A Comparative Study of Sample Dilution Techniques for the Analysis of Trace Metals in Whole Blood

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Introduction

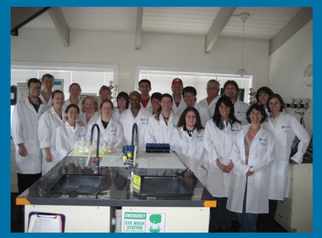
The role that trace elements play in both normal biological function and issues of toxicity has become of increasing interest[1]. As demand increases for analytical services to determine the concentrations of toxic metals in blood samples, through both industrial and environmental exposure, laboratories must develop methods that are accurate and precise for high-throughput, multi-elemental determinations. The use of inductively coupled - plasma mass spectrometry (ICP-MS) allows for fast, quantitative, multi-elemental analysis of biological samples with detection limits in parts-per-trillion (ng/L) levels. Due to the matrix complexity of blood samples, ICP-MS analysis can be prone to spectral and polyatomic interferences, which occur when unwanted ions possess the same nominal mass-to-charge ratio (m/z) as the analyte ion of interest.

Typical Polyatomic Interference

Isotope	Polyatomic interference
^{51}V	$^{35}Cl^{16}O$, $^{35}Cl^{14}N$, $^{34}S^{16}OH$, $^{38}Ar^{13}C$, $^{36}Ar^{15}N$
^{52}Cr	$^{40}Ar^{12}C$, $^{40}Ca^{12}C$, $^{37}Cl^{15}OH$, $^{38}Ar^{14}N$
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$
^{59}Co	$^{43}Ca^{16}O$, $^{42}Ca^{16}OH$, $^{24}Mg^{35}Cl$, $^{36}Ar^{23}Na$,
^{60}Ni	$^{23}Na^{37}Cl$, $^{24}Mg^{36}Cl$, $^{44}Ca^{16}O$, $^{43}CaOH$
^{63}Cu	$^{40}Ar^{23}Na$, $^{40}Ca^{23}Na$
^{65}Cu	$^{40}Ar^{25}Mg$, $^{40}Ar^{24}MgH$, $^{40}ArNH$
^{64}Zn	$^{32}S^{16}O_2$, $^{32}S_2$, $^{36}Ar^{14}N_2$, $^{40}ArNaH$, $^{40}Ar^{24}Mg$
^{75}As	$^{40}Ar^{35}Cl$, $^{40}Ca^{35}C$

Sample dilution (with matrix modification[3,4]) and sample digestion[5,6] are the most common ways to prepare blood samples for ICP-MS analysis. Different preparation methods have varying abilities to overcome potential interferences. Brooks Rand Labs performed a comparative study of three dilution techniques and concentrations of cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni) in whole blood samples were determined. A Perkin-Elmer Elan DRC II ICP-MS (in standard mode) was utilized for this analysis. Each sample was analyzed in duplicate and two spiked samples were prepared and analyzed at a 10% frequency. Five replicates of two standard reference materials (SRMs), Seronorm™ and NIST-966, were prepared for each dilution and preparation technique as well. Statistical analyses were performed on the data to determine trends. Additionally, background levels for these elements were established by analyzing blood samples from fourteen colleagues.

ICP-MS Perkin Elmer Elan DRC II



Colleagues

Dilution Techniques

The following dilution techniques were used in a comparative study of the analysis of trace metals in whole blood. Calibration standard solutions for Cu, Cd, Ni, and Pb were purchased from High Purity Standards, then diluted and matrix-matched in terms of acidity and reagent composition. For all samples, the results were density, method blank, and internal standard corrected.

Dilution Method #1) 1:50 dilution in 1% (v/v) HNO_3 in reagent water[7]

Dilution Method #2) 1:50 dilution in 1% (v/v) ethyl alcohol and 1% (v/v) HNO_3 in reagent water[7]

Dilution Method #3) 1:50 dilution in 0.05% (w/v) EDTA, 1% (v/v) TMAH, 1% (v/v) ethyl alcohol, and 0.05% (v/v) Triton-X 100 in reagent water[8]

Summary of Results

Figure 1

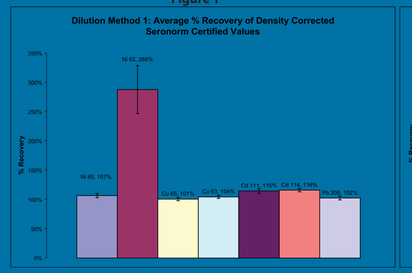


Figure 2

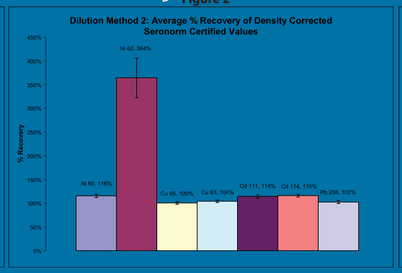


Figure 3

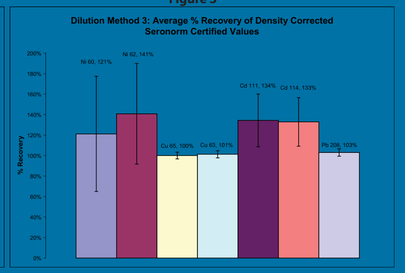


Figure 4

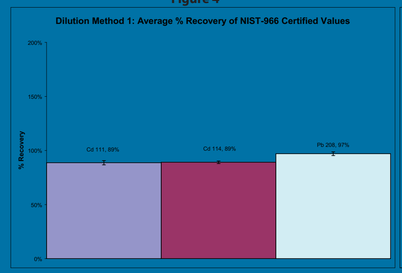


Figure 5

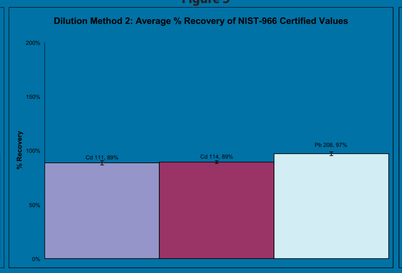


Figure 6

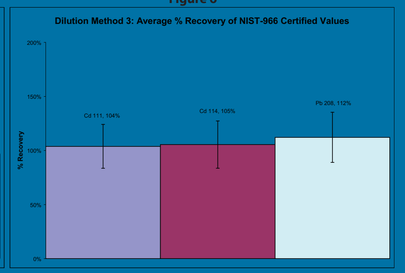


Figure 7

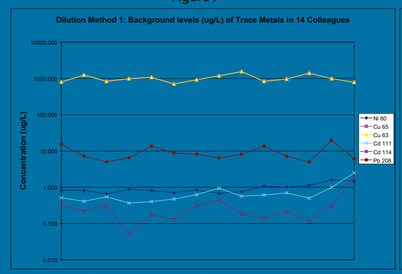


Figure 8

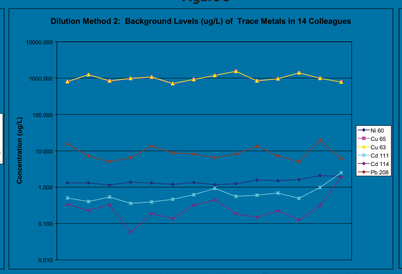
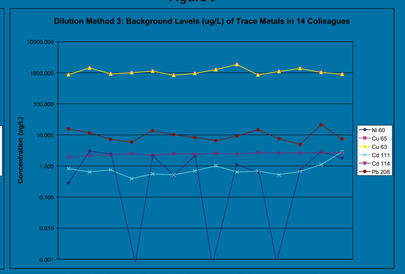


Figure 9



Average Background Concentration (ug/L) of 14 Colleagues' Whole Blood Levels

		Dilution Method 1			Dilution Method 2			Dilution Method 3		
Element	Isotope	Average (ug/L)	Std Dev	Duplicate RPD Precision	Average (ug/L)	Std Dev	Duplicate RPD Precision	Average (ug/L)	Std Dev	Duplicate RPD Precision
Ni	60	0.945	0.286	1%-25%	1.441	0.287	2%-16%	1.602	0.984	50%-709%
Cu	65	1004.5	246.0	0%-10%	1004.0	246.0	0%-10%	1097.3	285.8	1%-14%
Cu	63	1022.1	245.4	0%-10%	1021.5	245.4	0%-10%	1106.3	285.6	1%-14%
Cd	111	0.724	0.544	0%-17%	0.717	0.544	0%-18%	0.848	0.606	3%-151%
Cd	114	0.344	0.456	0%-17%	0.353	0.456	0%-16%	0.412	0.463	16%-170%
Pb	208	9.316	4.408	0%-9%	9.332	4.408	0%-9%	10.28	4.597	0%-54%

Discussion

The focus of this ongoing comparative study is to determine a simple and precise technique for the analysis of multiple trace metals in whole blood. For this comparison, three simple dilution techniques were utilized in the analysis of Cd, Cu, Pb and Ni. In these experiments Ni^{60}, Ni^{62}, Cu^{63}, Cu^{65}, Cd^{111}, Cd^{114}, and Pb^{208} were monitored. Multiple isotopes were measured to facilitate detection of interferences arising from the complex matrix of the whole blood samples.

Dilution Method #1: The 1:50 dilution in 1% (v/v) HNO_3 in reagent water[7] is the simplest method to overcome matrix effects used for the analysis of trace metals in blood. The results for the analyses of the CRMs Seronorm™ and NIST-966 showed excellent accuracy and precision (relative standard deviation [RSD] ≤ 5%) for all elements and isotopes, with the exception of Ni^{62}. It is likely that an interference was formed during the analysis, indicated by an average Ni^{62} recovery of 288%[Fig 1,4]. Furthermore, all of colleagues' blood samples, analyzed in duplicate, returned excellent relative percent differences (RPDs) of ≤ 25% for all isotopes, again with the exception of Ni 62.

Dilution Method #2: The 1:50 dilution in 1% (v/v) ethyl alcohol and 1% (v/v) HNO_3 in reagent water [7] was the second method investigated. Results from this experiment indicate similar trends as Dilution Method 1, producing acceptable recoveries and RSDs ≤ 5% for all CRMs. Interference on the Ni^{62} isotope was again observed, as average recoveries were 368%[Fig 2,5]. All colleagues' samples, analyzed in duplicate, returned RPDs ≤ 18%, again with the exception of Ni^{62}.

Dilution Method #3: The 1:50 dilution in 0.05% (w/v) EDTA, 1% (v/v) TMAH, 1% (v/v) ethyl alcohol, and 0.05% (v/v) Triton-X 100 in reagent water[8] is equivalent to the method quoted in an unpublished document from the Center for Disease Control (CDC) numbered "DRAFT ITB001A (modified)". This dilution method demonstrated terrific accuracy and precision for CRM Seronorm™ (RSD ≤ 5%) for the analyses of Cu and Pb, poor accuracy and precision (RSD ≤ 26 %) for Ni, and decent recovery but poor precision for Cd (RSD ≤ 26 %)[Fig 3]. Additionally, CRM NIST-966 returned good recoveries of Cd and Pb with RSDs of ≤ 25%[Fig 6]. Colleagues' blood duplicate precision was poor for Ni (RPD ≤ 709%), Cd (RPD ≤ 170%), and Pb (RPD ≤ 54%). Cu demonstrated the best duplicate precision (RPD ≤ 13%) throughout the experiment. This data indicates that many potential interferences can be formed by the presence of the additional reagents used in Dilution Method #3. The referenced method by the CDC was developed for use with a Dynamic Reaction Cell (DRC) ICP-MS, and this technology could reduce the level of interferences seen. All work for this investigation thus far has been analyzed in the standard mode, not DRC mode, of the instrument.

The results from this investigation indicate a preference for Dilution Method #1 and Dilution Method #2 for the analysis of Cd, Cu, Pb and Ni in whole blood. These methods are easy to perform, use relatively small amounts of reagents, provide good accuracy and precision, and have only one obvious isotope (Ni^{62}) that would be unusable due to interferences. The Dilution Method #3 utilized far more reagents and, in general, yielded poorer accuracy and precision. In conclusion, Dilution Method #1 would be recommended for use in the analysis of these four metals in whole blood. As this experiment continues, more trace elements will be studied, including arsenic (As) and selenium (Se), as well as the investigative use of DRC gasses. It has been cited that ethanol addition, as in the second dilution method, provides enhanced sensitivity and interference suppression for some metals, including Se[7]. Additionally, all experiments will be repeated to rule out any possibility of bias caused by instrument variances.

Health Information

Trace element Cu is essential for most animals, including humans. Cu is part of enzymes, which are proteins that help biochemical reactions occur in every cell, and are essential for humans. In addition, Cu is involved in the absorption, storage, and metabolism of iron. Cases of Cu toxicity are rare but may occur. Doses of 10 mg/day over several weeks may lead to toxic symptoms, such as weakness and nausea[9].

Ni occurs naturally in the environment at low levels. Nickel is an essential element for some animal species, and may be essential for human nutrition. However, negative respiratory effects have been reported in humans from inhalation exposure to Ni. Human and animal studies have reported an increased risk of lung and nasal cancers from exposure to Ni refinery dusts and Ni subsulfide[10].

Pb is not an essential element for humans. Almost all Pb in the body reflects exposure sources associated with human activities. Environmental, occupational, or residential exposure is the most common cause of elevated lead levels. Higher levels have shown a 2-3% reduction of cognitive performance, while the alkyl Pb species are highly toxic to the nervous system[9].

Occupational exposure is the most common cause of elevated Cd levels. Principal symptoms reported were respiratory distresses from workers exposure to high concentrations of fumes appearing from heated Cd metal or compounds. It has been estimated that eight hours exposure to 5 gm Cd/m³ will be lethal[9].

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