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Determination of Iodine in Low Mass Human Hair Samples by Inductively Coupled Plasma Mass Spectrometry

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As an essential trace element, iodine plays a crucial role in human metabolic processes. It is a major component of the thyroid hormones thyroxin (T4) and iodothyronine (T3), which are necessary for proper brain development and function (Hollowell et al. 1998). When dietary requirements for iodine are not met thyroid hormone synthesis is impaired, resulting in a series of developmental and functional problems referred to collectively as IDD, or iodine deficiency disorders (WHO 2004). Although iodine deficiency can adversely impact human health at all stages of life, it can be especially devastating to a developing fetus or newborn, causing brain damage, irreversible mental retardation, and other growth defects (Utiger 1999, 2006).

People living in areas with low environmental levels of iodine are more likely to develop IDD. Approximately one-third of the global population lives in such areas, including some of the large, industrialized countries of Europe (Dunn 1998; Jahreis et al. 2001; Bader et al. 2005). Foodstuff crops harvested from soil with low iodine content, or from soil with a composition unfavorable for botanical iodine bioavailability, do not provide adequate amounts of this trace element when consumed (Chandrajith et al. 2005). In addition, because marine seafood products are an important natural source of iodine in the human diet, populations that do not have ready access to these foodstuffs must find alternate iodine sources (Julshamn et al. 2001).

The serious health consequences of IDD, coupled with unequal and variable environmental iodine distributions, have fueled the demand for analytical methods capable of determining this element in a variety of matrices. Recently, inductively coupled plasma mass-spectrometry (ICP–MS) has become a popular technique for iodine measurements of foodstuff matrices because of its sensitivity and relative freedom from matrix interferences for the ^{127}I isotope (Knapp et al. 1998). The technique has also been applied to determine the concentration of iodine in human urine and in nominal 200 mg hair samples following acid digestion (Puchyr et al. 1998; Caldwell et al. 2003).

The primary goal of this investigation was to develop and demonstrate a method of determining the iodine concentration in very small (less than 25 mg) human hair samples by ICP–MS. Prior to analysis, samples were subjected to an alkaline extraction procedure to prevent the loss of volatile iodine species. The methodology was then applied to provide analytical support to a trace element and autism investigation. A biological marker is currently not available for autism, and diagnosis is based solely on observation of symptomatic behaviors (Adams et al. 2006). The developed analytical method would provide a valuable tool for investigators concerned with identifying iodine as an environmental trigger for autism spectral disorders and other related conditions. In addition, the low nominal mass could allow for the analysis of discrete hair segments. At present, a routine ICP–MS method for the determination of iodine in low-mass hair samples is not available.

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Materials and Methods

Hair study samples from an autism and environmental trace element investigation were received in the blind from by Professor James B. Adams (Arizona State University, Tempe, Arizona). Because samples were collected several years prior to the investigation's onset, the amount of hair available for testing was limited. In addition, a suite of other elements requiring different preparation techniques was also determined, further limiting the available hair. Sample preparation and analysis parameters were therefore chosen to maximize sensitivity for the determination of iodine in samples with a nominal mass of 25 mg or less.

The concentration of iodine was measured in approximately 60 study samples in several analytical batches. Prior to testing, samples were transferred to vials and were cleaned with acetone to remove external contamination using a procedure described in detail elsewhere (Pellizzari et al. 1999). Although some authors have reported that hair may be susceptible to iodine loss from washing, all samples were treated in the same manner, so results are reflective of iodine excretion (Zareba et al. 1995; Adams et al. 2006).

A nominal mass (25 mg) of each cleaned sample was transferred to a disposable 50-mL polypropylene tube (DigiTUBE, SCP Science). New tubes were used to minimize the potential for iodine memory effects from previously prepared samples (Knapp et al. 1998). Aliquots (2 mL each) of aqueous, iodine-free, 25% (w/w) tetramethylammonium hydroxide (TMAH, Electronic Grade, Alfa Aesar) and 18 MΩ deionized water (DI H_2O, Pure Water Solutions) were added, and the tubes were tightly capped. Samples were extracted in a DigiPREP MS block (SCP Science) by bringing the temperature to 80°C and holding for 4 h.

After digestion, a 10 mL aliquot of 0.1% (w/w) sodium hydroxide (NaOH, prepared by dilution of Certified Grade 50% (w/w) solution from Fisher Scientific with DI H_2O) was added, and samples were brought to a final 20 mL volume with DI H_2O, capped, and mixed by vortex action. Extracts were passed through 0.45-µm syringe filters (PALL Corporation) into new low-density polyethylene 30-mL bottles and stored at refrigerator temperature (2–8°C) until analysis. Throughout the extraction, alkaline conditions were maintained to prevent the loss of volatile iodine species which can form under acidic conditions (Julshamn et al. 2001).

Quality control samples were prepared to monitor method performance. Method blanks were extraction reagents processed with the samples. Method controls were method blanks fortified with iodine (NIST-traceable I 10 µg/mL, High Purity Standards) to contain 1.00 µg I/mL. Although a hair reference material with a certified iodine concentration was not identified, aliquots of a hair material (GBW 09101, The People's Republic of China) were processed because an information iodine value was provided. Aliquots of GBW 09101 were also fortified to contain nominal 5.00 or 10.0 µg I/mL to assess recovery. Finally, aliquots of a standard reference material with a certified iodine concentration (SRM 1549, non-fat milk powder, NIST) were prepared.

Table 1 ICP–MS configuration and operating parameters

Parameter	Value/configuration
Spray chamber	Polypropylene cyclonic
Spray chamber chiller	ESI PC^3 Peltier cooled, 2°C
Nebulizer	Concentric
Torch	Demountable with sapphire injector
Interface	HPI sampler and skimmer cones
Analyte isotope	^{127}I
Autosampler	CETAC ASX-500 series
Autosampler tubes	Falcon 14 mL polystyrene
Internal standard isotope	^{121}Sb
Uptake time	22 s
Rinse time	85 s
Dwell time	20,000 µs
Data collection mode	Peak jumping
Forward power	1,400 W
Ion lens settings	Tuned for optimal performance daily
Scans/analysis	3

All iodine data were collected using a Thermo X7 ICP-MS. The instrument configuration and operating parameters, presented in Table 1, were selected to maximize sensitivity and avoid sample memory effects. Between sample analyses, a rinse solution containing 1.25% (w/w) TMAH was used to flush the system. Because of the low study sample mass and matrix, extracts did contain high iodine concentrations and memory effects were not observed.

For each analysis, the ICP–MS was calibrated with nine external standards and a calibration blank. Standards ranged from 0.100 to 50.0 ng I/mL and were prepared in a matrix that approximated the samples [2.5% (w/w) TMAH and 0.05% (w/w) NaOH in DI H_2O]. Regressions included at least eight standards and the blank, had a correlation coefficient greater than 0.99, error less than 20% for the low standard, and error less than 10% for all other standards. An internal standard (NIST-traceable antimony, Sb, 10 µg/mL, High Purity Standards) was added to all standards and samples prior to analysis at 10 ng Sb/mL. The calibration blank and a mid-level standard were analyzed immediately after calibration, after a maximum of ten samples, and at the end of the analysis as a continuing check. For data bracketed by continuing checks to be valid,

Table 2 Quality control (QC) sample data

QC description	Replicates	Mean [I]	Precision (%RSD)	Mean recovery (%)[a]
GBW 09101 human hair CRM	18	1.11 μg/g	4.6	126
GBW 09101 5.00 ng/mL spike	3	4.74 ng/mL	2.1	94.8
GBW 09101 10.0 ng/mL spike	2	9.99 ng/mL	0	99.9
NIST 1549 non-fat milk powder SRM	9	3.24 μg/g	6.2	95.9

[a] Mean recovery calculated as: (mean [I]/nominal [I]) × 100; where nominal [I] = 0.875 μg/g for GBW 09101, 5.00 and 10.0 ng/mL for GBW 09101 spikes, and 3.38 μg/g for NIST 1549

the determined concentrations of the standards were required to be within ±10% of their nominal value and the iodine level for the calibration blanks was required to be less than 0.050 ng/mL.

Results and Discussion

For this investigation, the limit of quantitation (LOQ) was conservatively defined on each day of analysis as the lowest calibration standard included in the regression. For all study sample analyses, the 0.100 ng I/mL standard (equivalent to 0.08 μg I/g after correcting for nominal 20-mL extract volume, 25-mg mass, and unit conversion factor) met regression acceptance criteria and was defined as the LOQ. The lowest iodine concentration measured in the study sample extracts was 0.364 ng/mL. This value comfortably exceeded the defined LOQ, and indicated that the method had adequate sensitivity for the determination of iodine in low mass hair samples.

Twenty method blanks were processed with the study sample batches. The determined iodine concentrations in the method blanks ranged from <LOQ to 0.139 ng I/mL, and only the highest value exceeded the LOQ. Because these data indicated that the background analyte contribution from the reagents and the procedure was not significant, extraction method blank averages were used to correct study sample and quality control sample concentrations throughout this investigation.

The average iodine recovery for the 12 method control samples that were processed with the hair study samples was 93.8% of the nominal 1.00 ng I/mL concentration, with a percent relative standard deviation (RSD%) of 2.1%. These data indicate accurate and precise analyte recovery in the absence of sample matrix. They also indicate that volatile iodine species were not formed and then lost during sample preparation and analysis procedures.

Data from the eighteen GBW 09101 certified reference material (CRM) hair samples that were processed with the study samples are presented in Table 2. A certified concentration of iodine was not listed on the material's certificate of analysis, but an information value of 0.875 μg/g was provided and was used to estimate analyte recovery. Although the mean estimated recovery for these samples exceeded 100%, a high-level of precision was observed (RSD% less than 5%) throughout the investigation.

Five additional aliquots of the GBW 09101 CRM were fortified with iodine to assess analyte recovery in the presence of the hair extract matrix. The mean iodine concentration in these matrix spikes, prepared at nominal 5.00 and 10.0 ng/mL levels (equivalent to 4.00 and 8.00 μg/g after accounting for nominal 20 mL extract volume, 25-mg mass, and unit conversion factor), was corrected by subtracting the average recovery of unfortified GBW 09101 CRM samples processed with the same extraction batch. The accuracy and precision data, presented in Table 2, indicate that iodine signal suppression or enhancement from the extract matrix was not observed at either nominal spike concentration.

Because a hair CRM with a certified iodine concentration was not available, nine aliquots of a non-fat milk powder standard reference material (SRM) were also prepared to further assess method performance. The certified iodine concentration for this material (NIST SRM 1549) is 3.38 ± 0.02 μg/g. Non-fat milk powder SRM data, presented in Table 2, indicate a high-level of accuracy and precision for the sample preparation and analysis procedures.

This investigation's objectives were to demonstrate a method of determining the iodine concentration in very small human hair samples and apply the method to provide analytical support to a trace element and autism investigation. Data from the quality control samples processed during this study indicate that the method could be employed to collect accurate, precise iodine data from study samples. The described sample preparation procedure prevented the loss of volatile iodine species, and the instrument and labware memory effects often encountered when measuring this element were not observed. In addition, matrix suppression or enhancement was not observed, allowing for instrument calibration with external calibration standards. The applied method had adequate sensitivity to determine the iodine levels, even with the conservatively defined LOQ.

Because urinary iodine concentrations directly reflect dietary intake, urine is the primary biomarker used to

assess iodine nutrition (Utiger 2006). As a result, validated methods for the determination of iodine in human hair are presently not available. For this investigation, it was necessary to optimize analytical performance for the determination of iodine in hair samples with limited available mass. The employed alkaline sample preparation procedure minimized the loss of volatile iodine species that may have occurred from an acid digestion procedure for the determination of iodine and other trace elements in hair described elsewhere (Puchyr et al. 1998). In addition, the required 25 mg nominal mass for the current investigation was less than the nominal 200 mg mass required by this alternate analytical approach. Although several instrumental modes of analysis are capable of determining the concentration of iodine in biological matrices, the selectivity and sensitivity offered by ICP–MS made it an excellent choice for the current application. Other instrumental techniques, such as X-ray fluorescence and ion selective electrodes do not offer adequate sensitivity, and neutron activation analysis is only available to a few laboratories (Knapp et al. 1998).

Determined iodine concentrations from the blinded hair autism study samples ranged from 0.483 to 15.9 μg/g, with all of the measured values falling comfortably within the instrument calibration range. Of the 64 study samples, determined iodine concentrations were less than 1.00 μg/g for 10 samples, 1.00–1.99 μg/g for 20 samples, 2.00–2.99 μg/g for 16 samples, 3.00–4.99 μg/g for 8 samples, 5.00–9.99 μg/g for 9 samples, and greater than 10.0 μg/g for 1 sample. These data may prove to be important, because the mean concentration of iodine in the hair of children with autism has been reported to be lower than in the hair from control group children (Adams et al. 2006).

Because the health implications of iodine deficiency are serious, population urinary iodine excretion measurements are often collected to assess nutritional status. In the United States, the median urinary iodine concentration decreased by more than 50% between the National Health and Nutritional Examination Surveys (NHANES) conducted in 1971–1974 and 1998–1994. Of particular importance were the percentages of pregnant women and women of child bearing age with low urinary iodine concentrations, which increased by nearly seven times and four times between the NHANES studies, respectively (Hollowell et al. 1998). During the same time period, a large increase in the incidence of autism was observed (Adams et al. 2006). These trends make further iodine exposure investigations seem likely in the coming years. The described method provides researchers concerned with monitoring biological iodine levels another tool to facilitate their investigations.

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