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Elemental Analysis of Human Hair by LA-ICP-MS

The CETAC LSX-500 Laser Ablation System was used as a sample introduction system for the quantification of trace metals in human hair.

INTRODUCTION

Chemical analysis of hair can be used to evaluate the diet, exposure to contaminants and toxicants, and drug usage of an individual or population. In addition, hair has been used to assess metal body burden in occupational exposure cases. In their classic, comprehensive review of over 250 reports, the U.S. Environmental Protection Agency (Ho et al., 2001) concluded that hair is “a meaningful and representative tissue” for measuring high priority toxic metals such as lead, antimony, selenium, copper, and mercury. Although it is clear that hair metal content can provide meaningful information about exposure the use of this medium has not become widespread because of the limitations of available bulk digestion based analytical methods. These limitations include the lack of a suitable matrix standard, lack of accepted sample collection and preparation protocols, and lack of a standard method of analysis (e.g., Suzuki, 1988).

Here we present a laser ablation inductively coupled plasma mass spectrometric method for the quantification of trace metals in human hair. This method eliminates the need for sample preparation (e.g., washing) as well as provides high quality calibrated concentration data. This method was developed using matrix-matched human hair standard reference materials and provides highly precise data within minutes of sample collection.

EXPERIMENTAL METHODS

The Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometer (ICP-MS; DRC II, PerkinElmer Sciex) was used to quantify the trace element composition in hair standards. DRC II operating conditions are shown in Table 1. The DRC II, using dynamic reaction cell technology, eliminates or reduces polyatomic interferences. This is particularly important in the study of biological materials such as hair which are notoriously difficult matrices. In this case the DRC was not used to remove

interferences however even under gasless conditions the DRC quadrupole reduces background counts to less than 2.0 cps and reduces doubly charged and oxide species to less than 3%. Future applications of this method using NH₃ in the cell will allow for removal of carbon interferences (e.g., ArC on chromium).

The CETAC LSX-500 (266 nm) Laser Ablation (LA) System was coupled to the DRC II ICP-MS. The LSX-500 uses a high energy Nd:YAG (266 nm) laser to ablate the sample. The aerosol of ablated material is carried in a stream of carrier gas (ultra-pure Argon) into the ICP-MS for analysis. LA system operating conditions are shown in Table 2.

Table 1: DRC-ICP-MS operating conditions and measurement parameters (solution-based, see Table 2 for changes related to LA operation).

| | |
|----------------------------|-----------------|
| RF Power (v) | 1100 |
| Nebulizer Gas Flow (L/min) | 0.97 |
| Torch & Injector | quartz |
| Nebulizer | quartz Meinhard |
| Sample Cone | Nickel |
| Skimmer Cone | Nickel |
| Pulse Stage (v) | 1250 |
| Analog Stage (v) | -2100 |
| Lens Voltage (v) | 4.25 |
| Replicates | 3 |
| Sweeps per Reading | 10 |
| Readings per Replicate | 1 |

The goal of this study was to develop an analytical method that provides highly precise and accurate concentration data. Traditional LA-ICP-MS studies utilize reference standards such as NIST Glass, BCR-2 (USGS basaltic glass), or a combination of standardization by standard solutions and sample quantification by LA-ICP-MS. Whether using solid non-matrix

matched standards or using a combination of standardization by solution-based ICP-MS and quantification of LA-ICP-MS samples, neither approach meets the minimum criteria of an ideal analytical LA-ICP-MS method for quantification of metals in human hair. The standard reference material must be matrix matched and if the sample is quantified by LA-ICP-MS standard data must also be collected by LA-ICP-MS.

Table 2: LA-ICP-MS operating conditions and measurement parameters.

| | |
|-------------------------------|-----|
| LSX 500 Laser (266 nm Nd:YAG) | |
| Energy Level | 70% |
| Pulse Rate (Hz) | 20 |
| Spot Size (µm) | 100 |
| Bursts per Shot | 600 |
| Replicates | 1 |
| Sweeps per Reading | 1 |
| Readings per Replicate | 200 |

Results reported here are those based on human hair standard reference materials IAEA-085 and Chinese Hair Standard. We used IAEA-085 as a matrix-matched calibration standard and quantified, by LA-ICP-MS, the elemental composition of the Chinese Hair Standard run as an unknown. Future applications of the method presented here may rely on the two hair standards as standard reference materials for LA-ICP-MS to quantify metal concentrations in human hair samples.

Both hair standards are provided as semi-homogenized powders. Samples were homogenized in the lab by hand grinding in an agate mortar and pestle in a class 100 clean laboratory. One gram each of the powdered standards were pressed into 1 cm diameter disks using Carver press (Figure 1).

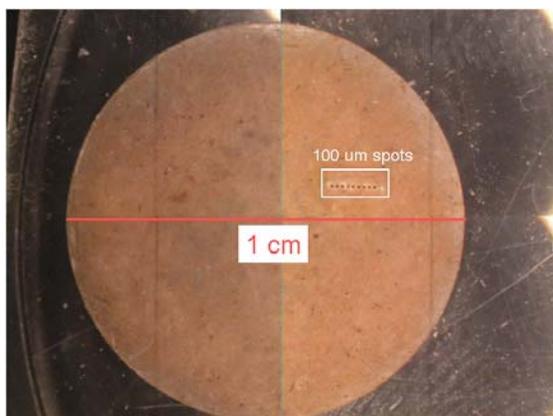


Figure 1. View of ablated pressed powder disk of IAEA-085 human hair standard.

Solution-based ICP-MS measurements of the Chinese Hair Standard, using IAEA-085 as a matrix matched calibration standard, followed EPA 6020 with digestion of 50 mg of powdered sample in 3 mL ultra-pure HNO₃ and 1 mL ultra-pure 30% H₂O₂ at 80°C for 6 hours. Samples were then dried down under laminar flow at 60°C until 0.1

uL of material remained and were then brought to 50 mL in 2% ultra-pure HNO₃ prior to quantification by ICP-MS. A calibration blank (2% HNO₃) and linear dilutions of IAEA-085 were used to calibrate measurements of the Chinese Hair Standard. Twenty replicate measurements were made of the Chinese Hair Standard with re-calibration every five samples.

RESULTS

Trace element abundances in the hair standards were easily resolved by using the LSX 500 in conjunction with ICP-MS. Using a matrix matched calibration standard (Figure 2) and processing the data using GeoPro it was possible to use IAEA-085 as a calibration standard and test the method quantifying the Chinese Human Hair standard as an “unknown” (Table 3). Calibrated analytes are those common to both hair standards (Cu, Fe, Hg, Mn, and Zn).

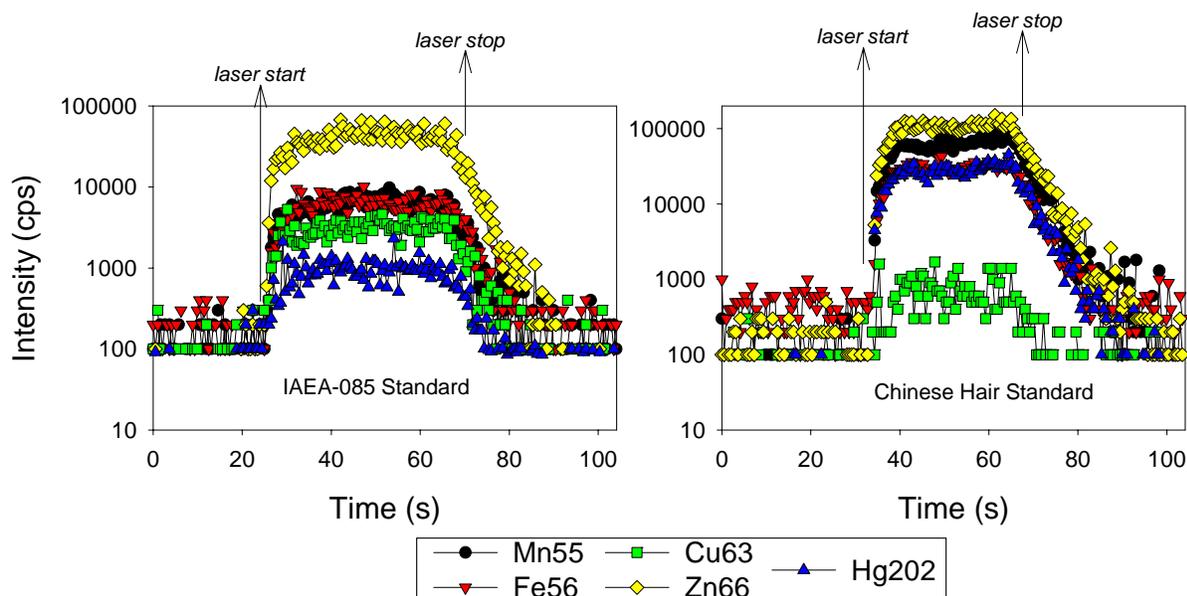


Figure 2: Time resolved ICP-MS spectra for analysis of the IAEA-085 hair standard.

Table 3: Measured concentrations (ppm) in the Chinese Human Hair Reference Standard using Human Hair Standard IAEA-085 as the calibration standard (n = 25; 5 spots per 5 samples). Standard deviation of measurements is shown (1-sigma).

| | <i>Measured</i> | <i>Known</i> |
|-----------|-----------------|----------------|
| <i>Cu</i> | 10.62 ± 0.93 | 10.60 ± 2.60 |
| <i>Fe</i> | 54.22 ± 1.03 | 54.00 ± 5.00 |
| <i>Hg</i> | 0.34 ± 0.07 | 0.36 ± 0.08 |
| <i>Mn</i> | 6.34 ± 0.07 | 6.30 ± 1.22 |
| <i>Zn</i> | 191.25 ± 1.12 | 190.00 ± 10.00 |

DISCUSSION

Comparison of solution-based to LA-ICP-MS results was done to explore the accuracy and precision of this method. One-way ANOVA was used to assess the differences between the two analytical techniques (solid vs. solution based sampling). Tukey's multiple components tests (95% confidence interval) were used to evaluate the differences between the samples analyzed by the two techniques. In order to determine

Quantification of trace metals in human hair samples by LA-ICP-MS will ultimately require analysis of individual strands or strand bundles collected from the human subject, or in the case of forensic applications, from a crime scene. Although Legrand et al. (2004) reported the potential use of single shot LA-ICP-MS for the detection of Hg in a single strand the method was not fully developed and no quantification was possible. In our experience using 266 nm Nd:YAG a single shot with an energy density sufficient to ablate the hair but not destroy the sample (<20%) did not provide sufficient analyte to the detector yielding intensities below 500 cps. Using strand bundles (10 strands per bundle) we were able to ablate enough material for a detectable and quantifiable

this, the concentrations of five (analyses) per sample were taken and compared to the other technique as well as the known standard. This gave a 95% confidence to the tests of difference between the results of the two techniques (Table 4).

After the comparison of concentrations, the mean, standard deviation, relative standard deviation and propagated error was calculated. Comparisons between the solution-based and LA-ICP-MS data with the known values are shown in Figure 3.

Table 4. Probability (p)-comparison of known standard concentrations (Tukey's t-test, ANOVA) using IAEA-085 as the calibration standard

| | Cu | Fe | Hg | Mn | Zn |
|-----------|-------|-------|-------|-------|-------|
| LA-ICP-MS | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| ICP-MS | 0.961 | 0.932 | 0.004 | 0.479 | 0.997 |

signal without ablating through the sample to the cell chamber floor. Ultimately the goal of LA-ICP-MS human hair analysis would be to develop a standard method using a line or series of discrete spots to study the long-term trend in trace metal chemistry however further study is needed to identify the appropriate wavelength for this complex matrix.

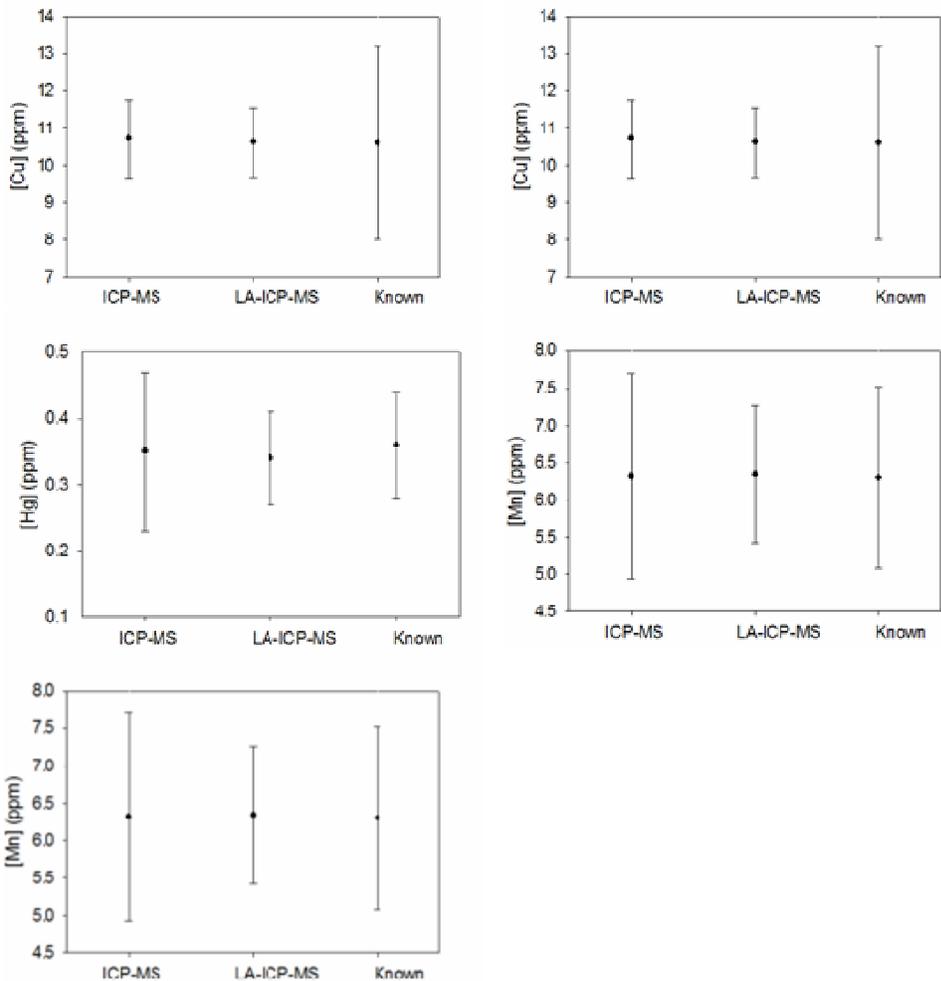


Figure 3: Comparison of measured and known concentrations of the Chinese Human Hair reference standard using the IAEA-085 matrix calibration standard for quantification.

CONCLUSION

The use of an Inductively Coupled Plasma Mass Spectrometer with a Laser Ablation system gives scientists versatility in application usage and biological sampling analysis. The method discussed above provides both accurate and precise concentration data.

Although hair is presently viewed as a complimentary matrix to blood & urine in medical trace metal studies, analysis via

laser ablation has shown that both quick and reliable results can be achieved. Previous studies performed by the Durrant (1999), Legrand et al. (2004) and Ho et al. (2001) have shown that hair is a biomarker that is both important and useful in environmental exposure analyses. Using LA-ICP-MS to study human hair trace element chemistry has far-reaching implications for clinical, forensic, and occupational health practitioners as it provided rapid, precise and accurate results.

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