Measurement uncertainty of Pb, Cd and Ni determination in human hair by electro thermal atomic absorption spectrometry

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Abstract: Today, in developing countries like Iran heavy metals are present everywhere which threatens the health of the people. The analysis of human hair is useful in monitoring the levels of certain trace elements in the body and assessing heavy metals environmental exposure.

In the present work, we propose uncertainty estimation for the analytical results we obtained from determination of cadmium, lead and nickel in human hair by electro thermal atomic absorption spectrometry (ETAAS). Prior to analysis, hair samples were washed with 1% (w/v) sodium diethyldithiocarbamate (DDTC), and 0.1M HCl. The hair samples were digested afterward in a mixture of HNO3 and H2O2. To estimate the uncertainty of analytical result obtained, we propose assessing trueness by employing spiked sample. Two types of bias are calculated in the assessment of trueness: a proportional bias and a constant bias. We applied Nested design for calculating proportional bias and Youden method to calculate the constant bias. The results we obtained for proportional bias are calculated from spiked samples. In this case, the concentration found is plotted against the concentration added and the slope of standard addition of curve is an estimate of the method recovery. Estimated method of average recovery in human hair is: (1.019±0.026), (0.918±0.014) and (1.059±0.017) for Cd, and Ni, respectively.

Keywords: Uncertainty; Hair analysis; Trace elements; Atomic absorption spectrometry

Introduction

Metal pollution and its health effects present a challenge currently facing the developing countries. Metal poisoning is usually difficult and expensive to assess or screen in these countries because of limited resources, which means that policies, guidelines, regulations and institutional managements are limited. So, in this regard the analysis of human hair is useful in monitoring the levels of certain trace elements in the body [1]. Several advantages were mentioned for the use of this biological material in monitoring studies, namely: hair is a biological specimen that is easily and no invasively collected, inexpensive, and easily

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stored and transported to the laboratory for analysis. These attributes make hair an attractive biomonitoring substrate, at least superficially [2]. In opposition, some limitations were described for hair analysis, mainly the occurrence of exogenous contamination that contributes to a differential increase in the total contents of different contaminants [3]. Pollutants due to the presence of toxic metals in environment not only enter the body by breathing, water, and food-stuff accumulates in hair, but they could be adsorbed directly on the hair from environment [4]. Actually these are the external contaminants which complicate interpretation of hair analysis results and make it difficult to determine levels of endogenous elements. In order to remove adsorbed elements and thus determine the internally bound elements correctly, hair sample must be washed [5, 6].

Analytical result must be validating because they are used as a peace of valuable information for a certain aim. Therefore, analysts are increasingly impelled to validate analytical procedures and to estimate the uncertainty associated with the results these procedures provide. Uncertainty can be obtained either by calculating all the sources of uncertainty individually (bottom-up approach) or by grouping all sources of uncertainty. However, the last one is not straightforward; other approaches based on calculating uncertainty using information from the validation process have been proposed [7, 8]. Approach proposed by Morato calculates uncertainty in wide range of concentration and assume proportional and constant bias may be present. In this approach recovery is estimated with the method of averaged recovery and constant bias with the Youden method [9, 10-12].

The main purpose of this study was to develop a procedure for human hair pre-treatment and a Morato method in uncertainty estimation of analytical results which obtained by assessing trueness and employing spiked samples in determination of Pb, Cd and Ni in samples by ETAAS. Also, the average composition of hair from Tehran (heavy industry dominates) was compared with the average composition of hair from the population living in a non-industrialized area of Iran (Takab province).

**Experimental**

**Instrumentation**

A Varian model Spectra AA-220 (Mulgrava, Victoria, Australia), atomic absorption spectrometer equipped with a GTA-100 graphite furnace atomizer and deuterium lamp background correction was used with hollow cathode lamps (Varian). The spectrometer parameters are tabulated in Table 1.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Wavelength (nm)</th>
<th>Spectral bandwidth (nm)</th>
<th>Lamp current (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Ni</td>
<td>232.0</td>
<td>0.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Pb</td>
<td>217.0</td>
<td>1.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Reagents**

Suprapure Merck HNO₃, H₂O₂, H₂SO₄ and HCl were used in the treatment of hair samples and preparation of standard solutions. All reagents used in the present study were analytical grade and deionized water of high purity (from a Millipore ultra pure water system). Standard stock solutions of Cd and Ni at a concentration of 1,000 g. l⁻¹ were prepared from Cd(NO₃)₂ and Ni(NO₃)₂. Pb stock standard solutions at a concentration of 1,000 g. l⁻¹ were prepared from lead metal strip (Merck). DDTC from Fluka was also used. Glassware, nalgene bottles and sealed plastic bags were all cleaned in 5% (v/v) nitric acid before use.

**Sample Collection**

A number of hair samples were collected from Tehran and Takab province (northwest of Iran) as control group. Individuals were invited to answer a small questionnaire designed to obtain information about age, sex, food ingestion (home grown fruits and vegetables, fish, and animals, breeding cattle and...
canned food), source of drinking water, smoking habits, health condition, medication, and workplace. The hair samples were cut from the nape of the neck with a stainless steel scissors and were stored individually in sealed plastic bags at room temperature.

**Influence of analytical parameter**

The influence of various analytical parameters including the amount of human hair sample, digestion procedures and washing factors (concentration and volume of washing solution), was investigated and after finding the optimum situation of analyse, all experiments runs and the uncertainty of analytical result estimated.

**Hair Digestion Procedures**

Preliminary studies showed that mixtures of HNO\textsubscript{3} – H\textsubscript{2}O\textsubscript{2} were better than HNO\textsubscript{3} or HNO\textsubscript{3} – H\textsubscript{2}SO\textsubscript{4} or HNO\textsubscript{3} – HCl in terms of complete dissolution in a short time. The effect of four important factors including; temperature, digestion time, volume of nitric acid and volume of hydrogen peroxide on the digestion efficiency of human hair samples were studied. All hair samples were digested by weighing the sample directly (about 1 g) into teflon bombs. Based on the results obtained, the optimum conditions for digestion were established as: temperature = 110 °C; digestion time = 90 min; volume of nitric acid (69%) = 8 ml; volume of hydrogen peroxide (30%) = 6 ml. After digestion, the sample was diluted to final volume with deionized water. Standard solution of each element was prepared in a mixture of HNO\textsubscript{3}, H\textsubscript{2}O\textsubscript{2} and deionized water. Detections were carried out using ETAAS.

**Washing Procedures**

Several washing procedures (organic and inorganic) were studied and the analytical results were compared [13]. In further experiments the following washing procedure was applied: washing with 0.1M HCl solution to remove Cd and Ni, with 1 % (w/v) DDTC solution to remove Pb, and, with deionized water. The washed hair samples were dried at 70 °C.

Data analysis. The values of the metal concentrations in human hair of the subjects were presented as arithmetic mean (µg g\textsuperscript{-1}) with standard error (±SE). The statistical significance of concentrations of heavy metals between different parameters was determined by ANOVA as appropriate. The level of significance was set at P<0.05. All calculations were performed using statistical packages SPSS (VERSION 14).

**Statistical Method**

Uncertainty and validation of analytical procedures. Analytical procedures should be validated before they are used to analyse routine samples. In this process, the systematic errors are estimated in the assessment of trueness. Uncertainty and trueness are much related concepts. This is because we can not guarantee the correctness of all the systematic errors if we have not previously assessed the trueness of the analytical method and, consequently, it is impossible to ensure that the true value is included within the interval estimated value ±U (where U is the uncertainty of the estimated result). Therefore every analyst should verify the trueness of the method before calculating uncertainty. Uncertainty can then be calculated using the information generated in the assessment of trueness. When dealing with spiked samples and recovery estimation, analytical results maybe corrected for these errors so that the final results are traceable. Moreover the uncertainty of these results should also be calculated as a measure of their reliability. Some component of this uncertainty can be obtained using information generated when the analytical procedure is validated within the laboratory. Uncertainty should then consider all the sources of error of the analytical results can calculated in a general way by grouping all these sources in four terms:

\[
U = \sqrt{u^2_{\text{precision}} + u^2_{\text{trueness}} + u^2_{\text{pretreatments}} + u^2_{\text{other terms}}}
\]

The first component of uncertainty precision \(u^2_{\text{precision}}\) depends on the intermediate precision of the procedure and also takes into account the fact that results depend on the matrix of the routine samples. The second terms, \(u^2_{\text{trueness}}\), consider the uncertainty caused
by systematic errors, i.e. constant and proportional bias in the assessment of trueness. The third term, \( u^2_{\text{pretreatments}} \), considers the uncertainty caused by the lack of homogeneity of the sample and pretreatment not carried out in the assessment of trueness. Finally, the forth component, \( u^2_{\text{other terms}} \), contains all the sources of uncertainty not considered in the former terms [7, 14]. In this study we calculate two terms (\( u^2_{\text{precision}} \) and \( u^2_{\text{trueness}} \)) and also consider two situation (with a spike uncertainty and without it) to estimate the final uncertainty in precision study.

Precision study

Precision can be estimated simply by test sample that lies within the concentration range studied. The within-laboratory precision of an analytical method should be characterized by the repeatability and the run-different intermediate precision. The experimental design we have proposed is a two-factor fully-nested design [15]. Here the factors studied are the p-run and n-replicate, one of which is inside the other. The use of the analysis of the variance provides the information about intermediate and the repeatability precisions.

Assessment of trueness

Trueness should be evaluated, in terms of bias, through the analysis of reference samples. We use spiked sample as a reference materials because the other references are not available. In the assessment of trueness, proportional and constant biases are calculated from spiked samples. Constant bias (when samples free from the analyte are available) must be calculated using the Youden method. The proportional bias can be expressed either as instrumental response or if a standard curve is used, as concentration [16]. We use the standard curve and concentration to express our results.

Standard addition method (SAM):
calculation of proportional bias and related uncertainty

1 g of each human hair (three samples) are spiked with analyte quantities 0.5, 1 μg for Cd, Ni and Pb, each spiked sample analysed for five time, so that the precision of the analytical procedure and the variability of results with the matrix can be obtained. For this purpose, appropriate amounts of metal standard solutions were added to hair samples before digestion. Fig.1 shows the proposed experimental design for obtaining information of the between-matrix variance, \( S^2_{\text{matrix}} \) and the variance associated with

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Figure 1  Experimental design for obtaining information about matrix variability and precision from the results obtained with spiked samples. Each measurement of metal concentration is denoted by \( y_{ijk} \), where i the number of the treatment, runs from 1 to a (a=2) represented the amount of analyte added; j, denoted the number of different samples used for i treatment, runs from 1 to b (b=3); and k the observation number from the jth samples on the i treatment, runs from 1 to n (n=5), so \( y_{ijk} \), represent the result for the kth analysis of the jth samples spiked an amount of analyte i.
precision, $S^2_{\text{precision}}$ [15].

SAM results expressed as a concentration when we use standard curve. Therefore, SAM curve performed by plotting concentration found versus concentration added. The slope of the SAM curve is an estimate of the method recovery ($R$). When we have obtained $R$, and its uncertainty $u(R)$:

$$u(R) = \sqrt{\left(s(b_{\text{SAM(conc)}})\right)^2 + \left(\frac{R}{b_{\text{sc}}}ight)^2 s(b_{\text{sc}})^2}$$

(These expression are given in the Appendix) we can evaluate whether the proportional bias is significant or not by t-test, that is, $|R-1| \leq t_{\alpha/2, \text{eff}} \times u(R)$ [16].

**Youden method**

Calculation of constant bias and related uncertainty. The Youden method consists of analysing two or more different amounts (weight or volumes) of a test sample under condition of repeatability or intermediate precision. Youden plot can be defined as a sample concentration curve plotted against sample amounts and the intercept of it shows the constant bias ($\delta_{ct}$). The uncertainty associated to $\delta_{ct}$ is given by,

$$u(\delta_{ct}) = \sqrt{s(a_{\text{YOU(conc)}})^2 + u^2_{\text{condition}} + u^2_{\text{sc}}}$$

where $s(a_{\text{YOU(conc)}})$ represents the standard deviation of the intercept of the Youden curve obtained when concentration is plotted against the amount of sample. $u_{\text{condition}}$, denote the uncertainty associated with how the amounts of sample and standards of the standard curve are analysed. If they are analysed under intermediate conditions, then $u_{\text{condition}} = 0$. If they are analysed under repeatability conditions, $u_{\text{condition}} = u_{\text{run}}$. Finally, $u_{\text{sc}}$ is the uncertainty associated with converting the instrumental response into concentration with a standard curve, $R$ is the method recovery and $\delta_{ct}$ denotes the constant bias. The concentration of future samples is obtained by correcting results by both biases. The standard uncertainty of the concentration, $u$, is obtained by applying propagation low to conc equation,

$$u = \frac{1}{R} \sqrt{\left(R \times u(R)\right)^2 + \delta_{ct}^2 + u(c_{\text{found}})^2}$$

The first two terms of this equation consider the uncertainty associated with the assessment of trueness, $u_{\text{trueness}}$: $u(R)$ represents the uncertainty of the method recovery and $u(\delta_{ct})$ denotes the uncertainty of constant bias. The third term, $u(c_{\text{found}})$, is the uncertainty of the concentration found for the routine sample with the standard curve and considers the uncertainty associated with precision uprecision. The practical estimation of the components of uncertainty and expression are referred to [8].

**Results and discussion**

**Uncertainty estimation for the analytical results**

The analysis of the spiked samples provides information about proportional bias and precision. Table 2 shows the estimated of variance component of variable in Nested – design, $S^2_{\text{matrix}}$, $S^2_{\text{Spike}}$, precision and calculated result obtained for metals in human hair. The results were expressed as concentration.
found after using a calibration curve.

Table 2  Estimated of Variance component of variable in Nested –design for Heavy Metals in human hair

<table>
<thead>
<tr>
<th>Metals</th>
<th>a S^2_{Matrix}</th>
<th>b S^2_{spikes}</th>
<th>c S^2_{precision}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.172</td>
<td>0.000</td>
<td>0.008</td>
</tr>
<tr>
<td>Pb</td>
<td>0.035</td>
<td>0.000</td>
<td>1.535</td>
</tr>
<tr>
<td>Ni</td>
<td>0.052</td>
<td>0.000</td>
<td>0.008</td>
</tr>
</tbody>
</table>

\[
a S^2 = \frac{MS_{Analyst} + MS_{Matrix}}{n}
\]
\[
b S^2 = \frac{MS_{spike} - MS_{Analyst} + MS_{Matrix}}{b}
\]
\[
c S^2 = \frac{c S^2_{precision} = MS_{precision}}{b}
\]

Table 3 shows the recovery, R and its uncertainty U(R), obtained when analytical results are expressed as concentration found. To compare the results of this method, recovery was calculated using the method of averaged recovery as others do [10, 11]. Recovery was calculated for each spiked sample and the overall recovery was estimated as the mean of the n recoveries calculated. The uncertainty of this average recovery was calculated using the precision information from the results of the spiked samples:

\[
U(R) = \frac{1}{n} \sqrt{\frac{S^2_{Matrix} + S^2_{spikes}}{b^2 c^2 \sum_{i=1}^{n} C^2_{ad.i}}}
\]

Table 3  Recovery, R, and its uncertainty, u(R), obtained with the standard addition curve. Constant bias, ct δct and its uncertainty, uδct obtained with Youden curve.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Standard addition</th>
<th>Average recovery</th>
<th>Youden curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>U(R)</td>
<td>R</td>
</tr>
<tr>
<td>Cd</td>
<td>0.939</td>
<td>0.110</td>
<td>1.019</td>
</tr>
<tr>
<td>Pb</td>
<td>0.999</td>
<td>0.058</td>
<td>0.918</td>
</tr>
<tr>
<td>Ni</td>
<td>1.008</td>
<td>0.083</td>
<td>1.059</td>
</tr>
</tbody>
</table>

Table 4  Concentration together with its uncertainty, obtained with the procedures for the metals analysed

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration Found*</th>
<th>Method of average recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With spike</td>
<td>Without spike</td>
</tr>
<tr>
<td>Cd</td>
<td>0.353 ± 0.039</td>
<td>0.353 ± 0.039</td>
</tr>
<tr>
<td>Ni</td>
<td>0.468 ± 1.357</td>
<td>0.468 ± 1.357</td>
</tr>
<tr>
<td>Pb</td>
<td>0.411 ± 0.252</td>
<td>0.411 ± 0.252</td>
</tr>
</tbody>
</table>

*Results are expressed in parts per million

Analysis of human hair samples of residents of Iran

The metals of ten different amounts (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0 and 1.5 g) of natural human hair sample were analysed under intermediate precision conditions. The analytical results were expressed as concentration found. Table 3 also shows the constant bias and its uncertainty when results are expressed as concentration found. The variance of the residuals of the Youden plot was compared with the variance associated with the intermediate precision of the method. Since the difference between the variances was not statistically significant for the metals determined, we assume that the matrix effect was the same for all the amounts of sample and, therefore, that a correct estimation of the constant bias was obtained from the Youden plot. The uncertainty related to real samples was calculated in two ways: (a) when results are expressed as a concentration found and (b) when recovery was estimated with the method of average recovery.

Table 4 shows the concentration, together with its uncertainty, for all the metals and for two procedures. As we can see in Table 4, results of two procedures are likely to be similar and constant bias which is not usually considered in the uncertainty budget is negligible for elements study and should not taken into account.

The metals of ten different amounts (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0 and 1.5 g) of natural human hair sample were analysed under intermediate precision conditions. The analytical results were expressed as concentration found. Table 3 also shows the constant bias and its uncertainty when results are expressed as concentration found. The variance of the residuals of the Youden plot was compared with the variance associated with the intermediate precision of the method. Since the difference between the variances was not statistically significant for the metals determined, we assume that the matrix effect was the same for all the amounts of sample and, therefore, that a correct estimation of the constant bias was obtained from the Youden plot. The uncertainty related to real samples was calculated in two ways: (a) when results are expressed as a concentration found and (b) when recovery was estimated with the method of average recovery.
drugs. Also, seasonal variations and other variables influence the level of elements in hair. The level of elements in hair can be also influenced by the level of other elements: synergistic and antagonistic effects were detected. However, factors (i) and (iv) were eliminated by the careful sampling procedure adopted in the present study. The average composition of hair from Tehran (heavy industry dominates) was compared with the average composition of hair from the population living in a non industrialized area of Iran (Takab province). The level of the elements in the hair sample from Tehran was higher in the hair sample from Takab: Cd (5.03 times), Pb (1.55) and Ni (1.95). We assumed that the content of these elements is rather related to the personal exposure than to the exposure of the whole population in the urban area of Tehran. The level of Ni is probably related to living habits and the level of Cd and Pb are related to local exposure or personal ability to accumulate a given element.

Conclusion
The aim of this study was to estimate the uncertainty of results obtained in determination of trace elements in human hair sample by ETAAS method. Hair is one of the best biomarkers for speciation of trace elements entering the body. We describe an estimation of measurement uncertainty for the analytical result, using the information generated when the trueness of analytical procedure is assessed using spiked samples. For this, we have developed Marota procedure which involves estimating the constant and proportional biases of the analytical procedure, produces lower uncertainties compared with other methods. In the present work also, the elemental composition of hair of the Tehran was compared with the average content of the population living in the Takab province. It was found that, the level of Cd, Pb and Ni changed significantly with changes of living habits or environmental exposure.

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