PREPARATION AND CERTIFICATION OF THE HUMAN HAIR REFERENCE MATERIAL*

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ABSTRACT

The preparation of the human hair reference material and the steps taken to confirm its homogeneity and stability as well as its certification are described. Certified values for 17 elements, which are of importance for human health, and reference values for the other 13 elements are provided for this material.

Keywords: Human hair Standard reference material Trace elements

1 INTRODUCTION

Human scalp hair mineral analysis is increasingly proposed as a method for the assessment of human contamination with environmental mineral pollutants and even as a diagnostic tool for related health problems[1- 2]. The use of scalp hair as a biopsy material has ideal advantages. It is easier to be collected and stored. It offers a good way of investigating long- term variation in trace element concentrations, and many minor and trace elements can be determined in hair samples with good precision and sensitivity by a variety of analytical techniques. An important requirement in such work is the application of suitable analytical quality control for which the availability of a hair reference material is of great importance.

In 1981, the IAEA prepared a hair reference material (HH- 1), but it was soon exhausted in an intercomparison study[3]. In Japan, the National Institute for Environment Studies issued a hair reference material (NIES- 5) in 1985[4]. However, only two kilos of such material was available. It is highly desirable to prepare a new suitable hair reference material with adequate amount in order to meet the ever growing need for analytical quality control on hair analysis in biological, medical and environmental studies.

On the basis of valuable experience gathered in two nationwide interlaboratory comparisons for the determination of trace elements in hair, which organized

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by the Chinese Nuclear Society, a new hair reference material has recently been prepared in a sufficient amount by Shanghai Institute of Nuclear Research, Academia Sinica. This paper describes the preparation procedures for this material as well as its certification.

2 MATERIAL AND PREPARATION

2.1 Sample collection and cleaning

Approximately 30 kg of human scalp hair was collected from normal male individuals (aged 18—30) in Wuxi area of Jiangsu province in 1983. After removal of visible contaminants, all black hair samples were washed by a mild detergent to remove surface exogenous contaminants, rinsed by distilled water, and then dried in an air– oven at 90 °C. The cleaned hair strands were clipped into segments of about 1 cm by a stainless blade.

2.2 Washing

The hair segments were washed in clean polyethylene container successively by dilute nonionic detergent (5 %), deionized water and acetone with stirring. After each wash, the washing solvent was decanted off. Finally, the hair samples were air– dried at room temperature between two clean filter papers in a dust– free room

2.3 Pulverization

The grinding of the hair sample into powder was accomplished by using a rotor– speed mill (Pulverisette– 14, Fritsch Gmbh, FRG). This mill combines grinding and sieving steps into one operation. A fine powder was usually obtained by repeating the operation. More than 99.8 % particles were less than 120 mesh.

2.4 Mixing and package

The hair powder was mixed together thoroughly in a polyester blender. The well mixed hair powder was then divided into 10 aliquots and tested for homogeneity by analysis of Cu, Zn, K and Mg with atomic absorption analysis. Results (see Tab.1) show no detectable difference between these batches.

Table 1

Results of homogeneity study by AAS* (in absorption)

Element	Cu		Zn		Mg		K	
	B	W	B	W	B	W	B	W
n	10	10	10	10	10	10	10	10
$\bar{x}$	0.183	0.184	1.72	1.73	1.25	1.26	0.110	0.112
S	0.00548	0.00485	0.017	0.011	0.0135	0.0158	0.0043	0.0062
C.V./ %	3.00	2.64	1.00	0.64	1.08	1.25	3.91	5.54
F	1.28		2.39		0.731		0.432	
$F_{\alpha,0.05}$	2.98		2.98		2.98		2.98	

* Sample size: 300 mg. B: For the between– bottle variation test. W: For the within– bottle variation test.

The 10 batches of hair powder were recombined, mixed, and dispensed in about 7 g amounts each into 2700 glass bottles of 50 ml that had been precleaned by nitric acid. Then these bottles were sterilized by ^{60}Co radiation at 20 kGy at the Shanghai Institute of Nuclear Research.

3 QUALITY ASSESSMENT

3.1 Homogeneity examination

X- ray fluorescence analysis (XRF) and instrumental neutron activation analysis (INAA) were chosen as the homogeneity evaluation techniques because they only need minimal preliminary sample operation and were capable of determining a number of elements simultaneously with high sensitivity and good precision.

3.1.1 XRF Forty bottles were randomly selected from 2700 ones for the between- bottle homogeneity examination. Samples of 300 mg each were taken. Another 10 samples were also taken from one bottle to examine the within- bottle homogeneity. Samples were pressed into 18 mm diameter pallets and then examined by XRF. The analytical results of Zn, Pb, Fe, Cu, Ca and S along with the results of F- test are presented in Tab.2. Values of F for any of the elements studied are not larger than that of the criterion ($F_{\alpha,\ 0.05,} = 2.42$), which indicate that for these elements the between- bottle homogeneities are not significantly greater than their within- bottle homogeneities.

Table 2

Results of homogeneity study by XRF

Element	Zn		Pb		Fe		Cu		Ca		S	
	B	W	B	W	B	W	B	W	B	W	B	W
n	40	13	40	13	40	13	40	13	40	13	40	13
x / kcps	0.7774	0.7758	1.032	1.032	0.8981	0.9011	0.8014	0.7983	4.066	4.066	62.705	62.73
$S\times 10^3$	6.782	5.26	7.13	6.16	12.1	8.90	10.7	8.35	23.8	25.3	354	304
C.V./ %	0.872	0.677	0.691	0.597	1.35	0.988	1.34	1.05	0.584	0.623	0.565	0.485
F	1.67		1.34		1.87		1.65		0.881		1.35	
$F_{\alpha,0.05}$	2.42		2.42		2.42		2.42		2.42		2.42	

3.1.2 INAA Another 12 randomly selected bottles were used for the examination of homogeneity by INAA. 100 or 200 mg portions were packed into polyethylene vials for the short or long irradiation, respectively. Analytical results for Al, Fe, Mg, Mn, Na, Zn and Cr as well as the results of F- test are summarized in Tab.3. The F criterion value at 95 % confidence level is 3.10, all the F values for these elements fall below this criterion.

From the results of XRF and INAA measurements, the conclusion is that this material satisfies the homogeneity criteria for a reference material.

3.2 Stability assessment

XRF analysis with fixed conditions was used to examine the stability of this material. Results of one- year examination indicate no significant difference on the element contents. Further observation on it will be carried out until the exhaustion of this material.

Table 3

Results of homogeneity study by INAA*

Element	Al		Fe		Mg		Mn		Na		Zn		Cr	
	B	W	B	W	B	W	B	W	B	W	B	W	B	W
n	12	10	12	10	12	10	12	10	12	10	12	10	12	10
$\bar{x}$/ μg g^{-1}	13.6	13.4	69.5	70.2	103.4	102.3	2.77	2.75	268	270	183	183	4.58	4.66
S	0.394	0.306	2.61	2.05	3.71	3.03	0.0627	0.0398	4.43	3.26	5.74	4.62	0.165	0.133
C.V./%	2.90	2.29	3.75	2.92	3.59	2.96	2.26	1.45	1.65	1.21	3.14	2.52	3.60	2.86
F	1.65		1.62		1.50		2.49		1.85		1.55		1.54	
$F_{\alpha,0.05}$	3.10		3.10		3.10		3.10		3.10		3.10		3.10	

* Sample size: 200 mg for long irradiation, 100 mg for short

3.3 Certification

The following aspects were taken into consideration in the collaborative certification on this hair material:

a. Before the certification analysis, participants were required to validate their analytical methods using a hair reference material prepared for intercomparison

Table 4

Certified values for the Chinese hair reference material* μg/g dry weight

Element	Content	Element	Content
Ag	(0.35)	La	(0.014)
Al	13.3±2.3	Mg	105±6
As	0.59±0.07	Mn	2.94±0.20
Ba	(5.41)	Mo	(0.58)
Br	(0.602)	Na	266±12
Ca	1090±72	Ni	3.17±0.40
Cd	0.095±0.012	P	(184)
Cl	(152)	Pb	7.2±0.7
Co	0.135±0.008	S	(4.69 %)
Cr	4.77±0.38	Sb	(0.21)
Cu	23.0±1.4	Sc	(0.00287)
Fe	71.2±6.6	Se	0.58±0.05
Hg	2.16±0.21	Sr	4.18±0.14
I	(0.875)	V	(0.069)
K	(11.8)	Zn	189±8

* Values in parentheses are reference values

studies and other biological reference materials.

b. Values required for certification should be obtained by at least 3 or more independent and established analytical techniques.

c. More than six analytical values were provided for each certified element.

Twenty- two laboratories were involved in the collaboratory certification analysis on this hair material. The analysis techniques include atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma emission spectrometry (ICP), polarography (POL), neutron activation analysis (NAA), proton induced X- ray emission analysis (PIXE), photon induced X- ray fluorescence analysis (XRF) and isotope dilution mass spectrometry (IDMS).

For the analytical results obtained, the Grubbs's outlier test was first applied to determine acceptability of results. The certified value was the mean of the acceptable values, the uncertainty of the certified value for each element was estimated by 2 SD from the mean of the acceptable values and as 95 % confidence intervals for the individual method. For those elements that their analytical data were derived with less than three analytical techniques or in which greater deviations were found, only reference values were provided for information. All the certified results are presented in Tab.4.

4 CONCLUSION

A human hair reference material has been prepared with sufficient amount and successfully certified. Certified values of 17 elements and reference values for the other 13 elements, which include most of essential and toxic elements are provided. Concentrations of these elements are comparable with the typical data for normals in literature[5,6]. This human hair reference material is practically useful for analytical quality control, verifying the accuracy of analytical procedures, calibrating instruments in laboratories engaged in biomedical and environmental studies.

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