

Development of a Routine Method for the Determination of Trace Metals in Whole Blood by Magnetic Sector Inductively Coupled Plasma Mass Spectrometry with Particular Relevance to Patients with Total Hip and Knee Arthroplasty

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Background: Joint-replacement surgery has revolutionized the treatment of osteoarthritis and is still the most effective therapy. A recent clinical trend reintroducing metal-on-metal bearing surfaces has in turn stimulated a requirement for accurate measurement of the concentrations of relevant metals in both pre- and postoperative patients. Thus, there is a need for cost-effective, multi-element methods for trace metal analysis in whole blood to monitor possible increases in wear metal concentrations.

Methods: A method was developed to allow routine analysis of whole blood samples for molybdenum, cobalt, chromium, and nickel. Sample preparation consisted of a simple 1:10 dilution of whole blood with a solution of 10 mL/L Triton X-100, 0.0002 mol/L EDTA, and 0.01 mol/L ammonium hydroxide. Final determination was performed by a double-focusing magnetic sector inductively coupled plasma mass spectrometer operated in medium-resolution mode (resolution, 3400). Online addition of rhodium was used for internal standardization.

Results: Detection limits in whole blood were 0.06 $\mu\text{g/L}$ for chromium, cobalt, and molybdenum and 0.30 $\mu\text{g/L}$ for nickel. Base concentrations of 0.22, 0.17, 0.62, and 0.99 $\mu\text{g/L}$ for chromium, cobalt, molybdenum, and nickel, respectively, in whole blood have been found.

Polyatomic interferences on all four elements have been shown to be resolved from the analyte masses by use of a resolution of >3000 .

Conclusions: The simple, rapid method of sample preparation is effective in minimizing potential contamination and enables 60 samples (run time, 8 h) to be analyzed before cleaning the instrument is necessary. A resolution >3000 was sufficient to separate polyatomic interferences from the masses of interest. The method was used to analyze a large number of blood samples taken from primary patients awaiting total hip arthroplasty. The method is sensitive enough to provide base concentrations for chromium, cobalt, and molybdenum in whole blood. The results for nickel were compromised by high signals for blank samples.

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Joint-replacement surgery has revolutionized the treatment of osteoarthritis and is still the most effective therapy. Initially, it was recommended that the technique be restricted to elderly patients. Because of the success of the procedure, however, increasing numbers of young patients are receiving joint replacements. For example, in the southwest region of the United Kingdom, one-third of all patients are <65 , whereas in the United States, 28% and 8% of joint replacements are performed on patients <60 and <40 years of age, respectively (1). It is estimated that 20% of joint replacements will fail within 20 years as a result of loosening associated with wear of the prosthesis. In younger patients (<30 years of age), loosening can be as high as 82% within 16 years (2). Particulate and soluble wear debris can be released from the orthopedic implants and systematically disseminated in the body (3). This dissemination is much greater if the prosthesis is loose (3). The long-term effects (if any) of this material have never been evaluated fully.

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Two main types of prostheses are used in current practice: a metal-on-plastic prosthesis, in which a metal stem articulates on a plastic cup; and a metal-on-metal prosthesis, in which both components are metallic. Three main metal alloys are in use: stainless steel; cobalt chrome; and titanium, vanadium, and aluminum.

Recently, there has been a clinical trend for reintroduction of metal-on-metal bearing surfaces, especially for young orthoplast patients, and an increasing popularity of modular porous-coated devices with large surface areas of exposed metal (4). A recent study found a ninefold increase in serum chromium and a threefold increase in serum cobalt in patients with long-term, well-functioning metal-on-metal implants (5). This and other studies have led to concern about the effect of long-term exposure to cobalt, chromium, nickel, molybdenum, and other metals and have highlighted the need for accurate biological monitoring of metal exposure. Effective monitoring can also provide insights into the mechanism of metal release. Corrosion is an important source of metal release, and it has been suggested that accurate monitoring could be a useful tool in the diagnosis of patients who are symptomatic after a total joint replacement (4) because the wear of metal-on-metal bearings cannot be measured on standard radiographs (1).

The main analytical problem in determining these metals in blood is they are present at extremely low (sub- $\mu\text{g/L}$) concentrations in a very complicated matrix. Electrothermal atomic absorption spectroscopy has been used successfully for such analyses (4, 5), but one of its disadvantages is that the analytical procedures are relatively time-consuming. This is because of the cycle time needed for electrothermal atomic absorption spectroscopic analysis and because the cuvette must be cleaned regularly to remove carbon, which is present in high quantities in the sample matrix. Most analyses are performed on serum samples because the matrix is less problematic than whole blood. There is evidence to suggest, however, that chromium, for example, accumulates in the red blood cells (6, 7), thus increasing the need for reliable methods of analyzing whole blood. Inductively coupled plasma mass spectrometry (ICP-MS) has sufficient sensitivity for such analysis, but quadrupole instruments suffer greatly from interferences in the mass range of interest (8, 9). A few methods have been reported that overcome these interferences, but the procedures are quite complicated, making routine analysis difficult (10–12). Newer magnetic sector ICP-MS (HR-ICP-MS) instruments are potentially very powerful tools for the analysis of samples with complex matrices. The strength of HR-ICP-MS lies in its ability to produce sufficient resolving power to separate polyatomic interferences from the analytes, which is of particular importance in the mass range of 18–80, where interferences from plasma gases are prevalent. In addition to resolving many of the common polyatomic interferences, HR-ICP-MS instruments

routinely have detection limits one order of magnitude lower than quadrupole ICP-MS instruments.

We recently published a report (13) demonstrating the ability of HR-ICP-MS to determine chromium, nickel, and cobalt in whole human blood based on the method originally developed by Delves and Sieniawska (14). Our method, however, was only used for a very limited number of samples. The aim of this present work is twofold: (a) to assess the effectiveness of the method as a routine, cost-effective technique for the analysis of a large number of samples; and (b) to determine baseline concentrations of metals associated with metal implants with particular reference to how metal concentrations might change as a result of an orthopedic surgery.

Materials and Methods

INSTRUMENT

A double-focusing HR-ICP-MS (Element; Finnigan MAT) was used in medium mode (resolution, 3400) for all analyses. Typical routine operating conditions and data acquisition settings are given in Table 1. The instrument was operated following a United Kingdom Accreditation Service (UKAS)-accredited method (LGC internal reference no. INS/A1-0008).

PREPARATION OF SAMPLES AND CALIBRANTS

Blood was withdrawn from a vein in the forearm using a standard Venflon cannula at the time of induction of

Table 1. Routine operating conditions of HR-ICP-MS for whole blood analysis.

Torch type	Fixed
Injector	Quartz 1.5 mm i.d.
Spray chamber	Scott-type double pass
Spray chamber temperature	5 °C
Cones	High-sensitivity platinum
Resolution	Medium, ~3000
Mass, m/z	
Chromium	52
Cobalt	59
Nickel	60
Molybdenum	95 and 98
Rhodium	103
Integration type	Average
Scan type	E-scan
Scan window	150%
Peak search window	80%
Integration window	50%
Sample time	5 ms
Samples per peak	60
Number of repeats (n)	6
Nebulization gas flow	1.05 L/min
Auxiliary gas flow	0.80 L/min
Carrier gas flow	14.0 L/min
Power	1100 W
Guard electrode	Yes
Nebulizer	Cross flow

anesthesia before joint-replacement surgery. The blood was drawn into standard plastic syringes, and the first 5 mL was discarded to avoid contamination from the trocar. The second 5 mL was then discharged into a lithium Teflon plastic container specifically designed for trace metal analysis (Teklab).

Blood samples (1 mL) were diluted 10-fold with a diluent consisting of 0.01 mol/L ammonium hydroxide solution (specific gravity, 0.88; BDH), 0.0002 mol/L EDTA (Aldrich), and 10 mL/L Triton X-100 (Scintran grade; BDH) in deionized water (18 Ohm; Elga Maxima).

A multielement, 10 mg/L stock solution was prepared in 0.7 mol/L ultrapure HNO₃ (Ultrex grade; J.T. Baker) from commercial single-element 1000 ± 3 mg/L calibration solutions (Spec CertiPrep Inc) from which daily calibrators were prepared by serial dilution in 0.14 mol/L HNO₃. Final calibrators were prepared in the diluent. An internal standard (rhodium; Spec CertiPrep Inc), was prepared in the diluent described above. The internal standard was added on-line to all calibrators and sample solutions via a T-joint at a concentration of 1 µg/L to correct for instrument drift and matrix effects in a continuous flow system. Calibration was achieved by external calibration using 0.1, 0.2, 0.5, 0.75, and 1.0 µg/L calibration solutions with the calibration curves calculated using the Linear Through Zero option. Samples were analyzed after dilution, and a dilution factor of 10 was applied to the results after analysis.

Method recovery was checked by adding to a diluted blood control sample the required amount of multielement solution to give a final concentration of 0.45 µg/L. After dilution, all samples were kept at 5 °C and analyzed within 48 h of dilution. The recovery was calculated by subtracting the concentration measured in the native blood sample from the concentration measured in the blood supplemented with the multielement solution and comparing that difference with the known amount added. The reference whole blood Seronorm Level 2 (Nycomed AS) was analyzed as an additional quality-control sample.

Results and Discussion

CONTAMINATION CHECKS

When sampling blood designated for trace-metal analysis, great care must be taken to minimize possible sources of contamination from the sampling procedure and materials. For this work, we used the Venflon cannula for blood sampling. This device consists of a stainless steel needle surrounded by an inert plastic sheath. After insertion into the vein, the needle is withdrawn and the blood is sampled through the plastic sheath. Experiments were performed with a variety of solution matrices from doubly distilled water to 0.7 mol/L HNO₃. Typical results using the severest solution tested (0.7 mol/L HNO₃) are given in Table 2. As can be seen, even with 0.7 mol/L HNO₃ no significant contamination was observed from the sampling device when used in the appropriate manner. However, if the sample was drawn through the

Table 2. Sampling device contamination results as determined by HR-ICP-MS

	Concentration, µg/L (n = 2)			
	Chromium	Cobalt	Nickel	Molybdenum
Detection limit	0.06	0.06	0.30	0.06
HNO ₃ blank (0.7 mol/L)	<0.06	<0.06	<0.30	<0.06
Plastic sheath	<0.06	<0.06	<0.30	<0.06
Stainless steel needle	0.83	0.09	3.50	0.08

stainless needle, substantial amounts of chromium and nickel were detected in the samples. This was also true for the stainless steel needles with 0.14 mol/L HNO₃ and doubly distilled water, although to a lesser extent. This highlights the importance of such sampling strategies, especially for the determination of sub-µg/L concentrations of these trace elements.

ISOTOPIC INTERFERENCES

Polyatomic interferences are known to occur in analysis by ICP-MS. However, when an analysis is directed to trace or ultratrace concentrations of metals, possible interferences that appeared to be insignificant or nonexistent can have an important impact on the validity of the analysis. A list of known and possible interferences for chromium, cobalt, nickel, and molybdenum in the whole blood matrix are given in Table 3, along with the resolution required to spectrally resolve them from the analyte isotope.

To evaluate possible interferences, scans of control blood samples from healthy preoperative patients were made under standard measurement conditions, covering the masses required. These scans are shown in Fig. 1. As can be seen, all of the isotopes used in this study exhibited substantial polyatomic interferences at the trace concentrations in whole blood and required a resolution >3000 to resolve. If a resolution >3000 had not been used (i.e., a quadrupole low-resolution ICP-MS was used), the interfering species shown in Fig. 1 could have caused substantial errors in the analysis.

The unidentified interference on ⁹⁵Mo (Fig. 1C) can cause problems if an instrument is not set up and opti-

Table 3. Possible isobaric and polyatomic interferences of the isotopes used in the method.

Element	Mass	Isotopic abundance, %	Interferent	Mass	Resolution required
Chromium	51.9405	83.79	⁴⁰ Ar ¹² C	51.9624	2376
			³⁵ Cl ¹⁶ O ¹ H	51.9716	1672
Cobalt	58.9332	100	⁴³ Ca ¹⁶ O	58.9540	2878
			⁴⁰ Ar ¹⁹ F	58.9608	2137
Nickel	59.9308	26.10	²³ Na ³⁷ Cl	59.9557	2410
Molybdenum	94.9058	15.92	⁷⁹ Br ¹⁶ O	94.9135	12803
	97.9054	24.13	⁹⁸ Ru	97.90529	829706
			Pt ²⁺	97.9825	1271

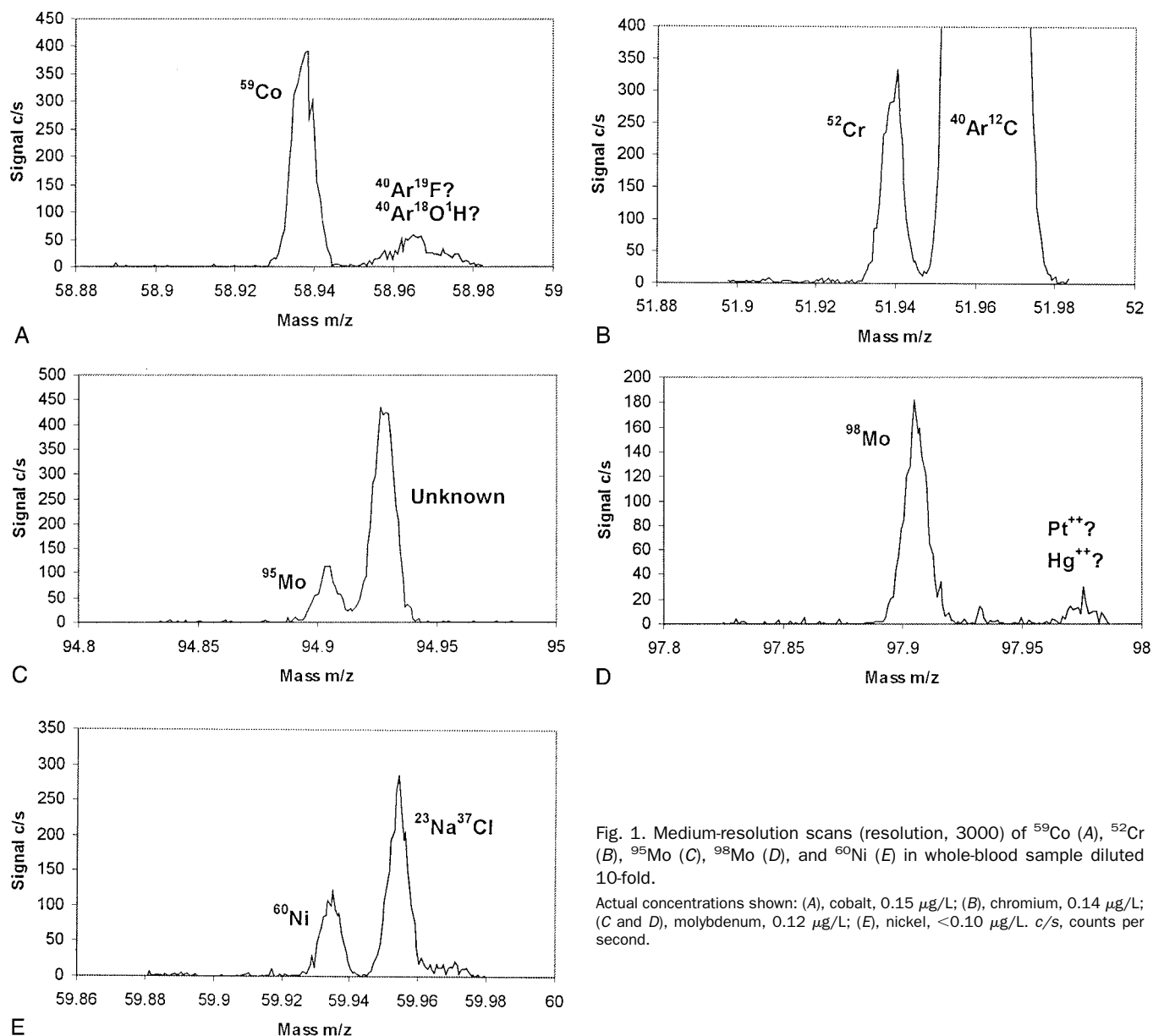


Fig. 1. Medium-resolution scans (resolution, 3000) of ^{59}Co (A), ^{52}Cr (B), ^{95}Mo (C), ^{98}Mo (D), and ^{60}Ni (E) in whole-blood sample diluted 10-fold.

Actual concentrations shown: (A), cobalt, 0.15 $\mu\text{g/L}$; (B), chromium, 0.14 $\mu\text{g/L}$; (C and D), molybdenum, 0.12 $\mu\text{g/L}$; (E), nickel, <0.10 $\mu\text{g/L}$. c/s, counts per second.

mized very carefully. The advantage of ^{95}Mo over ^{98}Mo is that the ^{95}Mo isotope does not suffer from direct isobaric interference (Table 3). However, we decided to use ^{98}Mo in future work despite the ^{98}Ru interference because we believe that the amount of ruthenium in whole blood is negligible. In addition, the abundance of ^{98}Ru is only 1.88%, so substantial amounts (with respect to molybdenum) would have to be present to cause substantial enhancement in the ^{98}Mo signal. For ^{98}Mo , the Pt^{2+} and possibly Hg^{2+} peaks are well resolved from the ^{98}Mo peak at medium resolution (resolution, 3400; Fig. 1D).

METHOD PERFORMANCE CHARACTERISTICS

Calibrations were linear over the calibration range with regression coefficients (R) >0.99 for all elements. Additional typical calibration parameters are given in Table 4.

Instrumental mass calibration was performed daily using a 1 $\mu\text{g/L}$ multielement calibrator and monitored during each analysis. If substantial mass calibration drift was detected, the instrument was recalibrated for mass calibration and the calibrations were run again before proceeding. Recoveries for the different trace elements (concentration of each element, 0.45 $\mu\text{g/L}$) in diluted blood samples are given in Table 4. Supplemented blood samples were analyzed within every batch along with replicates from previous batches to provide continuity data. An additional check on accuracy involved the analysis of the reference blood Seronorm Level 2. This had reference values for chromium, cobalt, and nickel with an indicative value for molybdenum. These data are also given in Table 4, in which good agreement can be observed for chromium, cobalt, and nickel, the three elements for which

Table 4. Method performance characteristics.

	Element			
	Chromium	Cobalt	Nickel	Molybdenum
Calibration				
Linearity, r^2	0.9975	0.9993	0.9970	0.9991
$S_{y/x}$	0.0096	0.0065	0.0033	0.0026
Slope	0.434	0.568	0.137	0.201
SE of the slope	0.011	0.007	0.004	0.003
Intercept	0.015	0.004	0.032	0.000
Imprecision (CV) at 0.5 $\mu\text{g/L}$	5%	5%	10%	5%
Accuracy				
Recovery, ^a %	94.9 \pm 5.0	102 \pm 3.3	96.7 \pm 10.4	105 \pm 4.3
CV	(5.2%)	(3.2%)	(11%)	(4.1%)
Seronorm expected, $\mu\text{g/L}$	7.1	5.2	5.0	5.0
Seronorm observed, $\mu\text{g/L}$ (n = 8)	7.17 \pm 0.63	5.27 \pm 0.46	4.48 \pm 0.63	6.86 \pm 0.19
CV	(8.8%)	(8.8%)	(14%)	(2.8%)

^a Diluted whole-blood sample (n = 17 replicates). Concentration of each element, 0.45 $\mu\text{g/L}$.

there was a reference value. For molybdenum, however, the ICP-MS method seemed to produce higher results, possibly because the value indicated for this Seronorm sample does not take into consideration the presence of endogenous molybdenum in the blood.

Whole-blood detection limits are given in Table 5. Detection limits were calculated as 3 SD above the value for a blank sample (n = 6) plus the dilution factor, which in this case was 10. The nickel detection limit was nearly always compromised by a relatively high signal for the blank sample due to Ni contamination. Care was taken to limit this nickel blank by use of a platinum sample and skimmer cones, ultrapure HNO_3 , 18 Ohm deionized water, and Scintran-grade Triton X-100, and soaking the quartz spray chamber and injector overnight in 0.7 mol/L HNO_3 . In this way, the results for the nickel blank were brought down to manageable concentrations.

Drift was corrected by analysis of an intermediate-concentration calibrator every six samples. If the drift was >10%, a simple linear drift-correction procedure was performed. This consisted of calculating the average drift for sequential drift-correction in-house calibrators. This average drift factor was then applied as a percentage

correction to the six samples that were analyzed between the two calibrators. Fig. 2 shows the typical drift encountered for a ^{95}Mo calibration solution over a 7-h run of 60 blood samples.

RESULTS OF PREOPERATIVE PATIENTS

The blood from 47 preoperative patients was analyzed using the described HR-ICP-MS method. The results are summarized in Table 5. The detection limits obtainable with the method are acceptable for the evaluation of the base concentrations of chromium, cobalt, and molybdenum in preoperative blood. This is illustrated by the fact that only four samples for chromium, two for cobalt, and none for molybdenum were below the detection limit; mean blood concentrations were $0.22 \pm 0.26 \mu\text{g/L}$ for chromium, $0.17 \pm 0.17 \mu\text{g/L}$ for cobalt, and $0.62 \pm 0.29 \mu\text{g/L}$ for molybdenum. In this respect, the multielement HR-ICP-MS procedure seemed to produce better data than reported by previous authors using the slower, single-element technique of electrothermal atomic absorption spectroscopy, as shown in Table 5. The baseline value for nickel in blood ($0.99 \pm 1.15 \mu\text{g/L}$) obtained in this study must be regarded as an overestimation because

Table 5. Summary of trace metal concentrations in whole blood.

Element	This study			Published data			
	Whole blood detection limit, $\mu\text{g/L}$	Percentage of samples below detection limit	Mean values of samples above detection limit, $\mu\text{g/L}$ (n = 44)	Reference	Detection limit, $\mu\text{g/L}$	Percentage of samples below detection limit	Mean values, $\mu\text{g/L}$
Chromium	0.06	8.5	0.22 ± 0.26	4	0.03	30.7	0.06
				16	Not reported		0.23
				17			0.19
Cobalt	0.06	4.3	0.17 ± 0.17	4	0.30	81.3	0.18
				16	Not reported		0.39
				18	0.30	54.5	0.19 ± 0.12
Molybdenum	0.06	0.0	0.62 ± 0.29				
Nickel	0.30	42.6	0.99 ± 1.15	16	Not reported		2.3

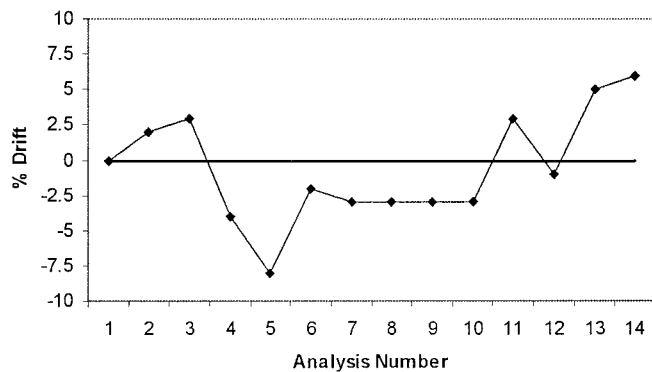


Fig. 2. Variation in recovery of a 0.75 µg/L molybdenum drift calibrator over a 7-h period.

only 57% of the samples had concentrations above the detection limit. This would concur with the conclusion of Templeton et al. (15), who stated in that the concentration of nickel in serum and blood is $\sim 0.1 \mu\text{g/L}$. The more abundant ^{58}Ni was not used in this work because it suffers from isobaric interference from ^{58}Fe . Although the abundance of ^{58}Fe is only 0.28%, the low concentrations of nickel in the samples would have been severely affected by the relatively high concentrations of iron in the whole-blood matrix.

In conclusion, we have developed a simple, rapid, and cost-effective method for the analysis of certain trace metals in whole blood. The method relies on the simple 10-fold dilution of whole blood with an ammonium/EDTA/Triton X-100 diluent with analysis by HR-ICP-MS using medium resolution (>3000). Substantial interferences for all the trace metals under study were found, indicating the limitations of low-resolution (quadrupole) ICP-MS for this type of analysis. The procedure has been applied in the study of trace metal concentrations in whole blood of preoperative arthroplasty patients. Baseline blood concentrations for chromium, cobalt, and molybdenum have been established. At the present time, baseline data for nickel are compromised by high signals for the blank. A large study is currently being performed to determine, in detail, what changes take place in these metals in whole blood, in particular with metal-on-metal prostheses at different postoperative intervals.

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