The aim of this study is to develop simultaneous multi-element graphite furnace atomic absorption methodology for the analysis of trace elements in biological samples.

EXPERIMENTAL

Instrumentation

Measurements were carried out by using a SIMAA 6000 electrothermal atomic absorption spectrometer with a longitudinal Zeeman-effect background correction system, Echelle optical arrangement, Solid-state detector and Standard THGA tube with pyrolytic coated integrated platform (Perkin-Elmer GmbH, Bodenseewerk). The rate of flow of the normal gas (Ar) was 250 mL.min⁻¹. Stopped flow during the atomization was used. The lamps used were hollow cathode lamps (HCL) for the determination of Cu and Mn and multi-element electrodeless discharge lamp for Bi and Sb. They were from Perkin-Elmer and the wavelengths used were: Cu 324.8 nm, Mn 279.5 nm, Bi 223.1 nm and Sb 217.6 nm. The sample injection volume was 20 µL. The integrated absorbance of the atomic absorption signal was used for the determination.

Reagents and samples

High purity water (18 MΩ cm) was prepared with de-ionized water system (Milli Q, Millipore Corp.). Analytical reagent-grade nitric acid (from KMF) was purified by sub-boiling distillation. AAS-Standard reference solutions of Cu, Mn, Bi and Sb (Brend Kraft GmbH) were used to prepare the reference

analytical solutions. The chemical modifier solutions (ICP-Standard Palladium and AAS-Standard Magnesium) were from Bernd Kraft GmbH.

The accuracy of the method was confirmed by the determination of Cu, Mn, Bi and Sb in trace elements urine sample from Seronorm (LOT 0511545) and Lyphocheck urine metals control level 1 from Bio-Rad (LOT 69061).

Contamination control

All sample containers, auto-sampler cups, etc. were acid washed with 10% v/v nitric acid for 24 h and then rinsed several times with de-ionized water before use. The analytical reference solutions were prepared daily by diluting with 0.2% nitric acid.

RESULTS AND DISCUSSION

Single-element mode optimization

When ETAAS is used for single-element determination, all experimental and instrumental parameters are optimized for only one analyte. Consequently, the best optimized pyrolysis and atomization temperatures are used in the heating program, minimizing condensed and gas-phase interference¹². The best pyrolysis and atomization temperatures were determined according to the pyrolysis and atomization curves and the absorbance peak for each element in the single-element mode. The heating program temperatures were summarized in Table 1. Fig. 1 shows the absorbance peaks for each element in the case of single-element mode at the optimum heating program. The heating program for each element was used to determine the detection limits and the characteristic mass for each element and they were summarized in Table 2.

Table 1: Temperature program for single-element determination of Cu, Mn, Bi and Sb with Pd + Mg modifier

Step	Temp. (°C)	Ramp time (s)	Hold time (s)	Gas Flow (mL min ⁻¹)
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Pyrolysis	Various ^a	10	20	250
Atomization	Various ^b	0	5	0
Clean-out	2450	1	3	250

^a1100, 1200, 1300°C for Bi, Cu and (Mn and Sb)

^b1800, 1900, 2000°C for Bi, Sb and (Mn and Cu)

Table 2: Detection limits and characteristic mass for single-element determination with Pd + Mg modifier

Element	Detection limit (µg L ⁻¹)	Characteristic mass (pg)
Cu	0.13	19.6
Mn	0.051	5.0
Bi	0.46	67.7
Sb	0.29	41.9

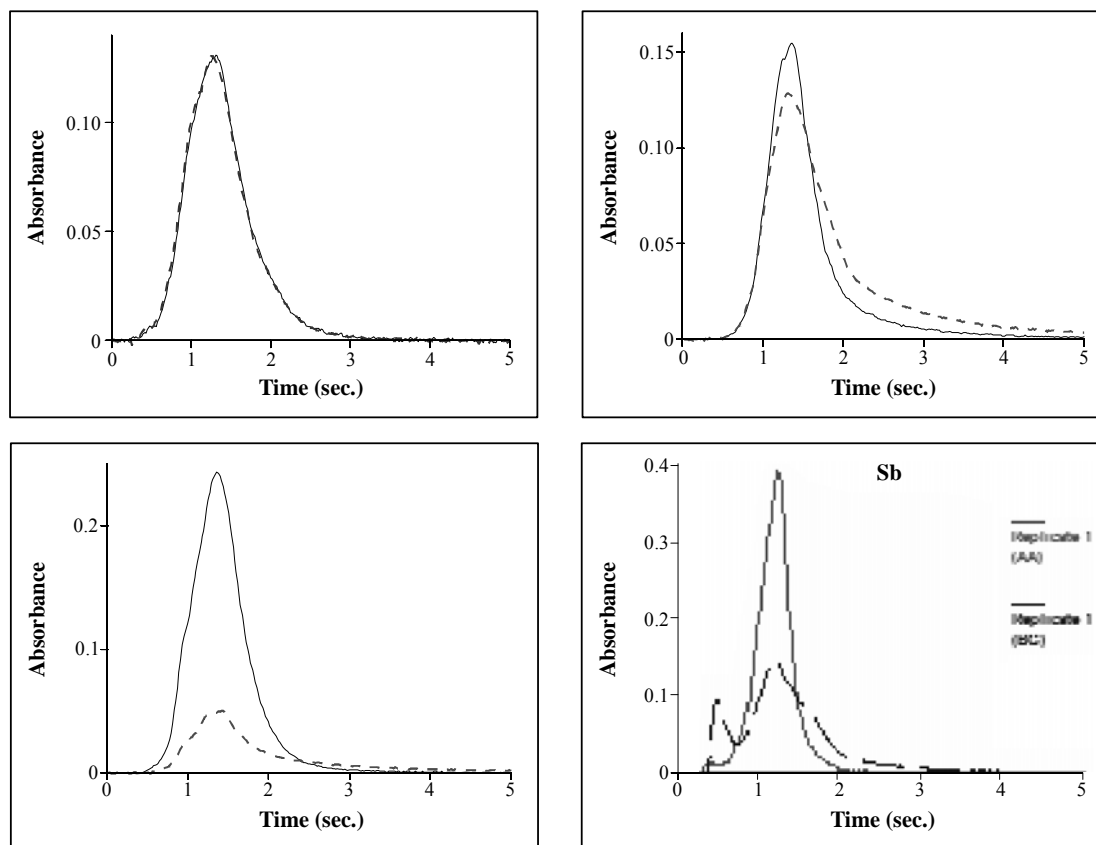


Fig. 1: Peak signal for single-element mode at the optimum heating program for each element

Multi-element mode optimization

In this case, the experimental and instrumental parameters are optimized for all elements in the multi-element mode. The heating program temperature and the chemical modifier must be carefully selected to achieve the best atomization efficiency for all the analytes. In general, the most volatile analyte determines the pyrolysis temperature while the least volatile one determines the atomization temperature.

The mixture of palladium and magnesium nitrate has been widely used for multi-element determinations by SIMAA¹²⁻¹⁵. It is considered as universal chemical modifier due to the thermal stability improvement for 21 elements. Therefore, this modifier has been used in this work for the multi-element determination of Cu, Mn, Bi and Sb. This modifier has also been used for the single-element optimization. The heating program temperature was summarized in Table 3. Fig. 2 shows the absorbance peak for each element in the case of multi-element mode at the compromised conditions.

Table 3: Temperature program for multi-element determination of Cu, Mn, Bi and Sb with Pd + Mg modifier

Step	Temp. (°C)	Ramp time (s)	Hold time (s)	Gas Flow (mL min ⁻¹)
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Pyrolysis	1100	10	20	250
Atomization	2000	0	5	0
Clean-out	2450	1	3	250

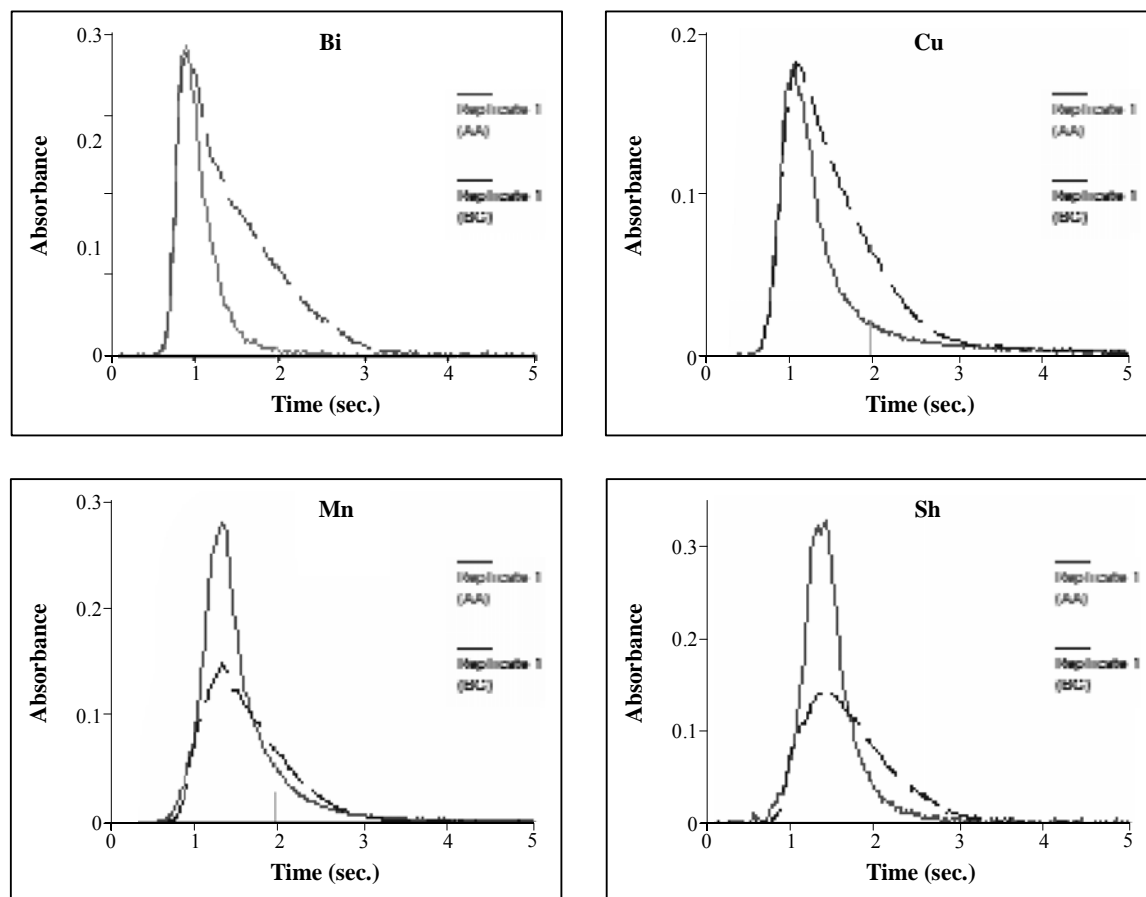


Fig. 2: Peak signal for multi-element mode at the optimum heating program for each element

The detection limits and the characteristic mass for each element were determined in the multi-element mode by using the compromised heating program and summarized in Table 4. The values of the characteristic mass are comparable with those of the single-element determinations. The higher detection limits for multi-element mode in comparison with single-element mode, can be related to the compromised conditions adopted for the simultaneous determination. Fig. 3 shows the pyrolysis and atomization curves for each element.

Study the effect of the matrix

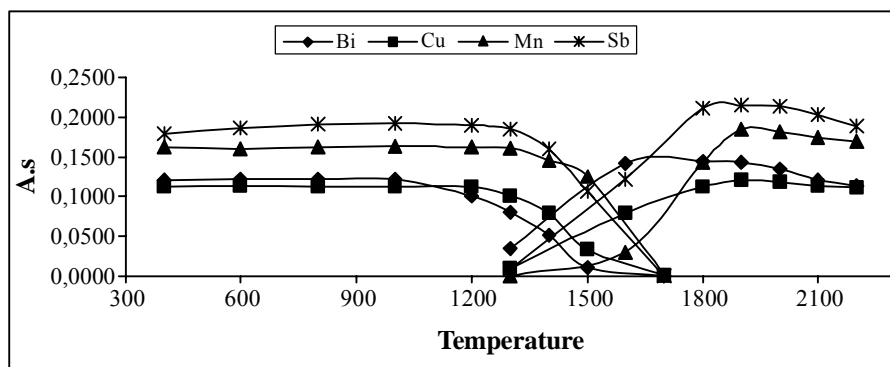
To study the effect of the matrix on the simultaneous determinations of Cu, Mn, Bi and Sb, we have used a reference urine sample from Seronorm. The urine sample was diluted (1 : 4) with 0.2% nitric acid. Mixture of 5 μg Pd and 3 μg Mg (NO_3)₂ was used as a chemical modifier. 20 μL diluted sample was injected into the graphite tube with the chemical modifier in one step.

Effect of pyrolysis temperature

The absorption signals were studied at an atomization temperature of 2000°C. The atomic absorption signals remained approximately constant as the pyrolysis temperature increased until 1000, 1200, 1300 and 1350°C for Bi, Cu, Sb and Mn, respectively. When the temperature increased above 1000°C, the absorption signal of Bi gradually decreased due to the volatilization of it. In order to determine all these elements together, 1000°C was chosen as an optimum pyrolysis temperature.

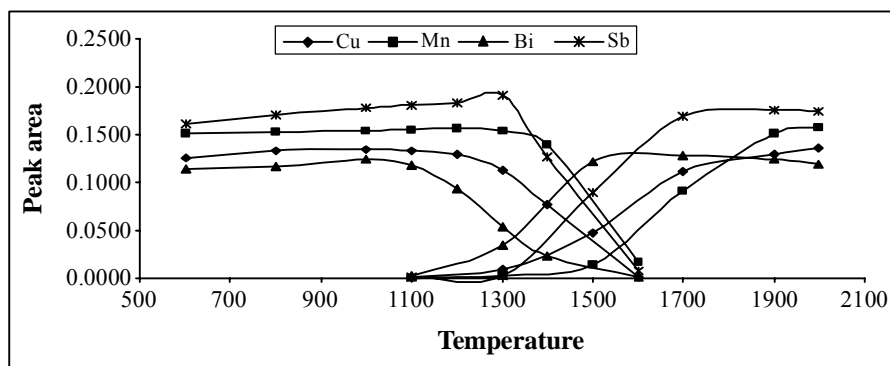
Table 4: Detection limits and characteristic mass for multi-element determination with Pd + Mg modifier

Element	Detection limit ($\mu\text{g L}^{-1}$)	Characteristic mass (pg)
Cu	0.28	20.5
Mn	0.087	5.1
Bi	1.4	80
Sb	0.90	44

**Fig. 3: Pyrolysis and atomization curves of Cu, Mn, Bi and Sb with Pd + Mg modifier**

Effect of atomization temperature

The absorption signals were studied at a pyrolysis temperature of 1000°C. For Bi, the atomic absorption signal decreased with increasing the atomization temperature above 1600°C. However, for Sb, it is increased and had its maximum value in the temperature range 1700-1800°C. For Cu and Mn, the maximum absorption signal was at 2000°C atomization temperature. Hence we decided to choose 2000°C as the optimum atomization temperature for all the four elements. Figure 4 shows the pyrolysis and atomization curves of each element in the diluted sample by using Pd+Mg as a modifier. The peak signal for each element at the optimum pyrolysis and atomization temperatures were shown in Figure 5.

**Fig. 4: Pyrolysis and atomization curves of Cu, Mn, Bi and Sb in diluted urine sample (1 : 4) with Pd + Mg modifier**

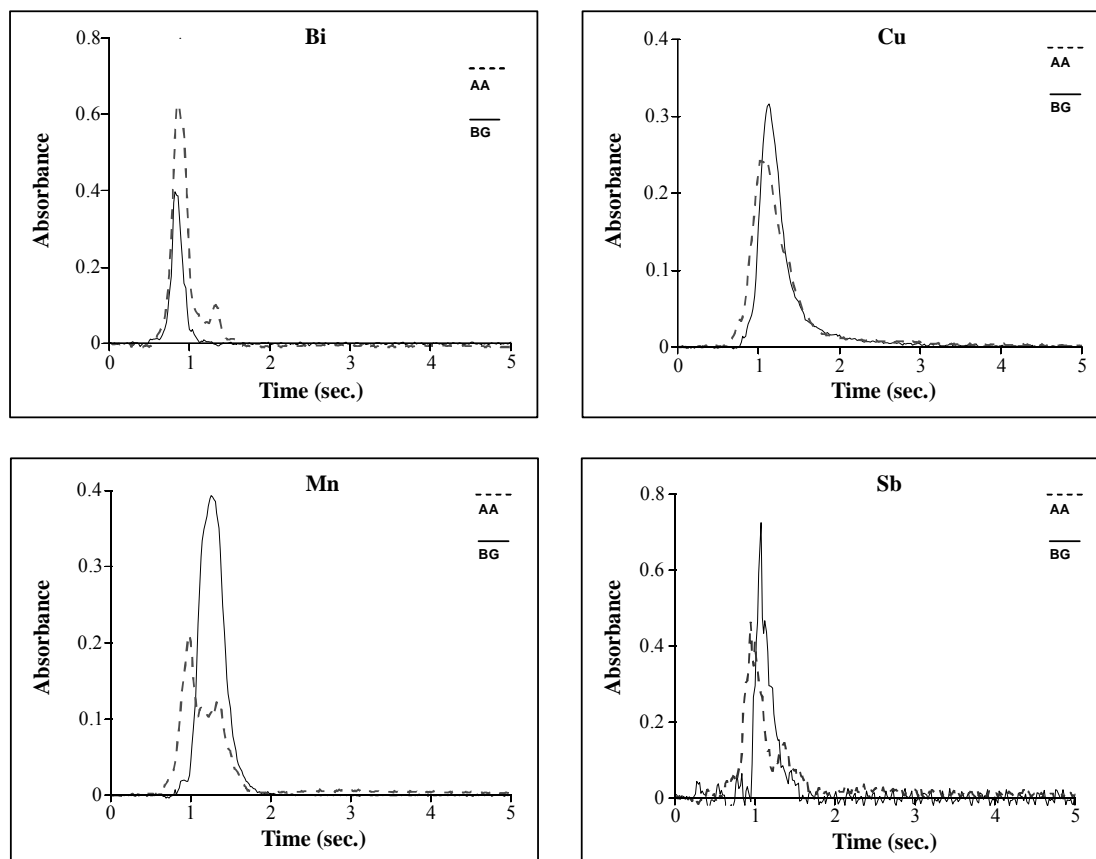


Fig. 5: Peak signals for multi-element determination in diluted urine sample with Pd + Mg modifier and at 1000 and 2000°C pyrolysis and atomization, respectively

Analysis of certified reference urine samples

The optimum temperature program is summarized in Table 5. Certified reference urine sample from Seronorm (LOT 0511545), Lyphocheck urine metals control level 1 from Bio-Rad (LOT 69061), Pig Kidney (BCR-CRM 186), and Bovine Liver (NIST-SRM 1577b) were used to confirm the accuracy of the developed method. The sample from Seronorm was diluted (1 : 4), Bio-Rad (1 : 1), Pig Kidney (1 : 29), while Bovine liver (1 : 99) with 0.2% nitric acid. The analyzed values for the four elements were within the range of the certified values. The results of the simultaneous determinations comparing with the certified values were summarized in Table 6.

Table 5: Temperature program for multi-element determination of Cu, Mn, Bi and Sb in diluted urine sample with Pd + Mg modifier

Step	Temp. (°C)	Ramp time (s)	Hold time (s)	Gas Flow (mL min ⁻¹)
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Pyrolysis	1000	10	20	250
Atomization	2000	0	5	0
Clean-out	2450	1	5	250

Table 6 Results of simultaneous determination of Cu, Mn, Bi and Sb in reference urine sample

Sample		Cu	Mn	Bi	Sb
Seronorm	Con. found ($\mu\text{g L}^{-1}$)	75.3	13.7	19.6	105.8
	Con. certified ($\mu\text{g L}^{-1}$)	78	12.3	20.1	99.9
	DL ($\mu\text{g L}^{-1}$)	0.29	0.12	1.38	0.90
	% RSD	2.8	2.3	2.7	2.1
Bio-Rad	Con. found ($\mu\text{g L}^{-1}$)	7.4	1.7	12.2*	16.3
	Con. certified ($\mu\text{g L}^{-1}$)	7.0	<3.5	12.0	16.4
	DL ($\mu\text{g L}^{-1}$)	0.34	0.11	1.50	1.13
	% RSD	0.5	3.1	5.8	4.6
Pig kidney	Con. found ($\mu\text{g L}^{-1}$)	15.9	4.38	14.5	14.6
	Con. certified ($\mu\text{g L}^{-1}$)	16.6	4.44	14.5*	14.5*
	DL ($\mu\text{g L}^{-1}$)	0.32	0.13	1.50	1.10
	% RSD	2.2	1.6	3.5	3.8
Bovine liver	Con. found ($\mu\text{g L}^{-1}$)	71.6	5.3	51.5	50.3
	Con. certified ($\mu\text{g L}^{-1}$)	82.1	5.4	50.0*	50.0*
	DL ($\mu\text{g L}^{-1}$)	0.41	0.13	1.50	1.10
	% RSD	1.9	3.2	3.4	3.6

* Added

Detection limits and precision

The detection limits (DL) and the relative standard deviations (RSD) were shown in Table 6. Detection limits were calculated as three times the standard deviation of ten replicate measurements of the blank. The RSD for the direct simultaneous determination of Cu, Mn, Bi and Sb in the urine sample were in the range (0.5-5.8%)

CONCLUSION

Simultaneous multi-element determination for a set of elements can be carried out using Simultaneous Multi-element GFAA Spectrometer (SIMAA 6000) under compromised conditions. These include pyrolysis and atomization temperature and the use of a suitable modifier. The sensitivity and the accuracy of this technique are comparable to those of the single-element determination. The detection limits were higher because of the set of the compromised conditions. Different combinations of elements can be directly determined by SIMAA 6000, if the compromised conditions have been carefully chosen.

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