Comparison between Digestion Procedures for the Multielemental Analysis of Milk by Inductively Coupled Plasma Mass Spectrometry

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Elements (As, Ca, Cd, Cr, Cu, Mg, Mn, Mo, Ni, Pb, Rb, and Zn) were determined by inductively coupled plasma mass spectrometry in Certified Reference Material (A-11 milk powder, International Atomic Energy Agency), human breast milk, and a representative variety of infant formulas. The effects of digestion procedures and the mass of reference milk samples on the recovery, precision, and accuracy of multielemental analyses for milk were examined. High-pressure microwave bomb and closed-vessel ashing on a hot plate were used as the means for digestion. Elements were either preconcentrated on a Chelex 100 resin or determined directly from the diluted digest. The closest comparisons between measured and reference values for the A-11 reference milk were obtained using a 0.05-g sample digested in 1.0 mL of concentrated HNO_3 on a hot plate set at 70 °C for 5 days and measured directly. Concentrations of elements in human milk and formula were, in general, in good agreement with literature values, where available.

Keywords: *Human milk; trace elements; ICP-MS*

INTRODUCTION

Considerable information has been amassed concerning the composition of human milk in terms of fat, protein, and vitamins (Arnold et al., 1987; Cockburn, 1983; Dawodu et al., 1990; Fomon et al., 1970; Sanders and Naismith, 1979; Widdowson, 1965), but less attention has been paid to the elemental composition of human milk. The importance of trace elements in nutritional management and the monitoring of toxins is now widely recognized. Of the trace elements investigated in the present study, Cr, Cu, Mn, Mo, and Zn are essential for the nutrition of humans; Cd, Pb, and As are toxins; and Ni and Rb are potential contaminants. The biochemical role of trace elements as enzyme components or cofactors, particularly in the first year of life, has led to increased interest in their study (Bougle et al., 1991; Nielson, 1991). Further, while some rarely determined trace elements (e.g., B, Li, V, and Ni) have important physiological roles in mammals, their essentiality in humans has not been clearly established (Lavi and Alfassi, 1990). Human milk is used as the reference standard for establishing nutrient requirements during infancy because it is the only food for many infants up to 6 months of age. It is essential, therefore, to quantify trace elements in human milk. This, however, requires a method that is capable of satisfying the requirements for low detection levels, specificity, and ease in order to generate reliable measurements.

Inductively coupled plasma mass spectrometry (ICP-MS) has been accepted as a means of multielemental and isotopic measurements (Akatsuka et al., 1992; Friel et al., 1990, 1993). However, this technique may suffer from a significant loss of sensitivity during analysis of imperfectly digested biological samples (Friel et al., 1990; Ridout et al., 1988). The detection capabilities of the technique are further compromised by high levels of total dissolved solids (TDS) in the analytical solutions and by polyatomic and isobaric interferences, especially from certain major elements (e.g., Ca). There is an urgent need, therefore, to develop a method that improves digestion of the organic component and minimizes levels of total dissolved solids and interfering elements.

Wet digestion hot-plate procedures for biological samples have been used extensively with HNO_3, H_2SO_4, and $HClO_4$ (Akatsuka et al., 1992; Durrant and Ward, 1989; Emmett, 1988). When used in an open system, these procedures, particularly with HNO_3, were found to be inefficient in destroying organic compounds (Krushevska et al., 1992). Microwave heating systems have replaced many hot-plate procedures since digestion time and reagent consumption can be reduced and the use of perchloric acid can be avoided (Würfels et al., 1989). We previously reported (Friel et al., 1990) a microwave digestion procedure for several biological tissues not including milk. At that time we were unable to obtain good results using 200 mg of certified milk powder and decided in the present study to (a) assay different sample sizes and (b) attempt preconcentration of trace elements using column procedures.

Therefore, the present study was undertaken with the following objectives: (1) to evaluate the effectiveness of various wet digestion procedures on the recovery, precision, and accuracy of major, minor, and trace elements in reference milk powder (A-11) and (2) to identify and quantify elements in digested reference milk material, human breast milk, and milk formulas using ICP-MS.

MATERIALS AND METHODS

Milk Powder and Reagents. Milk reference material (A-11 milk powder, International Atomic Energy Agency) was used in all of the digestion procedures. A variety of commercial formulas (premature and term) commonly fed to infants were obtained from a local pharmacy. Special care milk formulas were obtained from the Janeway and Grace hospitals in St. John's. A multielement solution (henceforth called synthetic milk) was prepared from salts (SPEX Industries) and standard

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Table 1. Concentration of Elements in the Synthetic Milk Solution

element	concn (ng g^{-1})	element	concn (ng g^{-1})
As	0.00065	Mg	50.0
Ca	260.00	Mn	0.002
Cd	0.002	Mo	0.02
Co	0.0002	Ni	0.022
Cr	0.015	Pb	0.012
Cu	0.080	Rb	1.15
Fe	1.90	Zn	0.40

solutions (Fisher Scientific). Literature values for human breast milk were employed to make up the concentration of each element in the synthetic milk solution (Table 1) (Durrant and Ward, 1989; Picciano, 1985).

Acids (HNO_3 and HF) were purified by sub-boiling distillation of reagent grade compounds in a Teflon still. High-purity distilled, deionized water (>18 MΩ-cm) was obtained by passing distilled water through a deionization system (Barnstead Co., Boston, MA). Addition of reagents, weighing of samples, and evaporation were performed in a clean laboratory with laminar-flow benches and fume cupboards providing a better than class 100 working environment.

Sampling for Human Breast Milk. Two lactating mothers with no known pathological conditions, living in the St. John's area, were chosen. Their full-term (40 weeks of gestation) and preterm infants (28 weeks), both female, were 1 week old at the time of milk collection. A strict protocol was established for sampling whereby the collector first cleaned the nipple and surrounding area with soft Kimwipes EX-L tissue and water wearing disposable talc-free gloves. About 15 mL of milk from one breast collection was taken between 10 a.m. and 2 p.m. during the day. The milk was collected during the regular feeding of the child using an electric breast pump. The milk collection kit was acid washed prior to the sampling date by repeated washing in 5% HNO_3. Immediately after collection, the milk was transferred into 50-mL polyethylene vials and placed in a styrofoam cooler for delivery to the laboratory. Milk samples were stored at −20 °C until analysis.

Digestion Procedures. A variety of different digestion and preconcentration procedures were investigated. For statistical purposes, four or five replicate analyses were made of each milk sample by each method tested. Each digestion procedure was repeated a minimum of four times. Reagent blank samples were also prepared for each procedure.

(1) Microwave Procedure. Different masses, 0.2, 0.4, 0.8, or 1.6 g, of dry A-11 milk powder were used. Samples were placed into a 23-cm^3 Teflon perfluoroalkoxy (PFA) cup of a high-pressure acid digestion bomb (Parr Instrument Co., Moline, IL). Five milliliters of concentrated HNO_3 was added, and the vessels were sealed. The maximum sample capacity for the digestion bomb was 200 mg. Therefore, for larger sample sizes, 200 mg was digested initially and, after cooling in a freezer, a further 200 mg was added to the same solution with an additional 1.0 mL of HNO_3. Four separate bombs were used, and digests were pooled to obtain the final sample weights of approximately 800 or 1600 mg of milk powder.

Samples were digested in a conventional microwave oven (Panasonic NE-6660C, 700 W; Toronto, ON) containing a rotating turntable. Each sample was heated for 2 min at the medium power setting (350 W). The digestion vessel was cooled in a freezer for 6 h to minimize loss of sample as an aerosol on premature opening (Friel et al., 1990). The sample was then transferred to a screw-top PFA vessel (ca. 23 mL) using ≈5 mL of water. It was then placed on a hot plate, evaporated at 150 °C until constant weight was attained, cooled, and made up gravimetrically with 253 μL of concentrated HNO_3 and water to ≈20 g. The concentration of HNO_3 in the final solution was 0.2 M. Solutions were either passed through columns using Chelex 100 as described below or introduced directly (henceforth called direct analysis) to ICP-MS for multielemental analysis.

(a) Preconcentration Method. Prior to use, empty columns (ca. 12-mL Poly-Prep column, Bio-Rad 731-1550, Bio-Rad Laboratories, Hercules, CA) were cleaned in 5% HNO_3 for 2 days followed by another 2 days in water and then air-dried in a clean laboratory. Prior to the resin being packed into columns, 15 mL of 5% HNO_3 was passed through the column, followed by water. A slurry of 1.0 g of Chelex 100 (100−200 mesh, Bio-Rad Laboratories) was added to the column. Alkali and alkaline earth metals were eluted with 1 M ammonium acetate, and then the trace elements were eluted with 2.5 M HNO_3 as described by Kingston et al. (1978). Samples were made up with 0.2 M HNO_3 to ≈20 g and introduced to ICP-MS for elemental analysis. To test the effectiveness of the column procedure, the synthetic milk solution and digested milk samples were passed through the resin.

(2) Hot-Plate Procedures. (a) Procedure A. A-11 milk powder (0.05 g) was weighed into acid-washed 30-mL screw-top PFA vessels (PFA Labware, Savillex Corp., Minnetonka, MN). A mixture of 1.5 mL of concentrated acids (HNO_3/H_2SO_4 3:1) was added to the sample, and the vessel was sealed. Vessels were heated at 150 °C for 24 h, cooled, weighed (to check for sample loss), and then evaporated at 200 °C until constant weight was attained. Samples were allowed to cool, and then 253 μL of concentrated HNO_3 was added to dissolve the residue. Samples were made up gravimetrically with water to ≈20 g, and solutions introduced to ICP-MS for elemental analysis.

(b) Procedure B. Closed vessels, as described in procedure A, containing 1.0 mL of concentrated HNO_3 and either 0.05 g of A-11 powder or ≈0.07 g of dried human breast milk (initial volume 0.5 mL) were heated at 70 °C for 5 days. Prior to the addition of acid to human milk, milk was dried at 50 °C until constant weight was attained. At the end of the digestion period, samples were cooled, weighed, and dried on a hot plate set at 150 °C. Samples were made up to volume with HNO_3 and water as described in method A.

(c) Procedure C. This procedure was similar to procedure B, except that the samples were digested at 150 °C for 24 h instead of at 70 °C for 5 days.

(d) Procedure D. This procedure was similar to procedure B, except that 1.5 mL of a concentrated HF/HNO_3 (1:3) mixture was used as the ashing reagent instead of HNO_3 alone.

Instrumentation and Data Acquisition. Analysis of milk samples was carried out using a SCIEX (Thornhill, ON) ELAN 250 ICP-MS. The operating conditions and data acquisition were similar to those described earlier (Friel et al., 1990) except that the internal standards used to correct matrix effects and temporal drift in sensitivity were Sc, Y, Tb, and Th. For elements heavier than Sc, the matrix/drift correction factors for each element were calculated by linear interpolation with mass between the correction factors measured for two bracketing internal standard, e.g., Sc and Y for Cu. For elements lighter than Sc, correction factors were calculated by linear extrapolation with mass of the correction factor to mass relationship for Sc and Y. A number of isotopes were used in the quantitation of elements including ^{27}Al, ^{75}As, ^{42}Ca, ^{43}Ca, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{53}Cr, ^{65}Cu, ^{25}Mg, ^{55}Mn, ^{98}Mo, ^{60}Ni, ^{208}Pb, ^{85}Rb, ^{118}Sn, and ^{66}Zn. USGS reference waters T-101, T-103, T-105, T-107, and T-109 were analyzed with the milk samples as a monitor of accuracy. Because ICP-MS lacks sensitivity for Fe, levels of Fe were not included in the present study.

Statistical Analysis. Sample concentrations were corrected for reagent contributions using the concentrations for reagent blanks prepared in the same manner as the samples. Statistical analysis was based on the study of at least four replicate analyses of each sample. Each digestion method was repeated at least four times (i.e., ≥4 trials). The ANOVA test was preformed to compare methods, and means were separated on the basis of the lsd test (SAS, 1982).

RESULTS AND DISCUSSION

Microwave Procedure. *(1) Effect of Mass.* The precision of the results, expressed as relative standard deviation (RSD), at all weights, was <5% for most elements and >20% for As, Cd, and Cr. It was expected that, by increasing the concentration of the elements

Table 2. Elemental Concentrations Obtained from Different Sample Sizes of A-11 Using the Microwave Digestion Procedure and the Direct Analysis

element	A-11 reference value range (ng g^{-1})	concn (ng g^{-1}) in processed sample of initial wt of 0.2 g	0.4 g	0.8 g	$lsd_{0.05}$
As	4.53–5.17	31 ± 22[a]	48 ± 10	37 ± 9	*b*
Cd	1.2–2.2	33.8 ± 11	<DL[c]	15.0 ± 7	*b*
Cr	13.7–21.7	368 ± 76	<DL	245 ± 68	*b*
Cu	354–402	789 ± 38	883 ± 41	645 ± 29	*b*
Mn	248–266	591 ± 12	<DL	332 ± 10	*b*
Mo	82–103	501 ± 26	185 ± 8	112 ± 6	*b*
Pb	29–79	107 ± 6	56 ± 2	103 ± 4	*b*
Rb	24500–37100	40594 ± 119	41395 ± 201	42504 ± 233	NS[d]
Zn	36600–41200	38083 ± 222	36799 ± 267	41701 ± 288	NS
Ca	12.1–13.7[e]	13.1 ± 0.02	12.6 ± 0.2	13.0 ± 0.1	NS
Mg	1.02–1.18[e]	1.0 ± 0.001	1.07 ± 0.0002	1.06 ± 0.0006	NS

[a] Value following ± refers to standard deviation of the mean. [b] Differences between samples and reference values are significant ($p < 0.01$). [c] Values below detection limit. [d] Differences between samples and reference values are not significant. [e] Values are in mg g^{-1}.

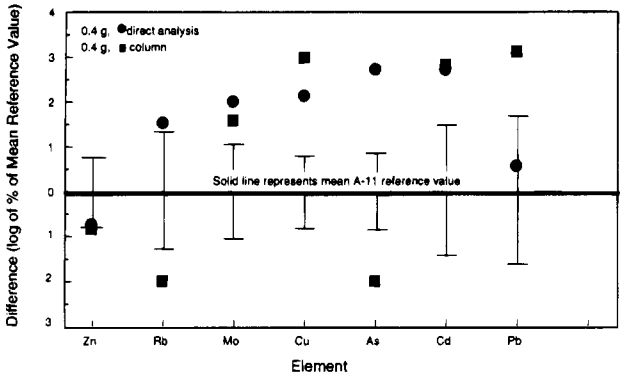

Figure 1. Comparison between column and direct analysis of elements from 0.04 g of A-11 milk digested with the microwave procedure (bars represent 95% confidence limit; experimental point represents a mean of three or four replicates). For each element, values on *Y*-axis were calculated as log *Y* = (mean experimental values − mean of A-11 reference values × 100)/mean of A-11 reference values.

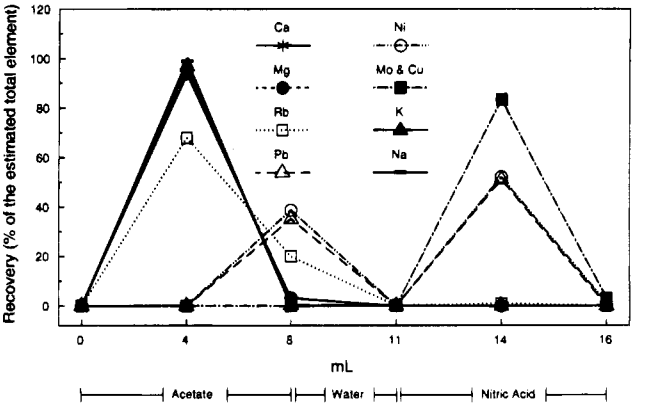

Figure 2. Separation of Ca, Mg, Na, and K from trace elements in the synthetic milk using 1.0 M ammonium acetate.

in solution by using larger sample weights, superior results might be achieved, particularly for those elements whose concentrations are close to the detection limit of the method. However, increasing the sample mass did not improve the results of most elements using direct analysis of digested milk samples (Table 2). For some elements the data are scattered erratically about the mean reference value at significantly overestimated levels (As, Cd, Cr, Mn, Mo, and Pb), while for others (Rb, Zn, Ca, and Mg) the data for different sample weights are within the range of the certified values.

(2) Column vs Direct Analysis after Microwave Digestion. It was thought that column separations should be a viable way to improve the data, by preconcentrating the analytes while lowering total dissolved solids and reducing polyatomic ion interferences from the major elements. However, the data after preconcentration 5–10-fold with the column method did not show improvement over the direct analysis. In fact, the precision was poor (>35%) for most elements with the column method. An example of the recovery with both column and direct analyses of 0.4-g samples is shown in Figure 1. Because the column method with milk samples was inconsistent, the synthetic milk was subsequently used to assess the recovery and accuracy of the method.

(3) Separation of Alkaline Elements on Chelex 100 with Synthetic Milk. Ammonium acetate was effective in separating Ca, Mg, Na, and K from the rest of the elements (Figure 2). Between 94% and 97% of these elements were eluted with the first 4 mL of ammonium acetate. This significantly reduced polyatomic interferences of these alkali and alkaline earth elements on minor and trace elements (i.e., Co, Cu, and Ni). However, a number of trace elements were also eluted with the ammonium acetate, a major drawback in this procedure. For example, significant amounts of Pb and Ni were eluted with ammonium acetate, which is inconsistent with the greater selectivity coefficients for these elements in comparison with ammonium.

The chelating potential of resin for transition metals was reduced in the presence of 2.5 M HNO_3. This is clearly illustrated in Figure 2 as recoveries with 2.5 M HNO_3 were 51%, 52%, 83.3%, and 83.3% for Pb, Ni, Mo, and Cu, respectively. There was no particular pattern, however, for the recovery of other elements; they either fell below the ICP-MS detection limit (Mn, Cr, Co, Cs, As, and Se) or showed a significant gain (recovered > applied), as was the case with Fe and Zn (data not shown). The gain with Fe and Zn indicates a possible contamination of these elements. In general, the preconcentration method using the resin as described in the present study either overestimated or underestimated trace element content and thus should be treated with caution. We think that with matrices such as A-11 milk powder, in which the concentrations of trace elements are so low, the addition of another step, i.e., column extraction, creates potential for elemental dislocation.

Hot Plate. *(1) A-11 Reference Material.* Because of the discrepancies in the values for most elements with the microwave digestion (column and direct), attempts were made to evaluate several hot-plate procedures. These procedures, employing closed-vessel digestion of a small mass (0.05 g) of milk at high acid:sample mass (≥20) ratio under prolonged heating, were evaluated for the recovery of elements.

Table 3. Comparison of Results Obtained by Four Hot-Plate Digestion Procedures with the A-11 Reference Milk

element	A-11 certified value range (ng g^{-1})	detection limit[a] (ng g^{-1})	amt (ng g^{-1}) recovered from the ascribed digestion procedure[b]				
			A	B	C	D	$lsd_{0.05}$
As	4.53–5.17	1.1	5.21 ± 3[c]	4.4 ± 0.5	2.73 ± 3	63.3 ± 7	*d*
Cd	1.2–2.2	0.3	<DL[e]	2.72 ± 0.8	<DL	6.7 ± 4	*d*
Cr	13.7–21.7	4.0	<DL	21.3 ± 44	<DL	73.3 ± 65	*d*
Cu	354–402	4.0	<DL	403 ± 8	<DL	610 ± 22	*d*
Mn	248–266	2.0	282 ± 26	283 ± 29	304 ± 39	396 ± 62	*d*
Mo	82–103	1.0	73 ± 14	81 ± 4	62 ± 6	90 ± 29	*d*
Ni	38.9–69.1	9.0	<DL	41.2 ± 2	44.4 ± 2	113.3 ± 8	*d*
Pb	29–79	2.0	22 ± 7	38 ± 2	49 ± 3	37 ± 11	NS[f]
Zn	36600–41200	3.0	39130 ± 110	39310 ± 428	37250 ± 117	39376 ± 509	NS
Ca	12.1–13.7[g]	0.052	12.1 ± 0.02	11.9 ± 0.2	12.1 ± 0.1	12.6 ± 0.02	NS
Mg	1.02–1.18[g]	0.07	1.0 ± 0.001	1.03 ± 0.0002	1.09 ± 0.0006	0.71 ± 0.0001	NS

[a] Mean of detection limit of four analytical runs. For each run, the detection limit was calculated for the average dilution of the samples in the run. [b] Procedure A was HNO_3/H_2SO_4 on a hot plate set at 150 °C for 24 h; procedure B was nitric acid on a hot plate set at 70 °C for 5 days; procedure C was nitric acid on a hot plate set at 150 °C for 24 h; procedure D was HNO_3/HF on a hot plate set at 70 °C for 5 days. [c] Value following ± refers to standard deviation of the mean. [d] Differences between procedures are significant ($p < 0.01$). [e] Values were below detection limit of ICP-MS. [f] Differences between procedures are not significant. [g] Values are in mg g^{-1}, in all digestion procedures.

Table 4. Comparison of Element Content in Breast Milk of Mothers of Full-Term and Preterm Infants[a]

element	content (ng mL^{-1}) in human milk in this work			content range (ng mL^{-1}) in the literature	
	detection limit	full term	preterm	full term	preterm
Al	4.4	62 ± 40	59 ± 43	16–392	NA[b]
As	1.0	<DL[c]	<DL	0.2–18.9	NA
Cd	4.0	<DL	<DL	2.0–24.0	NA
Co	1.0	<DL	4.5 ± 2	0.2–27.0	NA
Cr	24.0	106.3 ± 3	106.4 ± 20	0.2–105	NA
Cu	10.0	443 ± 14	598 ± 16	100–3050	422–1007
Mn	3.0	6.4 ± 0.3	6.5 ± 3	2.0–103	NA
Mo	2.0	4.9 ± 0.6	1.9 ± 0.2	0.1–24.0	0.3–8
Ni	5.0	15.8 ± 21	9.2 ± 4	3.6–420	NA
Pb	4.0	1.1 ± 0.5	2.8 ± 3	0.1–139	NA
Zn	24.0	4339 ± 244	4867 ± 267	140–11973	1775–4827
Ca[d]	72.0	170 ± 15	271 ± 23	97–610	218–390
Mg[d]	9.5	26 ± 0.5	23 ± 0.4	20–60	21–29

[a] Milk samples were digested using hot-plate procedure B. [b] Data not available. [c] Values below detection limit of ICP-MS. [d] Values are in $\mu g\ mL^{-1}$.

Table 5. Mean Value of Element Concentration in Milk and Special Care Formulas[a]

element	detection limit	range of concn of element in the ascribed formula		
		Fe-fortified[b] (ng g^{-1})	soy-based[c] (ng g^{-1})	special care[d] (ng mL^{-1})
Al	4.4	976–5186	6904–15515	513–595
As	1.0	<DL[e]	<DL	<DL–8.1
Cd	4.0	<DL	17.1–23	<DL–1.8
Co	1.0	<DL–201	<DL–196	<DL–104
Cr	24.0	716–2756	1072–1889	99–197
Cu	10.0	2498–5176	3880–6872	795–1786
Mn	3.0	514–1155	2734–3841	137–229
Mo	2.0	89.8–184.0	227–586	15.9–33.6
Ni	5.0	118–531	384–2814	9.9–153
Pb	4.0	22–74	27–93	1.3–3.9
Sn	54.0	366–2707	430–1227	17–172
Zn	24.0	32493–57541	41559–60691	6397–17104
Ca[f]	72.0	2.9–3.6	4.1–8.0	0.51–1.5
Mg[f]	9.5	0.3–0.5	0.5–0.9	0.02–0.13

[a] Milk samples were digested using hot-plate procedure B. [b] Formulas were Similac, Enfalac, and SMA (Wyeth Ltd). [c] Formulas were ProSobee (Mead Johnson), Isomil (Ross Laboratories), and Nursoy (Wyeth Inc). [d] Formulas were Enfalac (24 cal) premature formula, Similac (24 cal), Similac (20 cal), and Enfalac (24 cal). [e] Values below detection limit. [f] Values are in mg g^{-1} for commercial formulas and mg mL^{-1} for special care formulas.

Results for digestion of A-11 by four hot-plate digestion procedures are given in Table 3. For the majority of the elements measured in the present study, ANOVA showed significant difference ($p < 0.05$) among the four procedures, except for Pb, Zn, Mg, and Ca (Table 3). To illustrate these differences, results were plotted as percent difference from the mean of the reference value (Figure 3). For most elements, procedure B was less variable from the mean reference value than the other procedures. This suggests that elemental values can better be analyzed with procedure B than with the other hot-plate procedures used in the present study.

Measurement of the intensity of ^{12}C by ICP-MS in the digest showed that prolonged digestion with HNO_3 at 70 °C for 5 days (procedure B) was less effective in breaking down organics than when digestion was performed at 150 °C for 1 day (procedure C). ^{12}C intensity was 3 times higher in procedure B than in procedure C, although these analyses were performed on the same day. Digestion with a mixture of HNO_3/H_2SO_4 slightly

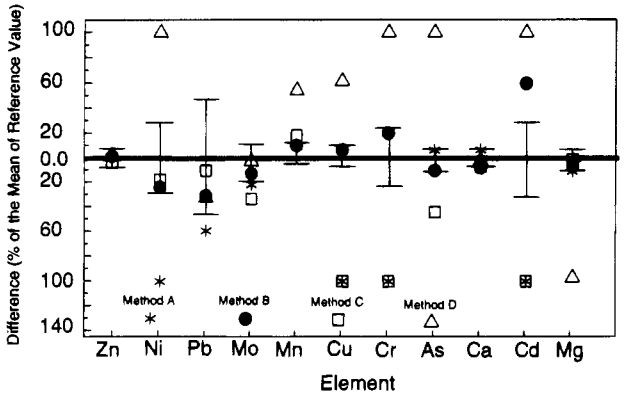

Figure 3. Comparison of different hot-plate procedures for element recovery from 0.05 g of A-11 milk (bars represent 95% confidence limit; experimental point represents a mean of three or four replicates). For each element, values on *Y*-axis were calculated as Y = (mean experimental values − mean of A-11 reference values × 100)/mean of A-11 reference values.

improved removal of ^{12}C over other digestion methods. For procedure A, evaporating at a temperature close to the boiling point of H_2SO_4 caused rapid evaporation of HNO_3, at which stage charring occurred. Nonetheless, carbon was not completely removed with the four hot-plate procedures described in the present study since ^{12}C intensities in the milk samples were 1−80-fold those of corresponding blanks. There was no significant difference in ^{12}C intensity between procedure B and procedure D.

Despite relatively high ^{12}C intensity for the A-11 reference milk powder, 10 of the 11 elements measured with procedure B are in a good agreement with certified reference values (Table 3). This indicates that organic matter that remained after digestion with procedure B did not interfere with quantification of the majority of the elements. The precision (RSD) of procedure B was <5.3% (for Cu, Mo, Ni, Pb, and Zn), 10.2−30% (for As, Mn, and Cd), and 105−206% (for Cr). Less precision with Cr may be attributed to an interference of carbon on ^{52}Cr (Friel et al., 1990).

(2) Human Milk and Formulas. In the present study, elemental concentrations in human milk samples were comparable to those in the literature, except for Co in the full-term milk and As and Cd in premature and full-term human milk (Table 4) (Bougle et al., 1992; Caroli et al., 1992; Dabeka and McKenzie, 1988; Durrant and Ward, 1989). The source of these elemental variations may be attributed to a number of factors including regional variations, socioeconomic factors, and the different methodology used in the present study compared to that in the literature. In the present study, we found greater amounts of Co, Cu, Zn, and Ca in preterm milk than in full-term milk.

Elemental concentrations in analyzed commercial infant formulas are shown in Table 5. Mean values were, in general, in agreement with the literature values (Dabeka and McKenzie, 1988; Koo et al., 1988; Krushevska et al., 1992; Milner, 1990). It is worth mentioning here that there are few data available in the literature on As, Ni, and Li in milk formulas (Asubiojo and Iskander, 1988; Gunshin et al., 1985).

In summary, the good agreement between reference values and results obtained by procedure B illustrates the viability of the described procedure as a means of preparing human milk and infant formula samples for analysis by ICP-MS.

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