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Determination of Total Arsenic Concentrations in Nails by Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT

The analysis of trace elements in biological samples will extend our understanding of the impact that environmental exposure to these elements has on human health. Measuring arsenic content in nails has proven useful in studies evaluating the chronic body burden of arsenic. In this study, we developed methodology with inductively coupled plasma–mass spectrometry (ICP-MS) for the determination of total arsenic in nails. We assessed the utility of the washing procedures for removing surface contamination. Four types of preanalysis treatments (water bath, sonication, water bath plus sonication, and control) after sample decomposition by nitric acid were compared to evaluate the digestion efficiencies. In addition, we studied the stability of the solution over 1 wk and the effect of acidity on the arsenic signal. Arsenic content in the digested solution was analyzed by using Ar–N_2 plasma with Te as the internal standard. The results suggest that washing once with 1% Triton X-100 for 20 min for cleaning nail samples prior to ICP-MS analysis is satisfactory. Repeated measurement analysis of variance revealed that there was no significant difference among the various sample preparation techniques. Moreover, the measurements were reproducible within 1 wk, and

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acidity seemed to have no substantial influence on the arsenic signal. A limit of detection (on the basis of three times the standard deviation of the blank measurement) of 7 ng As/g toenail was achieved with this system, and arsenic recoveries from reference materials (human hair and nails) were in good agreement (95–106% recovery) with the certified/reference values of the standard reference materials. ICP-MS offers high accuracy and precision, as well as high-throughput capacity in the analysis of total arsenic in nail samples.

Index Entries: Arsenic; nails; inductively coupled plasma–mass spectrometer (ICP-MS); washing efficiency; extraction efficiency.

INTRODUCTION

Arsenic is a widely distributed element to which humans may be exposed through ingestion of food, medicine, or water, and by inhalation. As arsenic compounds became more widely used in the production of glass, pesticides, fertilizers, steel, dyes, paints and electronic products, epidemiologic studies have demonstrated the carcinogenicity of inorganic arsenic in humans. Evidence from previous studies suggest that inorganic arsenic exposure is associated with lung, liver, kidney, bladder, skin, and lymphoid cancers in humans *(1–6)*. The ubiquity of arsenic in the environment enhances the difficulty to quantify human exposure to arsenic properly and accurately. Although measuring the concentrations of pollutants in the ambient air, water source, soil, or food has provided parameters for external exposure in ecologic studies, the measurement of external exposure does not necessarily represent the internal exposure status for individual subjects. Therefore, the need for establishing biomarkers is great. Many epidemiologic studies have suffered from lack of precision of estimates for arsenic exposure. As a consequence, biological measures of exposures and responses (termed biomarkers) may be very helpful in increasing the precision of exposure estimates, particularly on an individual level.

As monitoring arsenic levels in blood or urine reflects only short-term exposure, the development of other biomarkers reflecting a longer term of exposure to arsenic is desirable. Compared to blood and urine samples, nails have the advantage of facilitating specimen collection, storage, and transportation. Moreover, arsenic levels in nails reflect a longer-term exposure than does blood or urine. As a consequence, the concentration of total arsenic in nails has been proposed as an indicator of environmental exposure to arsenic in many studies, and positive associations between arsenic contents measured in the nails and the degree of exposure have been reported *(7–11)*. Although environmental arsenic exposure measurement is problematic, the above studies demonstrate that the nail assay would appear to have several advantages and is a potential biomarker to assess human exposure to arsenic.

Methods reported in use for nail arsenic analysis include atomic absorption spectrometer *(8,12–15)*, proton particle-induced X-ray emission *(16)*, and neutron activation analysis *(7,9,17–20)*. Nevertheless, some of the above techniques either could not reach a satisfactory low limit of detection or involved elaborate pretreatment procedures. Some studies lacked evidence to show that a proper preanalysis sample treatment process was performed before instrument analysis, such as an appropriate washing procedure to remove surface contamination, even though they provided techniques with a good limit of detection at the microgram per gram level. Because no standard procedures for trace element analysis in nails has been established, the goal of the present study was to develop a convenient, accurate, and precise method to determine the total arsenic concentration in nails.

In this study, the method of inductively coupled plasma–mass spectrometry (ICP-MS) has been employed to determine the arsenic contents in human nail samples. In people not chronically exposed to inorganic arsenic at elevated concentrations, the total arsenic concentration is below 0.2 μg/g. Hence, the methodologies and instruments used must yield a sufficiently low limit of detection to allow accurate detection of background concentrations. ICP-MS is being increasingly exploited in biological fields for trace element analyses of tissues and body fluids because it is a sensitive method and can detect trace metal concentrations at the nanogram per milliliter level. Another advantage of the ICP-MS technique is simplicity in preanalysis sample preparation. The procedures for sample preparation prior to measuring arsenic concentrations by ICP-MS include cleaning, drying, and wet ashing by nitric acid and dilution; these procedures can be easily performed in general chemical laboratories. Simplicity of the pretreatment procedures enhances the throughput of this technique, an attribute desirable for epidemiologic studies when a large number of samples are to be analyzed.

An ICP-MS instrument is composed of two parts: the inductively coupled plasma (ICP), which is a high-thermal-energy and electron-rich environment to convert sample atoms to ions, and a mass spectrometer (MS), which is designed to discriminate among ions with different masses and determine element concentrations. Although ICP-MS has several advantages over other analyzing techniques, it suffers in some cases from spectral interference and matrix effects *(21,22)*. The ICP is normally an argon plasma, and to eliminate spectral interference in this study, nitrogen gas was introduced into the outer gas stream of the ignited argon plasma. This argon–nitrogen mixed gas was employed to reduce the interference of $ArCl^+$ on arsenic signals at *m/z* 75. To overcome the matrix interference caused by high salt concentrations, samples were diluted after wet ashing and an internal standard is introduced in the analyses.

Because toenail samples contain a large concentration of proteins, some precipitate is formed after acid digestion and dilution. This can cause problems such as inhomogeneity of sampling solutions or blockage

of the pneumatic nebulizer. The goals were, first, to explore the influence of inhomogeneity on analysis, by studying the effects of various sample preparation techniques to evaluate digestion efficiencies; second, to investigate the utility of washing procedures to remove any arsenic contaminant deposited on the nail surface; and, finally, to assess the reproducibility of measurements and the influence of acidity on the arsenic signal of ICP-MS.

MATERIALS AND METHODS

Apparatus

A Perkin-Elmer (Norwalk, CT, USA) Sciex ELAN Model 5000 inductively coupled plasma mass spectrometer was used throughout this study. The mass spectrometer was set to sample ion intensities at the analyte mass *m/z* 75 and internal standard *m/z* 128. Nitrogen gas was introduced into the plasma argon flow using the 4th mass flow controller and a T connector. A peristaltic pump (Rainin Instrument Co., Woburn, MA, USA) was used for the sample introduction. Calibration standards and samples were mixed on-line with the internal standard using a T connection and a mixing coil.

Reagents

Samples were handled in a class 100 clean hood. All glassware was cleaned by soaking them in 10% HNO_3 for 24 h and rinsing several times with deionized water. Reagents used were arsenic (1000 µg/mL; High Purity Standards, Charleston, SC, USA), tellurium (1000 µg/mL; Baker Instra-Analyzed Reagent; J.T. Baker, Phillipsburg, NJ, USA), nitric acid (Optima, Seastar Chemical Co., Sidney, Canada), Triton X-100 (EM Science, Darmstadt, Germany), and deionized H_2O (Nanopure ultra water system; Barnstead, Dubuque, IA, USA). The total arsenic concentration in Triton X-100 was analyzed, and the value was below the limit of detection of ICP-MS.

Sample Collection

Toenail samples were obtained from healthy volunteers ranging in age from 20 to 50 yr old. Each subject was asked to collect toenail clippings from all 10 toes with a nail cutter. Standard reference materials include certified human hair reference material (The Office of Reference Material, Laboratory of the Government Chemist, Middlesex, UK, certified arsenic concentration = 0.59 ± 0.07 µg/g) and National Institute of Standards and Technology Standard Reference Material (NIST SRM) 1643d—trace elements in water (Gaithersburg, MD, USA, arsenic standard reference value = 56.02 ± 0.73 µg/L).

Preparation of the "In-House" Reference Sample

In order to investigate the influence of the extraction procedures on measurements, we first developed a homogeneous reference material with a constant arsenic concentration. As there is no suitable nail certified reference material at this time, an "in-house" reference material was prepared by collecting toenail samples from healthy volunteers. Toenails from 50 subjects were collected, cleaned, dried, and pooled together to build up a "toenail pool." We then ground the samples as a single batch in a mixer mill to fine powders, brushed the toenail powders through a 50-mesh sieve (size of hole: 300 μm), and collected the toenail powders to produce a homogeneous reference material. Before the "in-house" reference material was used as a quality control (QC) material, projected concentration levels of arsenic in the nail pool was obtained by analyzing them 40 times, using the analytic method of choice.

Sample Preparation of Toenail Specimens

Nails were kept in envelopes with proper identification until preparation. Any visible dirt on the surface of nails was removed manually before sample preparation. Toenail samples were prepared for analysis by washing them with a 1% solution of the surfactant Triton X-100 in an ultrasonic bath for 20 min. After this treatment, the toenails were rinsed five times with deionized distilled water. The washed samples were placed in glass beakers individually and allowed to dry at 60°C overnight in a drying oven. After cooling, the dried samples were weighed and transferred to 15-mL polyethylene tubes. Care was taken to avoid chances of external contamination.

After drying, samples were digested with nitric acid; 0.5 mL of Suprapure concentrated nitric acid (Fisher Scientific International Inc., Hampton, NH) was added to each sample with a weight less than or equal to 20.0 mg. In those weighing more than 20.0 mg, 1 mL of Suprapure concentrated nitric acid was added. Samples were covered partially and allowed to sit for at least 36 h. The resultant digest was made up to 2.5 mL (sample weight less than or equal to 20.0 mg) or 5.0 mL (sample weight larger than 20.0 mg) with distilled deionized water. The resultant solution was then ready for analysis.

Analytic Method

The solution was analyzed by ICP-MS under optimum instrumental conditions to determine its arsenic content. First, 1% nitrogen was introduced into the plasma gas flow. After modification of the plasma, an aqueous standard solution containing 10 ng/mL of arsenic was aspirated to optimize the instrument sensitivity. The signal intensity for arsenic was then maximized by adjusting the nebulizer gas flow rate; the torch position

remained constant. Once the machine was optimized, the settings were kept constant throughout all experiments. Sample solutions were introduced by conventional pneumatic solution nebulization into the ICP-MS. A solution of 250 ng/mL tellurium was used as an internal standard in all experiments presented (blank, sample, and standard). Afterward, the arsenic signal was normalized to the signal of the internal standard. The ICP-MS instrument was calibrated before each run by external calibration with standards at 0.0 (blank), 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 ng/mL arsenic. Five replicate analyses were done on each sample and the average concentration was used. A blank sample which contains the analyzed matrix was prepared as described above in each series of analyses. A detailed description of the method has been described by Amarasiriwardena et al. *(23)*. All the readings obtained from the ICP-MS were then calibrated by subtracting the concentration of the blank to yield net concentrations and then converted to microgram per gram (ppm) of dry weight.

Washing Efficiency

Comparisons to determine the utility of the washing procedures were evaluated with the use of two "identical" samples. Because arsenic concentrations can vary from one toenail to another, our comparison samples resulted from splitting each individual nail clipping into two equal portions vertically with respect to the axis of growth. It was reported that essentially identical samples could be prepared by this splitting technique *(24)*. To study the efficiency of the washing technique, one-half of a sample was washed by the methods reported above; the other half was not given any treatment. These two samples were then digested and analyzed to determine the change in arsenic concentrations. Eight pairs of samples were analyzed in the study. In addition, a set of experiments was performed to examine whether washing extracts arsenic from nail structure. We compared arsenic contents in paired samples, in which one-half of a sample was washed one time and the other half was washed two or three times prior to digestion. There were seven pairs of samples in the one wash vs. two washes and one wash vs. three washes experiments, respectively. The nonparametric Wilcoxson signed-rank test for paired data was used to test the difference between washed with unwashed, and one washed with multiple washed samples. As the sample size is small, the test statistics Z cannot be assumed to follow a standard Normal distribution. A distribution function of the smaller sum of ranks for samples of size less than or equal to 12 was used to determine whether to reject the null hypothesis *(25)*. Differences were considered to be significant if the p-value was less than 0.05.

Influence of Various Sample Preparation Techniques on the Analysis

We conducted a preliminary study to evaluate effects of various sample preparation techniques on the recovery of the analysis and the

stability of the resultant solution after acid digestion. Three different treatments were compared with the control group (no further treatment) to evaluate the digestion procedures, and the reproducibility of toenail arsenic analyses over 1 wk was also tested to examine the stability of solutions. In addition, a microwave digestion method was carried out to compare with the recovery of the room-temperature acid-digestion methods used in this study.

Recovery

There were 10 samples in each of 4 treatment groups. First, 60 mg of "in-house" reference material was placed into each polyethylene tube, digested, made up to 10 mL with distilled deionized water, and then subjected to (1) sonication for 10 min, (2) 60°C water bath for 1 h, or (3) 60°C water bath for 1 h plus 10 min sonication. No further treatment was applied in the control group. A schematic diagram of the analytic procedures is shown in Fig. 1. Blanks were carried throughout the whole procedures. As hair and nails are considered unique with respect to biological monitoring because of their similar structures and chemical compositions, the accuracy of the data was determined by using a certified human hair reference material (The Office of Reference Material, Laboratory of the Government Chemist, Middlesex, UK). The hair reference sample was digested as the control group. The total arsenic content was then determined by ICP-MS for each sample. Digestion efficiencies were evaluated by comparing the mean arsenic concentrations obtained from each treatment group with the mean arsenic level of subjects in the control group. The SAS Mixed procedure for repeated measurement analysis of variance was applied to examine whether a substantial difference exists among these techniques. Differences were considered to be significant for $p < 0.05$.

Three samples and one method blank were prepared for the microwave-assisted digestion method. Again, 60 mg of "in-house" reference material was transferred into each Teflon microwave digestion vessel (No. 243M, Parr Instrument Company, Moline, IL, USA). Then, the samples were treated with 2 mL of Suprapure concentrated nitric acid and sealed. The mixture was then digested using a household microwave oven for 3 min at 80% of maximum power. After solubilization, the samples were transferred to polyethylene tubes and made up to 10 mL with deionized water. The total arsenic level was determined in each sample by ICP-MS (Fig. 2). The average arsenic recovery of the microwave digestion method was compared with recovery of the room-temperature digestion method applied in this study. Statistical evaluation was performed by two-sample *t*-statistics.

Stability of the Digested Samples

The reproducibility of toenail arsenic measurements over a period of 1 wk was studied by analyzing each of the above subjects 1, 2, 3, and 6 d after sample preparation. For each sample, we calculated 95%

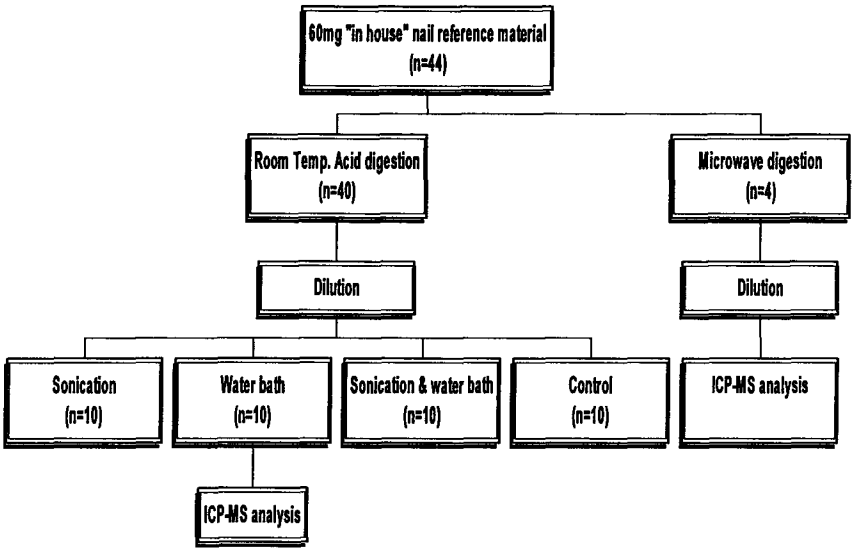

Fig. 1. The scheme used to evaluate extraction efficiencies of five pre-analysis sample preparation techniques (n = sample size).

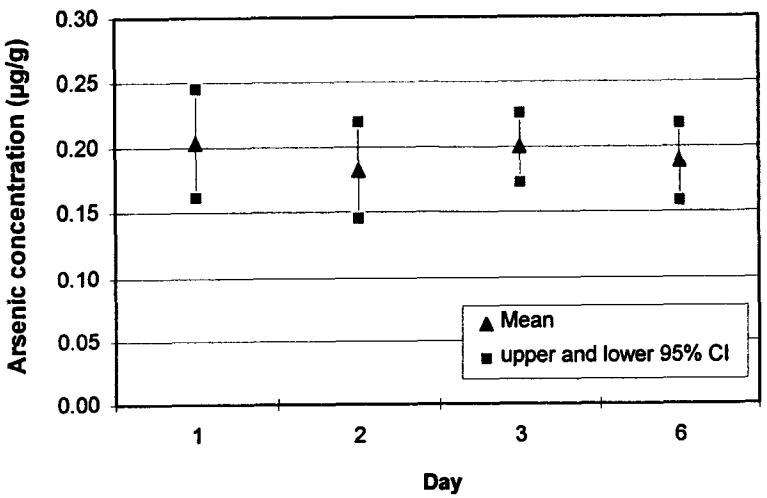

Fig. 2. Means and 95% confidence intervals (CI) of five repeated arsenic measurements for one subject analyzed 1, 2, 3, and 6 d after acid digestion.

confidence intervals according to five repeated measurements of every analysis to determine if the day-to-day variability was significant among the measurements.

Influence of Acidity on Arsenic Signal

Campbell et al. *(26)* reported that the presence of HNO_3 caused a decrease in arsenic sensitivity in the ICP-MS, although the authors could

not explain this phenomenon. To further explore this finding, we studied the influence of acidity on arsenic signal by preparing a series of solutions containing 5 ng/mL arsenic for the following acid concentrations: 5%, 10%, 15%, and 20% of HNO_3.

Limit of Detection

The minimum limit of detection was calculated from 3 times the standard deviation of the 10 replicate analyses of the blank count rate. Both the instrument and method limit of detections were estimated in each run to evaluate the performance of machine and the method. The instrument limit of detection was obtained based on 3 times the standard deviation of the 10 measurements of the calibration standard blank, whereas the method limit of detection was measured based on a reagent blank prepared by procedures mentioned previously, without adding any sample in it. The detection limit was then calculated in units of nanogram per gram in order to reflect what could be detected in the toenail samples. The value was calculated using the method limit of detection multiplied by the dilution factor.

Quality Control

In order to ensure the validity of our methodologies for analysis, several quality control procedures were employed. First, as nail certified reference material is not yet available, the "in-house" reference material mentioned previously was used as a reference sample in the analysis. Second, a certified human hair reference material (The Office of Reference Material, Laboratory of the Government Chemist, Middlesex, UK) was utilized as a laboratory control sample to confirm the accuracy of analysis, as the components of hair are similar to those of toenail. In addition, the National Institute of Standards and Technology (NIST) Standard Reference Material 1643d—trace elements in water was employed as a quality control sample to validate the calibration curve and to test the stability of the calibration curve during the day. The NIST water, toenail, and human hair reference samples were analyzed along with samples collected from study subjects to evaluate the accuracy of analyses. Also, spiked samples were prepared and measured to test the arsenic recovery of our experimental procedures.

RESULTS

Washing Efficiency

Of the eight pairs of samples studied, seven of them were reduced in arsenic concentration after being washed 20 min by 1% Triton X-100 (Table 1). Among these seven samples, the percentage of difference

Table 1
Evaluation of Washing Efficiency; Arsenic Concentrations Measured in Paired Toenail Samples With and Without Washing Prior to ICP-MS Analysis

	Unwashed		Once Washed			
Pair	Weight (mg)	Conc. ± SD [a] (μg/g)	Weight (mg)	Conc. ± SD [a] (μg/g)	Diff [b] (μg/g)	% Diff [c] (%)
1	64.3	0.058 ± 0.003	51.2	0.049 ± 0.005	-0.009	-17.7
2	25.4	0.017 ± 0.010	25.5	0.009 ± 0.006	-0.008	-85.2
3	73.5	0.093 ± 0.006	57.1	0.103 ± 0.003	0.010	9.3
4	65.6	0.079 ± 0.005	55.3	0.075 ± 0.007	-0.004	-5.6
5	44.8	0.081 ± 0.005	48.2	0.045 ± 0.005	-0.037	-81.4
6	34.2	0.090 ± 0.008	32.0	0.085 ± 0.013	-0.005	-6.3
7	31.4	0.343 ± 0.021	25.9	0.243 ± 0.021	-0.100	-41.4
8	27.6	0.187 ± 0.024	18.4	0.162 ± 0.035	-0.025	-15.3

[a]SD: standard deviation of five replicate analyses.
[b]Diff: difference in arsenic concentration between washed sample and unwashed sample.
[c]Diff: Diff/arsenic concentration found in unwashed sample.

between two treatment groups ranged from 5.6% to 85.2%. However, one pair of samples was found to have a higher arsenic level in the washed sample and the percentage of difference was 9.3%. The difference between washed and unwashed groups was significant ($p < 0.04$, Wilcoxon signed-rank test).

As for the one wash vs. multiple washes experiments, two samples in the twice-washed group and three samples in the thrice-washed group showed lower arsenic concentrations than their corresponding once-washed samples (Table 2). Most of the differences for paired samples were within 10.0% among the 14 pairs, except for pair 8 and 9, in which the two samples differed by 26.8% and 10.6%, respectively. The results of statistical analysis suggested the differences between arsenic levels in paired samples washed once and twice or once and thrice were not significant, indicating that employing the washing technique once would remove exogenous contaminating materials effectively. Therefore, the effect of washing procedures appears comparable.

Influence of Various Sample Preparation Techniques on the Recovery of the Analysis

The means, standard deviations (SD), and coefficients of variation (CV) are shown in Table 3. The mean and standard deviation of all samples in these four groups were 0.200 and 0.005 μg/g, respectively. The results for certified human hair reference material were in agreement with the certified value: 0.59 ± 0.07 μg/g. The results of the mixed

Table 2
Evaluation of Washing Efficiency; Arsenic Concentrations Measured in Paired Toenail Samples Washed Once and Twice, and Once and Thrice Prior to ICP-MS Analysis

	Once washed		Twice washed			
Pair	Weight (mg)	Conc. ± SD [a] (μg/g)	Weight (mg)	Conc. ± SD [a] (μg/g)	Diff [b] (μg/g)	% Diff [c] (%)
1	40.6	0.086 ± 0.004	42.6	0.083 ± 0.009	0.002	2.9
2	44.4	0.055 ± 0.009	40.8	0.058 ± 0.004	-0.003	-5.7
3	33.2	0.024 ± 0.004	33.0	0.023 ± 0.006	0.002	6.9
4	54.7	0.067 ± 0.003	53.3	0.073 ± 0.006	-0.006	-9.7
5	59.1	0.092 ± 0.004	56.9	0.097 ± 0.007	-0.004	-4.9
6	17.9	0.084 ± 0.008	19.6	0.090 ± 0.005	-0.006	-7.5
7	54.3	0.114 ± 0.007	53.9	0.118 ± 0.006	-0.003	-2.9

	Once washed		Thrice washed			
Pair	Weight (mg)	Conc. ± SD [a] (μg/g)	Weight (mg)	Conc. ± SD [a] (μg/g)	Diff [b] (μg/g)	% Diff [c] (%)
8	20.2	0.029 ± 0.006	21.8	0.036 ± 0.010	-0.008	-26.8
9	73.3	0.042 ± 0.003	72.8	0.037 ± 0.002	0.004	10.6
10	32.4	0.076 ± 0.004	32.4	0.076 ± 0.004	-0.001	-0.7
11	19.4	0.069 ± 0.008	17.6	0.065 ± 0.007	0.004	5.9
12	19.5	0.144 ± 0.010	20.3	0.156 ± 0.006	-0.012	-8.2
13	24.6	0.139 ± 0.014	21.2	0.133 ± 0.011	0.006	4.6
14	28.2	0.343 ± 0.015	22.5	0.363 ± 0.012	-0.021	-6.0

[a]SD: standard deviation of five replicate measurements.
[b]Diff: difference in arsenic concentration between one washed and two or three washed samples.
[c]Diff: Diff/arsenic concentration found in one washed sample.

model indicates that the various treatments investigated in the study show no significantly different influences on the toenail arsenic analysis by ICP-MS (p = 0.74, mixed linear model). The arsenic concentration of "in-house" nail reference material for microwave digested samples was 0.113 ± 0.006 μg/g. For room temperature (36 h) digested nail samples, the concentration was 0.107 ± 0.010 μg/g. From the two-sample t-test, we found the data gave no evidence that these two digestion methods made a substantial difference (p = 0.31, two-sample t-test).

Stability of the Digested Samples

We examined the time trends of various treatment effects on toenail arsenic levels measured by ICP-MS (Table 3). The mean concentrations of the four groups ranged from 0.196 to 0.211 μg/g (control), 0.188 to 0.207 μg/g (sonication), 0.198 to 0.204 μg/g (water bath), and 0.194 to 0.202 μg/g (sonication plus water bath). When we calculated and compared

Table 3
Means, Standard Deviations (SD), and Coefficients of Variation (CV) of the Results of the Study Evaluating Sample Preparation Techniques and the Reproducibility of Measurements

Treatment	Day	Mean ± SD (μg/g)	CV (%)
Control	1	0.211±0.012	5.7
	2	0.196±0.009	4.6
	3	0.201±0.010	4.8
	6	0.200±0.008	3.9
	1-6	0.202±0.007	3.3
Sonication	1	0.207±0.009	4.3
	2	0.188±0.011	5.8
	3	0.200±0.012	5.9
	6	0.202±0.012	6.1
	1-6	0.200±0.008	4.1
Water Bath	1	0.204±0.009	4.4
	2	0.198±0.006	2.8
	3	0.200±0.017	8.4
	6	0.198±0.006	2.8
	1-6	0.200±0.003	1.3
Sonication & Water Bath	1	0.202±0.011	5.3
	2	0.199±0.008	3.8
	3	0.194±0.005	2.7
	6	0.202±0.007	3.3
	1-6	0.199±0.004	1.8

Note: Sample size = 10.

the 95% confidence intervals based on five repeated measurements for each digested sample analyzed 1, 2, 3, and 6 d after sample preparation, the intervals overlapped. Figure 2 shows an example of the comparison of the four intervals. Hence, the reproducibility analysis demonstrated that the day-to-day variability of the measurements is not significant across a week after acid digestion.

Influence of Acidity on Arsenic Signal

The mean, standard deviation (SD), and 95% confidence interval (CI) were computed for each series of experiment and 5 ng/mL falls within all of them (Table 4). Therefore, acidity does not appear to have a substantial influence on the behavior of the arsenic signal.

Limit of Detection

An excellent limit of detection could be achieved by this analytical method. The limit of detection is calculated as three times of the standard

Table 4
Acidity Effects on the Arsenic Signal

HNO_3 Conc. (%)	Mean Conc.[a]± SD[b] (ng/ml)	95% CI[c] (low)	(up)
5	4.96±0.11	4.74	5.18
10	4.71±0.15	4.42	5.01
15	4.87±0.21	4.45	5.29
20	4.99±0.21	4.57	5.40

[a]Standard value = 5.0 ng/mL; sample size = 5.
[b]SD: standard deviation of five replicate measurements.
[c]CI: confidence interval.

deviation of the blank measurement. The instrument limit of detection of the ICP-MS was less than 0.06 ng/mL. Taking into account the uncertainty of the determination of blank values of the wet ashing procedure, the method limit of detection was 0.07 ng/mL. Incorporating the dilution factor, the limit of detection for toenail arsenic analysis was less than 7 ng As/g toenail.

Quality Control

Arsenic concentrations obtained by the ICP-MS technique were in good agreement with certified values of the reference materials. For the NIST water, the arsenic concentrations corresponded closely to the certified value with a relative error of less than 7%. For both hair and nail reference materials, the arsenic recoveries ranged between 95% and 106% and within the 95% confidence interval of the certified/reference values. Also, results obtained from spike samples were all satisfactory (recoveries were from 94% to 99%).

DISCUSSION

The washing procedure to remove external contamination from toenail samples is a crucial step in determining arsenic contents in nails. An ideal washing technique should completely remove surface contamination without depleting the arsenic within the nail structure. Although arsenic concentrations in nails have been analyzed in prior studies, there is no standard procedure for the washing of nail samples. Some studies did not report performing a washing procedure. For those that did, a variety of washing methods have been reported. Most of the protocols fall into two groups: use of detergents and use of organic solvents. Harrison and Tyree *(24)* compared these two types of procedures. They found that organic solvent washing rather consistently showed less reduction in elemental concentration than did detergent washing.

However, detergent washing appeared more advantageous in that it corresponds more closely to *in situ* washing. As a result, they concluded that the use of detergents is preferred when cleaning nails.

In our study, 1% Triton X-100 detergent was selected for all sample washing. Based on our results, arsenic levels were reduced in most of the samples washed with the detergent. The reduction ranged from a low of 5.6% to a high of 85.2%. This reduction could be due to a removal of surface contamination. Alternatively, the washing may have extracted arsenic from the nail structure. The nail plate is a tissue composed of multiple layers of horny cells firmly cemented together and filled with keratin. Keratin contains a complex mixture of very stable proteins and is remarkably resistant to chemical treatments, mainly because of its characteristic disulfide bonds. The studies of Ndiokwere *(8)* and Bate and Dyer *(27)* suggested that arsenic levels obtained for unwashed and washed hair and nail specimens showed little or no arsenic loss during washing. They concluded that heavy metals in hair or nail were not essentially influenced by a washing procedure because of their great tendency to complex with the disulfide groups in the keratin proteins. Because the toenail samples were soaked in detergent only 20 min for washing, it is not likely that the reduction in arsenic concentration could be explained by the leaching of arsenic out of toenails over the washing period. In addition, the results from one wash vs. multiple washes experiments demonstrated that nails washed once, twice, or three times did not vary substantially in arsenic concentrations. This indicates that further washes do not improve the effectiveness of the washing technique, and the washing procedures applied in the study are not likely to deplete arsenic in nail structures. Moreover, arsenic concentrations were lower in most of the washed samples than in the unwashed samples. These findings imply that the washing procedures remove most surface contamination effectively.

A higher arsenic concentration in the washed portion was obtained in one pair of samples (pair 3, Table 1). The causes of this discrepancy may arise principally from measurement errors and the inhomogeneity of the samples. Because the difference in weight between washed and unwashed samples for pair 3 was the highest among all of the samples studied, inhomogeneity could be identified as the major factor contributing to this inconsistency. Therefore, the washing method used in the study appears to be adequate.

For evaluation of sample preparation procedures, we prepared the "in-house" reference material. The coefficients of variance within each run were all below 6% (except d 3 of the water bath treatment), indicating that the criteria of the homogeneity of "in-house" reference material is met. Recoveries for arsenic in both room-temperature acid-digested and microwave digested reference nail samples were in good agreement, which suggests that nail specimens can be digested successfully using the room-temperature acid-digestion method described in the study. No

evidence of variability associated with the four treatments was observed. Because we arrived at the same results with these four techniques, we chose not to apply any treatment to samples after acid digestion. The flowchart summarizing recommended preparation procedures for toenail analysis is shown in Fig. 3.

The analysis comparing 95% confidence intervals revealed that the variability by days is not significant, although the mean values fluctuated. The variability by days might have resulted mainly from instrument error, such as errors introduced by operation and calibration of the ICP-MS machine, inhomogeneity of samples, and physical or chemical reactions occurred in the samples. As a result, we would not expect much day-to-day variability to be introduced by deterioration of digested samples. Nevertheless, in order to ensure the quality of the solution, we recommend analyzing samples within 5 d after acid digestion.

For the ICP-MS application, the use of H_2SO_4 or HCl is discouraged in general, as it gives rise to interference from polyatomic species. Campbell et al. *(26)* noted that the arsenic signal declined by about a factor of 2 in the presence of 2% nitric acid. The arsenic signal was found to be negatively correlated with acidity. There is no definitive explanation to this phenomenon. However, this will result in either underestimation or overestimation if the acidity between the samples and external calibration solutions are not the same. We examined the effect of 5%, 10%, 15%, and 20% nitric acid on the arsenic signal, but no decline was observed. The acidity of external calibrants and the study samples were different: 5% and 20%, respectively. However, as acidity does not affect the behavior of the arsenic signal, any error introduced by acidity would be negligible.

Accumulation of trace elements in nails may be influenced by both the environment and diet. Evidence to date has demonstrated that arsenic content in nails is elevated in individuals known to be exposed to arsenic. Thus, nail arsenic levels may be used as a biomarker of exposure. Nevertheless, the methodology for the determination of arsenic in nails has not been established. The work here indicates that ICP-MS has the potential of providing both accurate and precise measurements of arsenic in toenails. ICP-MS is a sensitive instrument with a relatively low limit of detection. Although it is subject to some limitations, such as interference and matrix effects, the addition of nitrogen gas to the nebulizer gas has succeeded in the removal of the $ArCl^+$ polyatomic interference on the arsenic signal and the matrix effects are minimized by proper dilution and use of an appropriate internal standard. In addition, the quality control protocols applied provide effective means to maintain reliable analyses. In terms of sample treatment, the simplicity in preanalysis preparation allows a high throughput for analysis. The high throughput will permit researchers to conduct large epidemiologic studies that have greater statistical power to identify associations between body burdens of toxicant and health outcomes.

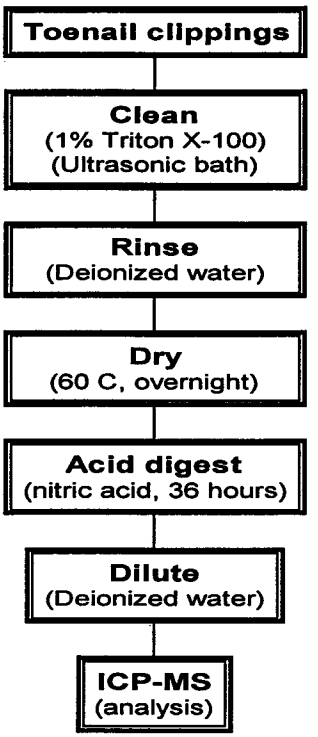

Fig. 3. Schematic diagram of the procedures for toenail analysis.

The methodology proposed here provides valuable features; furthermore, this technique can be applied to the analysis of other trace metals in nails.

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