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# The Determination of Heavy Metals Concentration in Hair by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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## Abstract

Heavy metals are non-biodegradable pollutants in environment that can enter our bodies through different routes and accumulated in the body. Hair analysis is increasing in studies due to the advantage of results of any homeostatic mechanisms unlike blood sample. The aim of this was to develop an analytical method to determine the concentration of nickel (Ni), arsenic (As), cadmium (Cd) and lead (Pb) in hair sample by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analysis was performed using ICP-MS ELAN 9000 (Perkin Elmer) that equipped with a Meinhard Concentric Quartz Nebulizer, Cyclonic Spray Chamber, Nickel Sampler and Skimmer Cones. The validation of each element was done using human hair Certified Reference Material (CRM), USA. Spike recovery of these elements was done by using CRM and the values were within 85% to 115% for validation. The linear calibration curves were established using concentration of 5, 10, 20, 50, 70 and 100 ppb of each element with good linearity ($r^2$>0.999). The lowest limits of detection for Ni, As, Cd and Pb were 61.18 µg/g, 2.34 µg/g, 6.15 µg/g and 112.95 µg/g. The results prove that ICP-MS can provide better sensitivity and detection limit can be achieved down to 2.34 µg/g. This approach offers the advantages of simplicity and ease of use as no pre-analytical steps such as digestion or excretion. In conclusion, ICP-MS can offer the capability to performed sub part per billion levels of multi-elements measurement. Hence this study demonstrated that ICP-MS can be effectively used for determination of heavy metal in human hair.

**Keywords:** Heavy metal; Human hair; ICP-MS

## Introduction

Heavy metals are one of toxicology aspect which are concern to human health and environment due to their toxic effects in recent years. It can be toxic for humans if they are not metabolized by the body and accumulate in the soft tissues. Toxicity of heavy metals can occur at the level above the background level where consuming high heavy metal concentration can cause either acute or chronic poisoning that can damaged or reduced mental and central nervous function, blood composition, lungs, kidneys, liver and other organs where long term exposure can cause slowly progressing physical, muscular and neurological degenerative conditions and cancer [1]. In general, heavy metals are also defined as those that have five times greater density than the water [2].

Heavy metals such as cadmium, arsenic and lead are extremely toxic even in small amounts because when these elements present in the environment in a high concentration, living organisms such as human are the subjected to the strong natural selection in tolerance [3]. For determination of heavy metals, hair analysis provided advantages because of its unique to reveal retrospective information of exposure in subjects 5 than blood or urine analysis where it easy to collect and transport, easy to handle, no need any special storage conditions, can detect high concentration of elements, non-invasive procedure, longer retrospective time represent and lower costs [4-6]. Hair is one of the important biological monitoring for environmental pollution and it can be used to sensitize individual towards maintaining healthcare lifestyle in their environment according to the Global Environmental Monitoring System (GEMS) of the United Nations Environment Program [3]. Heavy metals can enter human hair through different sources either exogenous or endogenous [7].

Literature has provided that inductively coupled plasma mass spectrometry (ICP-MS) can determine high sensitivity, good precision and accuracy where it can measure into parts per billion or even part per trillion for certain element versus the usual AAS where it only can measure up to parts per million [1,5,7-9]. ICP-MS is not only unique in term of sensitivity but also in term of ability to perform multi-elemental analysis where it able to test numerous elements up to 70 elements in single analysis so that it can reduce total analysis time and cost [1,3]. In general, atomic absorption can only detect up to 45 elements, cannot detect any metalloids or non-metals and unable to do simultaneously [9,10]. Now ICP-MS are one of the attractive and powerful technique that preferred and capable in scanning mass-to-charge (m/z) range 5-240 amu with minimum resolution of 0.9 [4].

Sample digestion process is an important step before determine the total element of mass concentration. There are various types of digestion methods that are been used to determine the mass concentration of trace elements. The most common types of digestion process used are open beakers that heated on the hot plates, using digestion tubes in a block digester and placed the digestion bombs in microwave oven to digest solid sample matrices [11].

The purpose of this paper is validation of method for the determination of metals in hair by inductively coupled plasma mass spectrometry (ICP-MS) method. Several parameters have been taken into consideration and evaluated for the method of validation such as linearity, the minimum detection limit, the limit of quantification, accuracy and uncertainty.

## Methods

### Materials and reagents

Ultra-pure de-ionized water (18 MΩcm$^{-1}$) from a Milli-Q analytical reagent grade water purification system (Millipore), 60% ultra-pure nitric acid, $HNO_3$, acetone and hydrogen peroxide, $H_2O_2$ were used. Meanwhile in order to validate the method to determine the concentration of metals, human certified reference material (CRM) was used. Internal standard mix calibration and special standard solutions were prepared by dilution of high purity ICP-multielement calibration standard (50 µg/mL from five element ICP-MS standard, matrix 2% $HNO_3$, Perkin Elmer Pure Plus). All laboratory equipment used for the sampling and sample treatment were new or cleaned are soaked using 10% nitric acid, $HNO_3$ for 24 hours and then rinsed using ultra-pure water.

### Sample collection and preparation

Ten hair samples were collected from the healthy individual. Hair samples were obtained from people who did not have colored or treated hair. Males had short hair, the hair was cut from all areas of the scalp. Meanwhile, for females only the distal portion of hair was cut. The hair samples were stored in polyethylene bags at room temperature until analysis. The present study was analyzed at the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. The aim of this study was to develop an analytical method to determine the concentration of nickel (Ni), arsenic (As), cadmium (Cd) and lead (Pb) in hair sample by ICP-MS. Hair samples were cut into pieces as small as possible and mixed to allow representative sub-sampling. Samples were then sonicated five times with 25 mL acetone-water-water-water-acetone [10], for 10 minutes each time and then dried in an oven overnight at 60°C. The purpose of hair washing is to remove any oily and greasy material from the hair surface [12].

### Analytical method

The dried samples approximately 0.1 gram was accurately weighed using four-decimal-place analytical balance and transferred to a screw cap digestion vessel. A mixture of 7 mL nitric acid and 3 mL hydrogen peroxide was added at room temperature. The vessels were then placed in the Microwave Digestion, Multiwave 3000, Anton Paar, XF 100-8 for 1 hour (Table 1). Once the digestion was complete, the vessels were allowed to cool to room temperature. The digest was diluted to 50 mL with Milli-Q water and ready for ICP-MS analysis. Based on digestion process, the cooling status are where the vessels were cooled to the point where the inserts could be safely removed [10].

The analysis of hair was performed using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS), ELAN 9000, Perkin Elmer (Figure 1) that was commercially introduced in year 1983. ICP-MS is a type of mass spectroscopy that is highly sensitive and capable in determination of high range of metals at concentration below one part per trillion. ICP-MS is equipped with a Meinhard concentric quartz nebulizer, cyclonic spray chamber, nickel sampler and skimmer cone. Meanwhile, nebulizer gas flow and the lens voltage were adjusted daily to give minimum count for oxide level and doubly charged ions and maximum count for indium. The running conditions for ICP-MS are summarized in Table 1. In order to analysis, ICP-MS were located in a temperature-controlled laboratory which allowed a sufficient period of time to stabilize before optimization procedures were carried out.

| Step | Time | Status | T1 | T2 | Pressure | Power |
|---|---|---|---|---|---|---|
| 1 | 00:30:00 | Ramp | 250°C | 60°C | 240 bar | 1200 W |
| 2 | 00:15:00 | Hold | 250°C | 60°C | 240 bar | 1200 W |
| 3 | 00:15:00 | Cooling | 250°C | 60°C | 240 bar | 1200 W |

**Table 1:** Digestion program for Multiwave 3000, Anton Paar, XF 100-8.

| Operations | Conditions |
|---|---|
| RF-power | 1200 Waltz |
| Nebulizer Gas Flow | 0.71-0.80 L/min |
| Lens Voltage | 6.00-7.00 Voltz |
| Analog Stage Voltage | -2100 Voltz |
| Pulse Stage Voltage | 1000 Voltz |
| Sweeps / Reading | 20 |
| Readings / Replicate | 1 |
| Replicates<br>Curve Type | 3<br>Simple Linear |
| Dwell Time | 100 ms |
| Integration Time | 2000 ms |

**Table 2:** Optimized Operating Conditions for the ICP-MS.

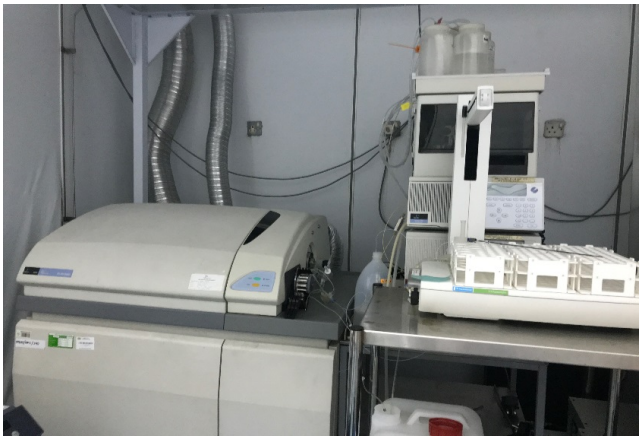

**Figure 1:** ICP-MS, ELAN 9000, Perkin Elmer.

### Quality assurance and control

The reagent blank samples were digested in the same way for hair samples and were used to correct the instrument readings. In order to

validate the method for determining the concentration of metals, human hair Certified Reference Material (CRM) from USA was used. Prior to analysis, the instruments were calibrated to ensure that it was operating within the acceptable ranges where the quality control (QC) standards and standard reference material (SRM) were analyzed. Then the instrument was allowed time to rinse between 1 to 2 minutes between each sample.

## Results and Discussion

Several parameters have been considered into account and evaluated for the validation of the method development for quantitative determination of heavy metals in hair samples. Table 2 gives a summary of statistical analysis of the concentrations of heavy metals in ten (10) hair samples. Mean concentration with standard deviation (SD) of each of the four (4) heavy metals under consideration have been listed.

| Sample No. | Metal Concentration (µg/g) | | | |
|---|---|---|---|---|
| | Ni | As | Cd | Pb |
| 1 | 22.67 | 1.99 | 3.86 | 87.25 |
| 2 | 26.35 | 0.98 | 8.34 | 125.91 |
| 3 | 11.43 | 0.11 | 3.69 | 121.41 |
| 4 | 34.08 | 0.60 | 2.33 | 55.37 |
| 5 | 9.67 | 0.96 | 2.52 | 49.67 |
| 6 | 6.55 | 0.66 | 0.96 | 6.55 |
| 7 | 2.31 | 0.78 | 3.32 | 99.66 |
| 8 | 67.65 | 0.60 | 2.13 | 67.65 |
| 9 | 1.24 | 0.57 | 2.63 | 95.16 |
| 10 | 35.19 | 2.76 | 1.42 | 42.11 |
| Max | 67.65 | 2.76 | 8.34 | 125.91 |
| Min | 1.24 | 0.11 | 0.96 | 6.55 |
| Mean ± SD | 21.71 ± 20.39 | 1.00 ± 0.78 | 3.12 ± 2.05 | 75.07 ± 37.65 |
| LOQ | 203.90 | 7.80 | 20.50 | 376.50 |
| LOD | 61.18 | 2.34 | 6.15 | 112.95 |
| Minimum limit of quantification and limit of detection | | | | |

**Table 3:** Metal concentrations (µg/g) using ICP-MS.

Lead (Pb) represent the highest mean concentration compared to the other elements in the samples where the mean and standard deviation concentration of Pb in the samples is 75.07 ± 37.65 µg/g. Pb is a toxic element even in a small concentration in the human body and one of the most popular heavy metal analysis due to it numerous health adverse effects of various organs in human body. Lead in toxic amounts can caused body inhibits of oxygen and calcium transport and alters nerve transmissions in brain. Meanwhile, nickel (Ni) concentration in the samples mean and standard deviation of 21.71 ± 20.39 µg/g and ranges between 1.24-67.65 µg/g.

The minimum limit of quantification (Table 3) is the lowest concentration that ICP-MS can quantitatively analyzed with an acceptable level of three times repeatability. Limit of quantification (LOQ) is one of the main analytical characteristic of the calibration curves to developed ICP-MS procedure. LOQ was calculated based on original samples (µg/g) by taking into account the amount of the sample that have been digested and the final volume that obtained by dilution. Meanwhile, LOD is defined by three times of standard deviation of the ten measurement. The LOD of these four elements studied are varies and shown that 112.95 µg $g^{-1}$ for Pb and 61.18 µg $g^{-1}$ for Ni.

### Calibration curve and range of linearity

The ICP-MS analysis was calibrated using external standard from Perkin Elmer, USA to increase the sample throughput. The calibration curves are evaluated although ICP-MS are well known as a wide working range of mass of concentration from ng $L^{-1}$ to mg $L^{-1}$ in a single analysis [13]. The calibration curves for Ni, As, Cd and Pb were linear in the range of 0.005 to 0.1 µg/g. Using calibration solutions curves were determined where a is the slope and b is the intercept (Table 4). The linearity was considered satisfactory and acceptable if the correlation coefficient, r exceeded 0.999 and in this study we found that that correlation coefficient, $r^2$>0.9999 in all 4 elements studied [14,15].

| Element | a | B | R |
|---|---|---|---|
| Ni | 0.0228 | 0 | 0.9999 |
| As | 0.0079 | 0 | 0.9999 |
| Cd | 0.0042 | 0 | 0.9999 |
| Pb | 0.0318 | 0 | 0.9999 |

**Table 4:** Calibration curves parameter (y=ax+b) for Ni, As, Cd and Pb.

## Conclusion

In this study a method of quantitative analysis for the determination of some metals (Ni, As, Cd and Pb) in ten hair samples by ICP-MS was developed and validated. The result provides sufficient evidence that can conclude this method are valid to use in toxicology aspect in order to determine the quantitative of toxic and acute level of heavy metal in hair samples. In addition, if time and money are not an issue, the microwave digestion procedure are recommended in sample preparation. Semi-quantitative analysis of hair samples by ICP-MS has been proven to be a powerful tool for rapid determination of heavy metal elements and the method is particularly useful for the analysis. It was characterized semi-quantitatively ten samples of hair from different peoples.

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