

The determination of copper, zinc, cadmium and lead in urine by high resolution ICP-MS

JAS

Journal of
Analytical
Atomic
Spectrometry

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Received 30th June 1998, Accepted 11th September 1998

High resolution ICP-MS was used to determine Cu, Zn, Cd and Pb in urine. The effect of sample dilution, preparation method and choice of internal standard were assessed. Sample dilution (1 + 9) with In as the internal standard was found to offer an acceptable compromise between analytical accuracy and sample throughput. A spectral resolution of 3000 was used to separate Cu and Zn isotopes from interferences commonly found in biological matrices, while a resolution of 300, offering increased sensitivity and lower detection limits, was used for Cd and Pb. The accuracy and precision of the analytical method were evaluated using two Bio-Rad Lyphohek standard urines. The concentrations of Cu, Zn, Cd and Pb in Bio-Rad Level 1 reference urine were determined by external calibration and were found to agree to within 0–17% of recommended values (Cu 48, Zn 710, Cd 6.5 and Pb 14.3 ng g⁻¹). Closer agreement of 0–7% was found for Bio-Rad Level 2 reference urine (Cu 63, Zn 1057, Cd 12.3 and Pb 69 ng g⁻¹). Forty-two urine samples from seven workers occupationally exposed to Cd were analysed and the results were compared with those obtained from 11 samples collected from four non-exposed volunteers. Similar average concentrations of Cu and Zn were found in both groups when the results were normalised to creatinine levels. Workers exposed to Cd were found to have an average urine Cd concentration elevated approximately 7–8-fold over that measured for the control group (about 2.2 compared with about 0.3 ng g⁻¹, or 1.7 and 0.2 µg per gram of creatinine when normalised). Urinary levels of Pb were slightly increased in the cadmium exposed workers (about 6 compared with about 4 ng g⁻¹).

Introduction

Cadmium poses a significant health risk when exposure is high, which can be from either industrial or environmental sources. Cadmium has a long elimination half-life and accumulates principally in the kidney and liver, leaving chronically exposed individuals at risk of renal tubular damage.¹ The accumulation is mediated by binding to a low molecular mass, cysteine-rich protein, metallothionein, which also tightly sequesters copper and zinc.²

The risk in the industrial setting was initially recognised during the 1940s and 1950s.³ Individuals occupationally exposed to cadmium fumes and dust in smelting, welding or electroplating situations are at risk of increased cadmium body burdens from uptake *via* the respiratory system. The past 50 years have seen a downward revision of recommended maximum exposure levels along with the introduction of respiratory protective equipment, improved ventilation, better work practices and improved monitoring techniques. Improved sensitivity of biological monitoring techniques has also contributed.

Cadmium in urine is a measure of total body concentration and is the most efficient means for regular biomonitoring.⁴ Current data indicate that renal tubular dysfunction may occur if the long-term normalised concentration of urinary cadmium exceeds 10 µg g⁻¹ creatinine, thus the present threshold limit is 5 µg g⁻¹ creatinine.⁵

Atomic absorption spectrometry has commonly been the analytical method used for routine clinical assays and screening studies.^{6–8} More recently, quadrupole based ICP-MS has become the technique of choice for many applications, combining multi-element analysis and fast analysis times with excellent detection limits.^{9–17} However, ICP-MS is not without its

limitations. In the clinical laboratory, the high levels of organics and inorganic salts in blood, serum and urine samples often lead to matrix interferences and isobaric overlap from polyatomic ions. Nixon and Moyer,¹³ Hsiung *et al.*¹⁵ and Barany *et al.*¹⁶ used quadrupole ICP-MS instruments to determine the concentrations of elements such as As, Cd, Pb, Tl, Cu, Zn, Co, Ni and Se in urine and blood samples. In these studies, much discussion focused on the problems caused by spectral interferences, particularly found for elements with atomic mass lower than 80 u (*e.g.*, Cu, Zn, As).

High resolution (HR) ICP-MS is a relatively new technique employing a magnetic sector mass spectrometer. Many polyatomic interferences typically encountered in biological applications can be overcome with this type of instrument by using a higher resolution setting, while the combination of high sensitivity and low background results in extremely low detection limits.^{18–21} With regard to clinical applications, HR-ICP-MS has been used to determine precious metals in blood,^{22,23} trace elements in serum,^{8,20,24} uranium in urine²⁵ and Ca isotope ratios in urine.²⁶

It was the aim of this study to use HR-ICP-MS to quantify cadmium concentrations in urine samples collected from workers exposed principally to cadmium dust and fumes from an industrial electroplating and casting process. Copper and Zn are refined at the same industrial site, although they are largely removed from the process before cadmium purification. These elements were studied because of their association with metallothionein in the kidney. Lead was monitored because of its close industrial relationship and affinity with these elements, although the relationship between urinary Pb levels and toxicological effect is incompletely understood.²⁷ A simple sample

preparation method involving acidification and dilution is reported, and some of the advantages of HR-ICP-MS over quadrupole based instruments are demonstrated.

Experimental

Reagents and standards

High purity HNO₃ (Mallinckrodt, Paris, KY, USA) was used as received for urine digestion and solution acidification. Deionised water (≥ 18 M Ω) obtained using a Milli-Q system (Millipore, Bedford, MA, USA) was further purified in a quartz sub-boiling still prior to use.

Mixed standard solutions of Cu, Zn, Cd and Pb were prepared from a 100 $\mu\text{g g}^{-1}$ multi-element solution (QCD Analysts-Environmental Science Solutions, Spring Lake, NJ, USA). More concentrated individual Cu and Zn standards were also prepared from 1000 $\mu\text{g g}^{-1}$ single element standards (Plasma Chem, Farmingdale, NJ, USA). Scandium and In internal standards were obtained from 1000 $\mu\text{g g}^{-1}$ single element solutions (High Purity Standards, Charleston, SC, USA) and Bi was prepared separately from the solid oxide (High Purity Standards, Spex, Metuchen, NJ, USA). All prepared standard solutions were acidified with 1% HNO₃.

Two Bio-Rad (Munich, Germany) reference urines (Lyphochek Urine Metals Control Level 1-69011 and Level 2-69022) were extensively used for method development. The lyophilised references were reconstituted with 25 ml of ultra-pure water as recommended by the manufacturer. Both standard urines contained Cu, Zn, Cd and Pb, and were supplied with recommended values and an acceptable concentration range ($\pm 20\%$).

Calibration

Aqueous standard solutions covering the concentration ranges Cu 0–50, Zn 0–100, Cd 0–10 and Pb 0–10 ng g^{-1} were used for external calibration. Between four and seven standards were used for each element, providing correlation coefficients in excess of 0.995. Standards were prepared daily in a laminar flow hood. Calibration by standard additions was also employed for a limited number of urine samples, for comparison and verification. For this method four samples of the same urine were spiked with increasing amounts of the elements to be analysed. All standards were acidified with 1% HNO₃.

Urine preparation

Prior to ICP-MS analysis, urine samples were diluted (1+9), acidified (to 1% HNO₃ final concentration) and spiked with internal standard (typically In, 10 ng g^{-1} final concentration). This simple preparation method is designated 'cold dilution'. For comparison purposes, other preparation methods were tested using reference urine samples. The 'hot digestion' method involved warming a small volume of urine at 40–50 °C to incipient dryness, before being reconstituted in concentrated HNO₃ and diluted to final volume. Reference urine was also prepared by the 'microwave digestion' method in the presence of HNO₃. A Milestone (Soriso, Italy) MLS-1200 Mega microwave digestion system using medium pressure vessels was used for the digestion. The standard procedure suggested for urine samples was followed and consisted of three 5 min power stages (250, 400 and 600 W). In comparing the three digestion methods, the same urine dilution factor (1+9), final acid strength (1% HNO₃) and internal standard concentration (In at 10 ng g^{-1}) were used.

Urine samples

Urine samples were collected from seven males with industrial exposure to cadmium. All employees working in this section

used respiratory protective equipment. The mean age (\pm standard deviation) of the group was 53 ± 7 years and the median time spent working with exposure to cadmium was 4 years (range 1.5–22 years). All subjects had been smokers for an average of 23 ± 9 years, although six had since quit (on average 15 ± 10 years previously). The concentration of cadmium in air at the time of the study was $13 \mu\text{g m}^{-3}$, based on site monitoring data. The control group consisted of four males (mean age 56 ± 3 years) who were university based and not exposed to high levels of cadmium. Two of these were former smokers, one was a current smoker and one had never smoked. Average years spent smoking were 25 ± 13 years (for the three individuals who had smoked). All subjects gave informed and written consent.

Early morning urine samples were collected by the exposed workers in their homes over a 6 d period. Collection commenced 1 d prior to their 4 d working shift and finished 1 d after their shift. In addition, one spot urine sample was collected on-site from each exposed worker on day three of their shift. The non-exposed group collected early morning samples over a period of 3 d. Urinary creatinine was quantified using the alkaline picrate (Jaffé) method.²⁸

Samples were collected in 120 ml polycarbonate containers (Labserv, Auckland, New Zealand) which were acid washed (10% HNO₃) and rinsed with Milli-Q de-ionised water before use. Urine samples were acidified within 12–48 h of collection (1% HNO₃ final concentration). Samples were stored at 4 °C until analysed (within 2 weeks).

A pool urine was prepared from exposed and non-exposed worker samples. The pool was acidified (1% HNO₃), aliquoted into several tubes and frozen (-20 °C). The pool urine was used to measure the long term reproducibility of the analytical method over a 6 month period.

Instrumentation

Measurements were carried out on an ELEMENT HR-ICP-MS system (Finnigan MAT, Bremen, Germany). This instrument has predefined resolution settings ($m/\Delta m$ at 10% valley definition) of 300 (low), 3000 (medium) and 7500 (high). Instrument settings are outlined in Table 1. A standard Meinhard nebuliser and Scott double pass water cooled spray chamber were employed. Isotopes of interest were analysed using electric scanning, with the magnet held at fixed mass. The secondary electron multiplier detector was operated in

Table 1 Typical instrument settings

Instrument	ELEMENT (Finnigan MAT)
Resolution ($m/\Delta m$)	Low = 300, medium = 3000, high = 7500
Rf power	1250 W
Gas flows rates	
Plasma gas	12–13 l min^{-1}
Auxiliary	0.9–1 l min^{-1}
Sample gas	1.0–1.2 l min^{-1} (optimised daily)
Torch	Fassel type
Nebuliser	Meinhard
Spray chamber	Scott-type (double pass), cooled to 3.5–5 °C
Cones	Ni sampler (1.1 mm orifice id) and skimmer (0.8 mm orifice id)
Sample uptake	Pumping <i>via</i> a Spetec peristaltic pump
Instrument tuning	Performed daily using a 10 ng ml^{-1} multi-element solution
Ion transmission	$\sim 100\,000$ counts s^{-1} per ng g^{-1} In
Scan type	Magnetic jump with electric scan over small mass range
No. of sample scans	40
Ion sampling depth	Adjusted ^a
Ion lens settings	Adjusted ^a

^aAdjusted to obtain maximum signal intensity.

counting mode. Instrument tuning and optimisation was performed daily using a 10 ng g⁻¹ multi-element solution containing the elements of interest. Further details concerning the ELEMENT instrument have been reported previously.¹⁸⁻²¹

The isotopes used for analysis in this study are given in Table 2, along with some potential interferences reported when analysing urine samples. For comparison purposes, two or three isotopes were monitored for each element. The isotopes tested as internal standards are also noted in Table 2. Quality control samples were run every 5-10 samples during the course of an analytical sequence to monitor instrument performance.

Cadmium levels were independently determined by the Clinical Chemistry Department of the Royal Hobart Hospital. These analyses were carried out by ETAAS (Model 640Z, Varian, Melbourne, Australia) with Zeeman background correction. Briefly, 14 acidified urine samples were diluted 1+4 with a modifier consisting of 0.4% m/v (NH₄)₂HPO₄, 5% v/v HNO₃ and 0.2% v/v Triton X-100. Furnace conditions consisted of an ashing stage between 450 and 550 °C with atomization at 1600 °C. Results were calculated using standard additions to a control urine sample.

Results and discussion

Interferences and choice of instrument resolution

Most clinical studies using ICP-MS to measure elements below 80 u have reported spectral interferences arising from the presence of relatively high concentration of organic species.^{8,10,11,13,15,24} This problem has often been minimised by limiting the analysis to (relatively) interference-free isotopes. As an example, Hsiung *et al.*¹⁵ recently determined Cu and Zn in urine using quadrupole ICP-MS. ⁶³Cu was found to suffer greatly from spectral overlap from ⁴⁰Ar²³Na, so ⁶⁵Cu was chosen for analysis as interferences from S and Ca species were noted to be low at this mass. Similarly, ⁶⁴Zn, ⁶⁶Zn and ⁶⁷Zn isotopes suffered (to various extents) from interference by S and O based polyatomics (see Table 2). Based on H₂SO₄ spike studies, Hsiung *et al.*¹⁵ suggested that ⁶⁸Zn was relatively free from these interferences. Similar Cu and Zn interference

trends were noted in an early study by Vanhoe *et al.*¹⁰ HR-ICP-MS provides increased resolution to separate common polyatomic interferences from the analyte of interest. Fig. 1 shows the mass spectrum of ⁶³Cu in the pool urine (diluted 1+9) obtained using HR-ICP-MS at a resolution of 3000. The signal corresponds to about 11.5 ng g⁻¹ in the original sample. Copper is clearly separated from the suggested polyatomic interferences ⁴⁰Ar²³Na, ³¹P¹⁶O₂ and ²³Na₂¹⁶O¹H.²⁹ The determination of ⁶⁵Cu in the pool urine is shown in Fig. 2, also at a spectral resolution of 3000, and probable interferences from ³³S¹⁶O₂, ³²S¹⁶O¹⁷O and ³²S¹⁶O₂¹H are noted.²⁹ Small interferences were measured at masses close to ⁶⁸Zn, present in the pool urine at a concentration of about 390 ng g⁻¹ (Fig. 3). It is difficult to assign these interferences unequivocally, but possible species may be ³⁶Ar³²S, ³⁴S³⁴S, ³²S³⁶S, ³⁶Ar¹⁶O₂, SO₂ (various isotopic combinations), ³⁵Cl¹⁶O₂¹H and ⁴⁰Ar¹⁴N₂, which have been reported previously.^{15,30} For each of these three examples, the polyatomics could not be resolved on quadrupole based instruments (or in the low

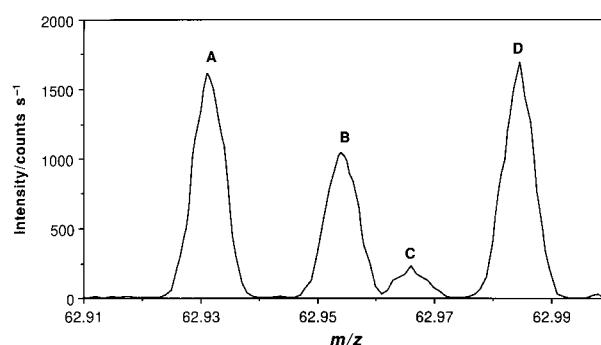


Fig. 1 Mass spectrum of ⁶³Cu and nearby interferences in the 1+9 diluted pool urine. The average Cu concentration measured using this isotope with a spectral resolution of 3000 was found to be 11.5 ng g⁻¹. Analyte and suggested interferences are (A) ⁶³Cu, (B) ⁴⁰Ar²³Na, (C) ³¹P¹⁶O₂ and (D) ²³Na₂¹⁶O¹H. The Na based interferences may arise from Na in the sample or from blank contributions.

Table 2 Isotopes of interest, internal standards used and some potential interferences when analysing urine samples. The majority of the interferences noted have been reported previously.^{8-11,13,15,24,29,30} Method detection limits are also given

Isotope	Abundance (%)	Resolution ^a	Example problematic interferences ^b	Method detection limit pg g ⁻¹ (n = 10)
<i>Isotopes of interest—</i>				
⁶³ Cu	69.17	3000	⁴⁰ Ar ²³ Na, ²³ Na ₂ ¹⁶ O ¹ H, ²³ Na ₂ ¹⁷ O, ³¹ P ¹⁶ O ₂ , ³⁵ Cl ¹⁴ N ₂	29
⁶⁵ Cu	30.83	3000	³³ S ¹⁶ O ₂ , ³² S ¹⁶ O ¹⁷ O, ³² S ³³ S, ³² S ¹⁶ O ₂ ¹ H, ⁴⁸ Ca ¹⁷ O, ⁴⁸ Ca ¹⁶ O ¹ H, ³⁷ Cl ¹⁴ N ₂ , ³¹ P ¹⁶ O ¹⁸ O, ³¹ P ¹⁷ O ₂	49
⁶⁶ Zn	27.90	3000	³² S ¹⁶ O ¹⁸ O, ³² S ¹⁷ O ₂ , ³³ S ¹⁶ O ¹⁷ O, ³³ S ¹⁶ O ₂ ¹ H, ³⁴ S ¹⁶ O ₂ , ³³ S ₂ , ³² S ³⁴ S, ⁴⁸ Ca ¹⁸ O, ⁴⁸ Ca ¹⁷ O ¹ H	95
⁶⁷ Zn	4.10	3000	³³ S ¹⁷ O ₂ , ³³ S ¹⁶ O ¹⁸ O, ³² S ¹⁷ O ₂ ¹ H, ³² S ¹⁶ O ¹⁸ O ¹ H, ³⁴ S ¹⁷ O ¹⁶ O, ³³ S ³⁴ S, ³⁶ Ar ³¹ P, ³⁵ Cl ¹⁶ O ₂	282
⁶⁸ Zn	18.80	3000	³⁶ S ¹⁶ O ₂ , ³⁴ S ¹⁶ O ¹⁸ O, ³⁴ S ¹⁷ O ₂ , ³² S ¹⁸ O ₂ , ³³ S ¹⁷ O ¹⁸ O, ³² S ³⁶ S, ³⁴ S ₂ , ³⁶ Ar ³² S, ³⁵ Cl ¹⁶ O ¹⁷ O, ³⁵ Cl ¹⁶ O ₂ ¹ H, ³⁶ Ar ¹⁶ O ₂ , ⁴⁰ Ar ¹⁴ N ₂	111
¹¹¹ Cd	12.80	300	⁹⁵ Mo ¹⁶ O	3.7
¹¹² Cd	24.13	300	¹¹² Sn (0.97% ^c), ⁹⁶ Mo ¹⁶ O	1.6
¹¹⁴ Cd	28.73	300	¹¹⁴ Sn (0.65% ^c), ⁹⁸ Mo ¹⁶ O	1.4
²⁰⁶ Pb	24.10	300		4.2
²⁰⁷ Pb	22.10	300		7.8
²⁰⁸ Pb	52.40	300		6.6
<i>Internal standards—</i>				
⁴⁵ Sc	100	3000	²⁹ Si ¹⁶ O, ²⁸ Si ¹⁶ O ¹ H, ¹³ C ¹⁶ O ₂ , ¹² C ¹⁶ O ₂ ¹ H	
¹¹⁵ In	95.70	300	¹¹⁵ Sn (0.36% ^c)	
²⁰⁹ Bi	100	300		

^aLow resolution corresponds to $m/\Delta m = 300$ and medium resolution to $m/\Delta m = 3000$. ^bThis interference list is not exhaustive and should be used as a guide only. ^cIsotope percentage abundance.

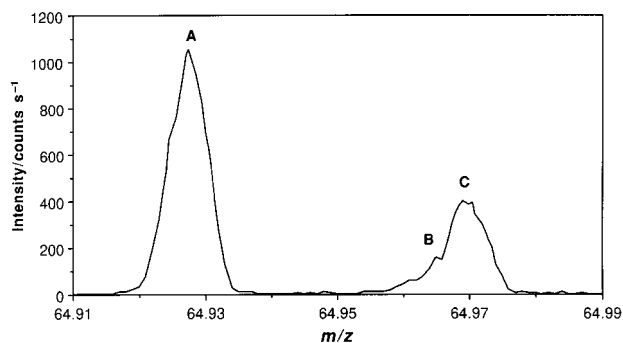


Fig. 2 Mass spectrum of ^{65}Cu and nearby interferences in the 1+9 diluted pool urine (resolution 3000). The average Cu concentration was found to be 11.6 ng g^{-1} using this isotope. Analyte and suggested interferences are (A) ^{65}Cu , (B) $^{33}\text{S}^{16}\text{O}_2$ and $^{32}\text{S}^{16}\text{O}^{17}\text{O}$ and (C) $^{32}\text{S}^{16}\text{O}_2^1\text{H}$.

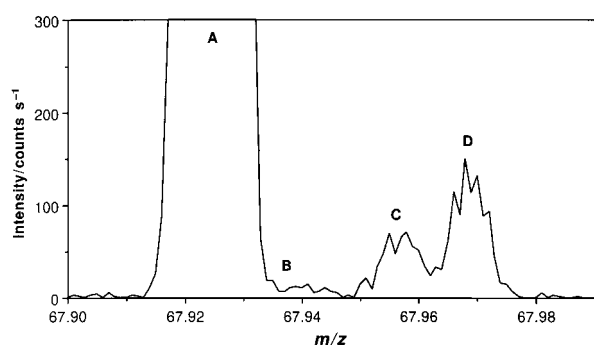


Fig. 3 Mass spectrum of ^{68}Zn and nearby interferences in the 1+9 diluted pool urine (resolution 3000). The Zn signal (shown truncated) corresponds to about 12 000 counts s^{-1} and an average concentration of 391 ng g^{-1} . Analyte and possible interferences are (A) ^{68}Zn , (B) $^{36}\text{Ar}^{32}\text{S}$ and $^{34}\text{S}^{34}\text{S}$, (C) $^{36}\text{Ar}^{16}\text{O}_2$ and $^{36}\text{S}^{16}\text{O}_2$ and (D) SO_2 (various isotopic combinations), $^{35}\text{Cl}^{16}\text{O}_2^1\text{H}$ and $^{40}\text{Ar}^{14}\text{N}_2$.

resolution mode for HR-ICP-MS instruments), leading to higher than expected values for urine samples. This would be problematic for Cu isotopes, but not so apparent for Zn, which is present in urine at higher concentrations. The ^{65}Cu and ^{66}Zn isotopes have typically been used for the analysis of clinical samples using ICP-MS.^{9,10,15,16,24}

Isobaric overlap of ^{112}Cd and ^{114}Cd by two weak isotopes of Sn (^{112}Sn 0.97% and ^{114}Sn 0.65%) was of concern for the determination of Cd. These interferences are unresolvable using any of the resolutions available on our HR-ICP-MS instrument. However, as the concentration of Sn was found to be low for the urines analysed in this study (typically much less than 1 ng g^{-1} from measurement of ^{118}Sn 24.22%), no correction for Sn was made for ^{112}Cd and ^{114}Cd . The ^{111}Cd isotope, which suffers no interference from Sn, was also monitored. Generally, this is the preferred isotope for the determination of Cd in urine by ICP-MS;^{9,15,17,24} however, some studies have also utilised ^{112}Cd and ^{114}Cd .^{11,13} Little difference in concentration was noted between the three Cd isotopes for the urines analysed (usually in agreement to within 2–5%), further suggesting that any interference by Sn on Cd was small.

The other potential interference on Cd arises from the oxides of Mo, which interfere with all three Cd isotopes considered, and which again are unresolvable using the resolutions available. Molybdenum oxide formation was measured with our HR-ICP-MS system and was found to be 0.1–0.2% of the signal arising from the original Mo isotope, much lower than that noted in a previous study.¹³ As the

concentration of Mo in the urine samples collected was found to range from 1.9 to 302.5 ng g^{-1} [mean 59.3 ng g^{-1} , median 69.0 ng g^{-1} , measured using ^{95}Mo (15.92%)], Cd values were corrected for MoO. Nixon and Moyer¹³ did not correct for MoO on Cd as the Mo concentrations were found to be $< 50 \text{ ng g}^{-1}$.

As low Cd concentrations were expected in the urine samples (even more so after 1+9 dilution), and interferences could not be overcome using higher resolutions, the low resolution mode, offering greater sensitivity, was employed for the determination of this element. Lead is interference free in urine samples and was also determined in the low resolution mode. At these higher masses polyatomic interferences from matrix ions are reduced or non-existent.

For confirmation, a number of urine samples were analysed using both resolution 300 and 3000. Excellent agreement was found between data obtained in the low and medium resolution modes for Zn, Cd and Pb, while elevated Cu concentrations were found using a resolution setting of 300. These findings reinforce the interference problems noted above when measuring Cu in urine using quadrupole ICP-MS instruments, while the agreement between Zn data obtained with the two resolutions also suggests that any Zn interferences were relatively small.

Detection limits

The detection limits for each isotope, based on three times the standard deviation of 10 consecutive measurements of the method blank (3σ), are given in Table 2. The method blank used consisted of ultra-pure water, 1% HNO_3 and internal standard, while measurements were made under routine operating conditions. Lower detection limits were found for Cd and Pb isotopes measured using a resolution of 300 ($< 8 \text{ pg g}^{-1}$) compared with Cu and Zn isotopes determined using a resolving power of 3000 ($30\text{--}300 \text{ pg g}^{-1}$). Using HR-ICP-MS in the low resolution mode, superior detection limits were found compared with quadrupole-based instruments, owing to the lower background and greater sensitivity of the sector field instrument.²¹ Detection limits were higher using a resolution of 3000 owing to the decrease in sensitivity when changing from resolution 300 to 3000²¹ (on our instrument the sensitivity was found to decrease to about 8–10% of its original value). Isotope abundance was also found to have a large influence on measured detection limit [e.g., 95 pg g^{-1} for ^{66}Zn (27.9%) compared with 282 pg g^{-1} for ^{67}Zn (4.1%)]. With such low detection limits available using HR-ICP-MS, accurate analysis is often limited by the quality of the blank solution.

Urine preparation

The analysis of urine by ICP-MS for biomonitoring purposes typically requires minimal sample preparation. Simple dilution (usually by a factor of 10) is often used to minimise matrix effects.^{10–16,24} For more complex clinical samples such as serum or blood, which contain significant levels of protein and other organics, acid digestion (often with modifier addition) has often been employed prior to a final sample dilution.^{12,16,24}

In this work, the effects of sample dilution and digestion procedure were investigated. Using the Bio-Rad Level 1 reference urine, three dilution factors were investigated, namely 1+4 (number of replicates $n=5$), 1+9 ($n=10$) and 1+19 ($n=5$), while a 1+49 sample ($n=1$) was analysed for Zn only. The results are shown in Fig. 4 for the Cd and Zn isotopes, along with accepted Bio-Rad Level 1 values and concentration ranges. For the comparatively low concentration of Cd, increasing the dilution factor produced values higher than the target value, and outside the recommended

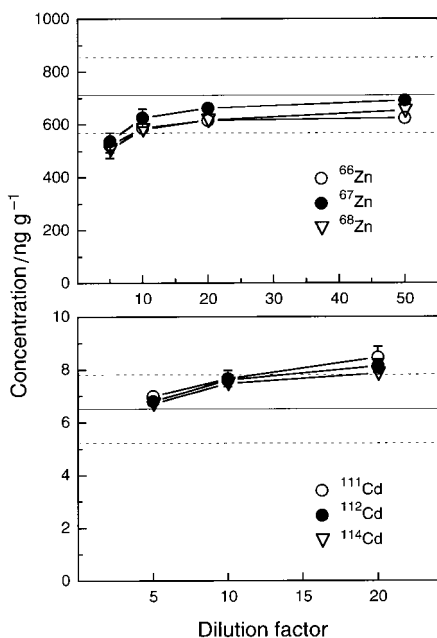


Fig. 4 Measured Zn and Cd concentrations in Bio-Rad Level 1 reference urine as a function of sample dilution. Indium was used as the internal standard. Mean results, recommended values and acceptable ranges are shown.

range when diluted 1+19. However, the opposite effect was found for Zn which was present at higher concentrations. Greater dilution gave concentrations closer to the average value supplied. A similar trend was found for Cu, whereas the Pb values were relatively constant with dilution factor. Based on these results, and in agreement with other studies,^{8,10-14} a dilution factor of 1+9 was taken as an acceptable compromise for the four elements under consideration in this work.

The results of the three digestion methods using Bio-Rad Level 1 reference urine are shown in Fig. 5. All urine preparation procedures resulted in concentration values lying within the acceptable range. For Cu and Zn the microwave digestion method provided values closer to the accepted average, whereas cold dilution and hot digestion methods gave marginally more accurate values for Cd and Pb. It may be that the elevated temperatures and pressures involved with microwave digestion help to break down the urine matrix, in particular the protein content. However, for ease of handling and to aid in sample throughput, a simple cold dilution by 1+9 was found to be acceptable for routine screening tests.

Internal standards

The choice of internal standard for ICP-MS analysis of urines was investigated in some detail by Nixon and Moyer.¹³ Considering the mass and ionisation energy of any potential internal standard, Nixon and Moyer tested Rh, Ag, In and Sb as internal standards for Cd, and Re and Bi for Pb. Accurate and precise results were found using Rh and In for Cd, along with Bi for Pb. A variety of internal standards have also been used in other urine studies, including Co, Tl, Sc, Ir, Ga and Y.^{8,10-17,24} In other work in our laboratory, In has been found to be an acceptable internal standard for a wide variety of elements in geological matrices.³¹ Using Bio-Rad Level 1 as the reference urine, In alone as an internal standard was compared with a three internal standard mixture, namely Sc, In and Bi; ⁴⁵Sc was used as the internal standard for Cu and Zn, ¹¹⁵In for Cd and ²⁰⁹Bi for Pb. At a spectral resolution of 3000, the signal for the ⁴⁵Sc isotope could easily be separated from those of the polyatomic species ¹³C¹⁶O₂, ¹²C¹⁶O₂H and

²⁹Si¹⁶O. Although the concentration of Si in urine may be expected to be very low, the use of quartz torches and glass nebulisers and spray chambers makes this interference worth noting. As has already been discussed for Cd, ¹¹⁵In also has a weak isobaric interference from ¹¹⁵Sn (0.36%), which is unresolvable with the resolutions available. However, compared with the concentration of internal standard, only low Sn concentrations were measured in the urines analysed in this work. The possible effect of Sn on the internal standard In for urine analysis has not been noted in earlier studies. Both internal standard methods were found to agree to within 1.5%, providing similar analytical precision (results not shown). In subsequent work In was used as the sole internal standard.

Analysis of reference urines

Two Bio-Rad reference urines were extensively analysed in this study and the results are presented in Table 3. Copper, Zn, Cd and Pb in both references were quantified by comparison with aqueous standards (external calibration, $n=10$), and the method of standard additions was also used for Bio-Rad Level 2 ($n=3$). Agreement to within 0-17% and 0-7% of recommended values was found using external calibration for Bio-Rad Level 1 and Level 2 reference urines, respectively. Values determined for Bio-Rad Level 2 by standard addition analysis were found to lie within 0-15% of target values. Improved accuracy was found using the external calibration method for the Bio-Rad Level 2 reference urine, in contrast to the study by Hsiung *et al.*¹⁵ who found that the method of standard additions provided results closer to the accepted average. It should be noted that both calibration methods gave values lying within the quoted acceptable range for both standard urine samples. For speed of analysis, external calibration was used for further urine measurements.

Short and long term precision

The short term precision of the analytical method was evaluated using the Bio-Rad standard urines. Ten consecutive sample preparations and measurements were undertaken and the results are given in Table 3. Quantification was by external calibration. For Bio-Rad Level 1 the precisions obtained (RSD for 1 σ) ranged from 1.3% for ²⁰⁸Pb to 6.0% for ⁶⁵Cu. The precisions obtained for Bio-Rad Level 2 were slightly lower, reflecting the elevated elemental concentration of this urine. The RSDs ranged from 0.9% for ²⁰⁸Pb to 3.3% for ⁶⁶Zn. In

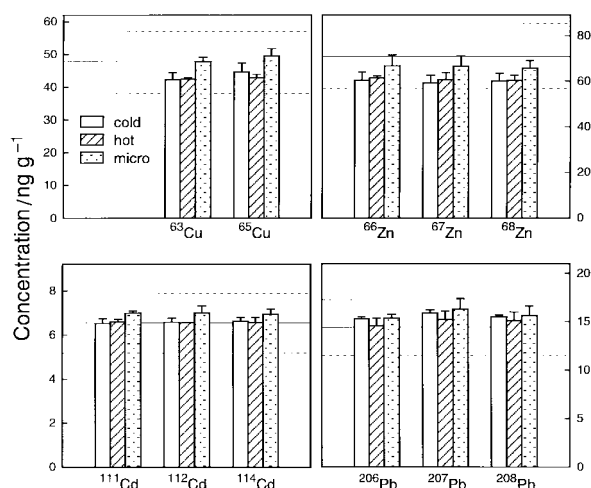


Fig. 5 Effect of sample preparation method on the analysis of Bio-Rad Level 1 reference urine. Samples were diluted 1+9 with In as the internal standard. Mean results, recommended values and acceptable ranges are shown.

Table 3 Comparison of results (ng g^{-1}) for Bio-Rad Level 1 and Level 2 reference urines obtained by external calibration and standard additions

Isotope	Bio-Rad Level 1-69011			Bio-Rad Level 2-69022			
	Recommended value	Acceptable range	Values found by external calibration ^a (n = 10)	Recommended value	Acceptable range	Values found by external calibration ^a (n = 10)	Values found by standard addition ^a (n = 3)
⁶³ Cu	48	38–57	42.3 ± 2.2	63	50–75	61.2 ± 1.5	68.4 ± 1.9
⁶⁵ Cu	48	38–57	44.7 ± 2.7	63	50–75	59.8 ± 1.2	67.9 ± 2.3
⁶⁶ Zn	710	568–852	605 ± 36	1057	846–1269	1020 ± 23	1180 ± 66
⁶⁷ Zn	710	568–852	591 ± 35	1057	846–1269	1060 ± 34	1160 ± 98
⁶⁸ Zn	710	568–852	600 ± 34	1057	846–1269	1010 ± 24	1210 ± 108
¹¹¹ Cd	6.5	5.2–7.8	6.52 ± 0.21	12.3	9.9–14.8	13.0 ± 0.2	13.6 ± 0.2
¹¹² Cd	6.5	5.2–7.8	6.57 ± 0.19	12.3	9.9–14.8	13.1 ± 0.2	13.6 ± 0.5
¹¹⁴ Cd	6.5	5.2–7.8	6.61 ± 0.19	12.3	9.9–14.8	13.1 ± 0.2	13.8 ± 0.7
²⁰⁶ Pb	14.3	11.5–17.2	15.3 ± 0.2	69	56–83	69.8 ± 0.7	68.6 ± 4.4
²⁰⁷ Pb	14.3	11.5–17.2	15.9 ± 0.3	69	56–83	72.7 ± 0.8	67.5 ± 3.5
²⁰⁸ Pb	14.3	11.5–17.2	15.5 ± 0.2	69	56–83	71.8 ± 0.7	64.5 ± 1.9

^aErrors shown are one standard deviation of the mean.

both cases the RSD values were higher for the isotopes measured using a resolution of 3000.

The long term precision was monitored using a prepared pool urine consisting of a homogeneous mixture of exposed worker and unexposed control urines. This urine mixture was stored at -20°C and analysed 21 times using 11 individual sample preparations over a 6 month period. Mean values, concentration ranges found and RSD values (based on 1σ) are given in Table 4. Median concentration values were also calculated and were found to correspond to the mean values shown. The long term precisions obtained for this sample ranged from about 5% for Pb to about 15% for Cu. This range is in agreement with Schramel *et al.*,¹⁷ who prepared and analysed a pool urine for a variety of elements over a 10 d period using quadrupole ICP-MS. Example precisions found in that study were 2% for Pb, 4.8% for Cd, 9.8% for Sn and 14.8% for Sb.

Comparison of cadmium results by HR-ICP-MS and ETAAS

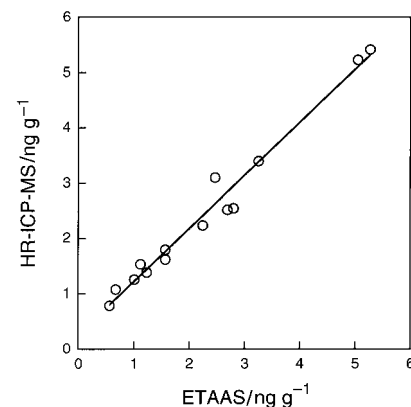
The determination of Cd was of particular interest in this work. To confirm further the reliability of the HR-ICP-MS data for Cd, selected samples were also analysed by ETAAS. Comparative data are shown in Fig. 6. Each sample was analysed once by both methods. The two techniques were found to be in agreement ($[\text{Cd}]_{\text{HR-ICP-MS}} = 0.959[\text{Cd}]_{\text{ETAAS}} + 0.255$, $n = 14$, $r^2 = 0.976$). The Cd values obtained using HR-ICP-MS were found to be slightly elevated. This effect

Table 4 Results from the long term analysis of a prepared pool urine. The sample was analysed 21 times using 11 different sample preparations over a 6 month period. The sample was diluted 1+9, with In as the internal standard

Isotope	Range of values/ ng g^{-1}	Mean value ^a / ng g^{-1}	RSD (%) ^b
⁶³ Cu	8.40–15.3	11.5 ± 1.7	15
⁶⁵ Cu	8.8–15.5	11.6 ± 1.9	16
⁶⁶ Zn	334–442	382 ± 32	8.4
⁶⁷ Zn	335–438	386 ± 35	9.1
⁶⁸ Zn	318–444	391 ± 42	11
¹¹¹ Cd	3.25–4.90	3.80 ± 0.49	13
¹¹² Cd	3.26–4.39	3.77 ± 0.40	11
¹¹⁴ Cd	3.14–4.32	3.72 ± 0.40	11
²⁰⁶ Pb	54.4–66.3	61.4 ± 4.3	7.0
²⁰⁷ Pb	59.6–70.4	65.2 ± 3.3	5.1
²⁰⁸ Pb	56.4–67.7	63.6 ± 3.8	6.0

^aErrors shown are one standard deviation of the mean.

^bBased on standard deviation values.

**Fig. 6** Comparison between HR-ICP-MS and ETAAS for the determination of Cd in 14 urine samples from cadmium-exposed workers. HR-ICP-MS results were obtained from analysis of ¹¹¹Cd. Samples were analysed once by both methods, and were prepared as described in the text. Values were not corrected for creatinine concentration.

was most pronounced at low Cd concentrations, and may reflect the superior blanks and detection limits of the HR-ICP-MS method.

Analyses of urine samples from cadmium-exposed workers

A total of 48 samples were collected from the seven cadmium-exposed workers and 12 from a non-exposed control group. Two worker samples (both collected on site) were excluded owing to anomalously high cadmium levels ($> 100 \text{ ng g}^{-1}$), indicating contamination. Urine samples were excluded from further analysis if creatinine levels indicated excessive dilution. Other studies have discarded results from urines with creatinine values less than 0.3 or 0.5 g l^{-1} .^{32,33} In this study, a lower limit of 0.3 g l^{-1} was imposed, excluding four samples from the exposed group and one from the control group. Spot urine samples were included as diurnal variation in Cd excretion has been shown to be small.³²

Average results from the analysis of the remaining urine samples (42 cadmium-exposed workers, 11 non-exposed controls) are given in Table 5. The results are expressed both as the concentration in urine (ng g^{-1}) and the concentration of Cd normalised to that of creatinine ($\mu\text{g g}^{-1} \text{ Cr}$). There was little difference in urinary copper and zinc concentrations between the exposed and control groups, suggesting that exposure to these elements was not greater for the workers. Also, cadmium is evidently not altering the balance of

Table 5 Average Cu, Zn, Cd and Pb concentrations in urines from cadmium-exposed and non-exposed individuals. Analyses of 42 samples from cadmium-exposed workers and 11 samples from the non-exposed controls are shown as both concentration in urine (ng g^{-1}) and concentration normalised to creatinine ($\mu\text{g g}^{-1}$ Cr). Errors shown are one standard deviation of the mean

Isotope	Cadmium-exposed		Non-exposed	
	ng g^{-1}	$\mu\text{g g}^{-1}$ Cr	ng g^{-1}	$\mu\text{g g}^{-1}$ Cr
^{63}Cu	7.35 ± 3.03	5.86 ± 2.35	7.72 ± 2.54	6.02 ± 2.84
^{65}Cu	7.33 ± 3.06	5.65 ± 2.13	7.46 ± 2.48	6.08 ± 2.67
^{66}Zn	371 ± 209	280 ± 128	398 ± 280	277 ± 128
^{67}Zn	364 ± 205	273 ± 122	390 ± 274	275 ± 130
^{68}Zn	388 ± 212	293 ± 133	397 ± 280	275 ± 126
^{111}Cd	2.25 ± 1.38	1.74 ± 0.98	0.33 ± 0.15	0.27 ± 0.15
^{112}Cd	2.14 ± 1.30	1.66 ± 0.92	0.34 ± 0.18	0.28 ± 0.16
^{114}Cd	2.14 ± 1.34	1.65 ± 0.92	0.32 ± 0.17	0.26 ± 0.16
^{206}Pb	5.49 ± 1.84	4.44 ± 2.23	3.76 ± 1.88	2.71 ± 1.55
^{207}Pb	6.22 ± 2.13	5.00 ± 2.42	4.34 ± 2.14	3.14 ± 1.74
^{208}Pb	5.94 ± 1.98	4.80 ± 2.41	4.00 ± 2.00	2.88 ± 1.64

excretion between these two elements. Considering the concentrations of Cu and Zn relative to Cd, this is not surprising. There was a small difference in urinary Pb levels measured for the two groups, with the cadmium-exposed group being slightly higher. As expected, there was a significant increase in average cadmium levels for the exposed worker urines, based on both the uncorrected and creatinine corrected results.

Conclusion

A simple procedure for the determination of Cu, Zn, Cd and Pb in urine using HR-ICP-MS was developed. Sample dilution (1+9) with In as the internal standard, in combination with external calibration using aqueous standards, was found to provide the best analytical results for the isotopes considered over a wide range of concentrations. This method minimised sample preparation while maximising sample throughput. HR-ICP-MS was also shown to offer a number of advantages over quadrupole based instruments for the analysis of urines. The capacity to separate isotopes of interest from some interferences using higher instrumental resolution was demonstrated for the determination of Cu and Zn, while increased sensitivity and lower detection limits in the low resolution mode were utilised for the determination of Cd and Pb. Elevated Cd and Pb concentrations were found in urine samples from workers occupationally exposed to Cd. Similar average Cu and Zn concentrations were found in urines from both worker and control groups.

We thank Stephen Cook of the Clinical Chemistry Department of the Royal Hobart Hospital for providing the Bio-Rad Lyphochek Level 1 and Level 2 urines and for comparison analyses by ETAAS. We are grateful for the assistance provided by Pasmenco Hobart Smelter and the employees of the cadmium section. The study was supported by a grant from the Workers (Occupational Diseases) Relief Fund of Tasmania.

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