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Determination of total mercury in human hair and animal fur by combustion atomic absorption spectrometry

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Abstract

A commercially available mercury (Hg) analyzer based on sample combustion, gold amalgamation, and atomic absorption spectrometry (AAS) was evaluated for the direct determination of Hg in composites of human hair and individual samples of horse fur. Results for human hair reference material (NIES No. 13) were within the certified range. Analyses of "blind" samples from an international interlaboratory ($n > 16$) comparison study produced results within 1S.D. of the consensus means. Precision (%R.S.D.) was found to be <5% and total analyses time per sample was <10 min. This study demonstrated that analyzers based on combustion-AAS are suitable for wide-scale monitoring of Hg in human hair and animal fur.

Keywords: Mercury; Hair; Fur; Horse; Combustion; Atomic absorption spectrometry

1. Introduction

Methylmercury (MeHg) is a neurotoxic compound that is produced in the environment by biotic and abiotic methylation of inorganic mercury (Hg) and readily biomagnifies up the aquatic food chain [1]. Because MeHg is almost completely absorbed in the gastrointestinal tract and transported throughout the body; humans, particularly fetuses and young children and wildlife consuming fish are potentially affected [2]. Hair is useful to estimate an individual's exposure to MeHg because: (1) it captures temporal exposure history as Hg is incorporated into growing hair; (2) it is easily collected in a non-invasive manner; and (3) MeHg is concentrated in the hair at least 150–200 orders of magnitude higher than the corresponding concentrations in the blood [3]. Consequently, hair has been used in a variety of toxicological studies in both individuals and populations [4,5]. Likewise animal fur, here used to indicate hair from species other than humans, can be used to assess environmental Hg exposure [6].

Once collected, hair is generally washed and rinsed with acetone to remove external contamination. Then, a variety of methods are employed to digest the hair sample and measure the Hg in the resulting solution. In a recent report on results from a multiyear international interlaboratory comparison program for Hg in human hair, at least 15 different digestion schemes and five different detection techniques were used [3]. The most frequently used instrumental techniques were cold-vapor atomic absorption spectroscopy (CV-AAS), cold-vapor atomic fluorescence spectroscopy (CV-AFS), and inductively coupled plasma mass spectrometry (ICP-MS). All of the above-mentioned analytical techniques involved, to some extent, are time-consuming and potentially contaminating sample pretreatment.

An alternative approach which can measure total Hg directly is available in commercial instrumentation. The AMA-254 and the DMA-80 are two technically similar models manufactured by Leco Corporation (St. Joseph, MI, USA) and Milestone Inc. (Monroe, CT, USA), respectively. These techniques integrate sample combustion, pre-concentration of Hg by amalgamation with gold, and atomic absorption spectrometry (AAS). The advantages are: (1) no sample pretreatment is needed; (2) a rapid technique that essentially eliminates reagent waste; and (3) lower potential for contamination. The cost for the instru-

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ment is approximately $30,000 (US) and the manufacturers estimate a total of at least 700 instruments world-wide.

Although these analyzers are certainly being used for determination of Hg in a variety of matrices, there is little information in the peer-reviewed literature evaluating combustion-AAS performance. Several groups have published on combustion-AAS application to fish [7–9], geological materials [10], waterfowl [11], and to assess Hg emissions from coal-fired power plants [12]. Another group used the AMA-254 to analyze mixtures of biological materials in order to model potential sources of uncertainty in trace atomic absorption measurements [13]. In this short article, we present results evaluating the performance of the combustion-AAS technique for hair and fur matrices.

2. Experimental

2.1. Overview of instrumentation

A schematic showing the features of the AMA-254 is given in Fig. 1. Samples are weighed to the nearest 0.1 mg in a nickel boat which is automatically introduced into the instrument's quartz combustion tube. The instrument self-seals and oxygen begins flowing over the sample at a rate of ~200 mL min^{-1}. The sample combusts as the temperature is raised to ~550 °C for a pre-specified period. Gaseous combustion products are carried through a Mn_3O_4/CaO-based catalyst (kept at 750 °C), where oxidation is completed; different chemical forms (species) of Hg are converted to elemental Hg vapor; and sulfur oxides, nitrogen oxides, and halogens are trapped. Elemental mercury (Hg^0) and other decomposition products are carried to a tube containing gold-coated sand. There, Hg^0 is selectively trapped (forms an amalgam with gold) while other products are flushed out of the system. Later in the cycle, the trap is rapidly heated to ~700 °C and Hg^0 vapor is carried in a pulse through a spectrophotometer. Because there are two measuring cells (Fig. 1), the instrument can be calibrated over two ranges (typically, 0.05–40 and 40–500 ng of Hg). The Hg^0 concentration is then calculated by the software based on the absorbance measured at 253.7 nm and the weight of the sample.

2.2. Samples

Composites of human hair were obtained from Health Canada as part of a round-robin quality assurance analytical program for Hg in human hair [3]. Horse fur samples were collected from a population of wild horses from southern Nevada (Oliver Ranch near Red Rock National Recreation Area, Las Vegas) and domestic horses from Fallon in northern Nevada. The wild horses were rounded up for evaluation and treatment due to their poor health conditions resulting from drought related decreased food sources. The domestic horse hair samples were collected as part of a separate study investigating potential environmental links to a recent childhood leukemia cluster. Fur samples were cut from the tail of the horse and placed in plastic zip-lock bags until analysis. At the laboratory, fur samples were rinsed with

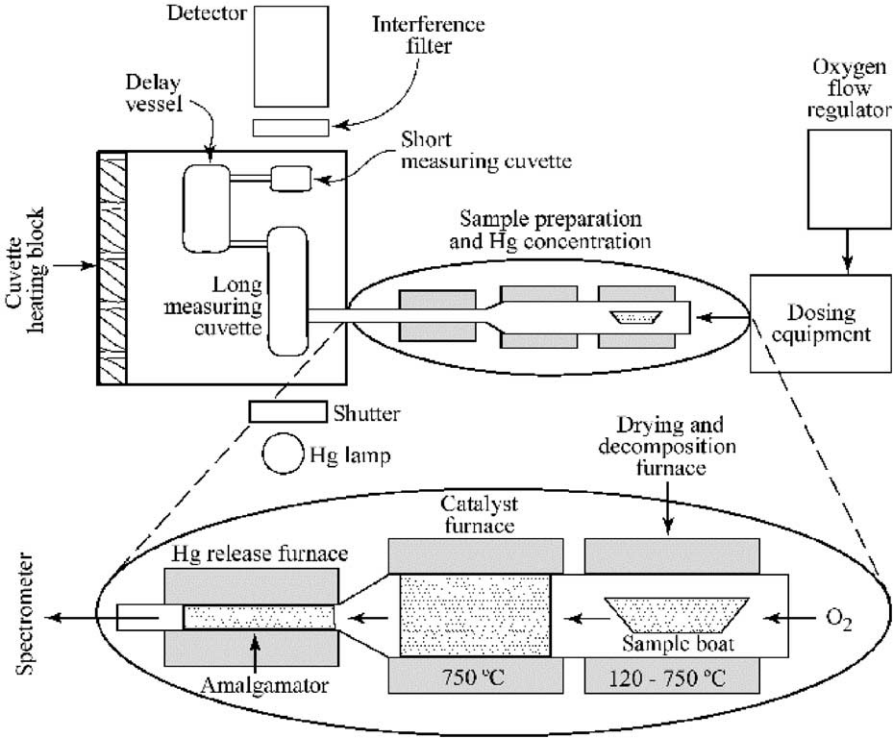

Fig. 1. Schematic of the combustion-AAS Hg analyzer.

deionized water (>18 MΩ), followed by a wash with high purity acetone to remove external contamination and then were allowed to air dry in a laminar flow hood.

2.3. Analysis and quality assurance

The instrument was calibrated with a NIST-traceable Hg standard solution (AccuTrace Single Element Standard; AccuStandard Inc., New Haven, CT, USA). Calibration checks using certified reference materials, including human hair (NIES No. 13), oyster tissue (NIST 1566a), and dogfish muscle (DORM-2), were performed at the beginning and end of each set of samples (typically, 10) to verify that the instrument remained calibrated during the course of the study.

Approximately 2–20 mg of hair or 20–100 mg of fur was weighed into combustion boats for analysis. Instrumental operating times for the drying, combustion, and waiting (post-combustion flushing) periods were 30, 150, and 45 s, respectively. Total analysis time was <10 min per sample. Blanks (i.e., an empty sample boat) were analyzed periodically (particularly after samples containing a relatively high amount of Hg) to confirm that Hg was not being carried over between samples. Blank readings were typically <0.003 absorbance units corresponding to <0.1 ng of Hg. The precision was generally less than 5% relative percent difference (RPD) for human hair and 10% for wild-horse fur, which contained over an order of magnitude less Hg.

3. Results and discussion

3.1. Human hair

The combustion-AAS analyzer results were in agreement with the round-robin consensus mean results (Table 1). Recoveries relative to the consensus mean averaged 100.7 ± 4.2% and ranged from 92.4 to 110.4%. In addition, results (mean, $4.55 \pm 0.04\ \mu g\ g^{-1}$; $n = 3$) for human hair reference material NIES 13 (Japan) were within the certified range ($4.42 \pm 0.20\ \mu g\ g^{-1}$). Since MeHg accounts for 86% of the total Hg in the reference material, these results demonstrate that the analyzer accurately measures this form of Hg in the total Hg measurement. Moreover, the results indicate that the high sulfur content of the hair matrix did interfere with the analyses (i.e., the SO_2 trap was not overwhelmed during combustion). Thus, this CRM can be used as a calibration check or even to calibrate the instrument for human hair analyses using varying amounts of the hair.

The precision of the technique was similar to other techniques [3] with relative standard deviation (R.S.D.) averaging about 5% (range, 0.78–9.30%). The instrumental detection limit (3 sigma criteria) was previously determined at 0.02 ng total Hg [9]. This corresponds to ~4.5 μg of hair (using the average value for the NIES composite human hair reference material). Since individual hair can weigh significantly more than this, it is theoretically possible to subdivide and analyze a single hair strand to gain temporal Hg exposure information. But due to difficulty in handling and accurately weighing such small fibers, the usefulness of that approach is questionable. It is far simpler to obtain multiple hair strands noting the position relative to the scalp and then to cut them into 1 cm lengths (~1 month worth of growth) for analyses.

3.2. Horse fur

Compared to the composite human hair samples, the total Hg concentrations in the horse fur were much lower (Table 2), reflecting the different diets (i.e., Hg uptakes) between the species. Humans are mostly omnivorous and gen-

Table 1
Total mercury in human hair by combustion-AAS vs. other methodology

Sample	This study mean ($\mu g\ g^{-1}$)	n	S.D.	R.S.D.	Consensus mean ± 1S.D. ($\mu g\ g^{-1}$)[a] (n)	Recovery (%)
1998-1-1	7.00	7	0.46	6.56	6.9 ± 1.4 (22)	101.4
1998-1-2	10.72	5	0.91	8.51	10.7 ± 2.2 (22)	100.2
1998-1-3	13.26	11	1.08	8.18	13.7 ± 3.0 (22)	96.8
1998-2-1	15.09	7	0.83	5.48	14.9 ± 1.5 (19)	101.3
1998-2-2	10.20	7	0.91	8.92	10.2 ± 2.1 (19)	100.0
2000-2-1	3.79	3	0.35	9.30	4.1 ± 0.3 (5)	92.4
2000-2-2	4.00	9	0.10	2.45	4.2 ± 0.4 (5)	95.2
2000-2-3	16.33	9	0.65	3.99	15.9 ± 1.0 (5)	102.7
2002-1-1	10.27	3	0.60	5.86	9.8 ± 0.5 (16)	104.8
2002-1-2	14.22	3	0.40	3.11	14.2 ± 0.5 (16)	100.1
2002-1-3	9.21	3	0.60	6.44	9.6 ± 0.4 (16)	95.9
2002-2-1	4.93	3	0.13	2.67	5.0 ± 0.4 (18)	98.6
2002-2-2	2.08	3	0.07	3.38	2.0 ± 0.4 (22)	103.9
2002-2-3	5.10	3	0.10	2.05	5.0 ± 0.4 (18)	101.9
2003-2-1	7.73	5	0.06	0.78	7.4 ± 0.5 (19)	104.5
2003-2-2	11.77	5	0.35	2.97	11.6 ± 1.0 (20)	101.5
2003-2-3	5.3	5	0.21	3.96	4.8 ± 0.5 (20)	110.4

[a] Data for 1990–2000 is the consensus mean from a round-robin study [3]. Data for 2002–2003 is the mean from the "mercury in hair" interlaboratory comparison program, FNIHB, Health Canada. S.D.: standard deviation and R.S.D.: relative standard deviation.

Table 2
Mercury in horse fur from Las Vegas and Fallon, Nevada

Las Vegas					Fallon			
Horse ID	Hg (ng g^{-1})	Sex	Age (years)	Condition[a]	Horse ID	Hg (ng g^{-1})	Sex	Age (years)
1	61.9	m	0.2	3	1	127	f	7
2	31.1	f	2	2	2	45.1	f	4
3	86.7	m	16	2	3	85.2	f	2
4	78.6	f	1	3	4	45.7[b]	m	3
5	96.7	f	4	2	5	126	m	16
6	77.8	f	4	4	6	112[b]	m	1
7	60.6	m	9	3	7	55.5	m	28
8	66.8	f	3	3	8	50.6	m	5
9	75.7	f	3	4	9	68.7	m	6
10	64.6	m	4	4	10	42.7	m	16
11	81.8	m	12	3	11	26.9[b]	m	11
12	60.6[b]	m	7	3	12	55.9	m	24
13	64.4	m	11	3	13	40.9	m	25
14	73.9	m	4	3	–	–	–	–
15	103	f	15	2	–	–	–	–
16	82.6	m	9	3	–	–	–	–
17	88.7	f	1	2	–	–	–	–
Mean	73.8	–	6.2	3	–	67.8	–	11.4
Median	75.7	–	4.0	3	–	55.5	–	7.0
S.D.	16.7	–	5.0	1	–	33.8	–	9.5

[a] 1: near death and 5: ideal.
[b] Duplicate mean (mean relative percent difference = 5.7 and range = 1.3–15.2).

erally include fish containing relatively high levels of Hg in their diet, whereas horses are primarily herbivores. There were poor correlations for total Hg concentrations with horse age, sex, and health condition. This was not surprising considering that the fur probably only reflects the horse's exposure to Hg over the prior few months and the diet of the adult horses would be similar. No statistical differences between the population of horses from northern Nevada (domestic) and southern Nevada (wild) were observed. Replicate measurements ($n = 4$) provided similar results with RPD values of 1.3, 2.6, 3.7, and 15.2%.

4. Conclusions

The combustion-AAS instrument is an affordable and accurate alternative technique for measuring total Hg concentration in human hair and animal fur. Compared with other standard analytical techniques such as CV-AAS, CV-AFS, and ICP-MS, the system reduced the likelihood of contamination or loss from volatilization during multiple sample preparation steps. The instrument can be equipped with an autosampler to increase productivity to as high as 15 samples per hour making it useful for laboratories that analyze large numbers of samples of hair for Hg. Due to the non-invasive nature of hair sampling, this technique has application for threatened or endangered species as well as human cohort studies.

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