Sample Preparation for Quantitation of Tritium by Accelerator Mass Spectrometry

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The capability to prepare samples accurately and reproducibly for analysis of tritium (3H) content by accelerator mass spectrometry (AMS) greatly facilitates isotopic tracer studies in which attomole levels of 3H can be measured in milligram-sized samples. A method has been developed to convert the hydrogen of organic samples to a solid, titanium hydride, which can be analyzed by AMS. Using a two-step process, the sample is first oxidized to carbon dioxide and water. In the second step, the water is transferred within a heated manifold into a quartz tube, reduced to hydrogen gas using zinc, and reacted with titanium powder. The 3H/H ratio of the titanium hydride is measured by AMS and normalized to standards whose ratios were determined by decay counting to calculate the amount of 3H in the original sample. Water, organic compounds, and biological samples with 3H activities measured by liquid scintillation counting were utilized to develop and validate the method. The 3H/H ratios were quantified in samples that spanned 5 orders of magnitude, from 10−10 to 10−15, with a detection limit of 3.0 × 10−15, which is equivalent to 0.02 dpm tritium/mg of material. Samples smaller than 2 mg were analyzed following addition of 2 mg of a tritium-free-hydrogen carrier. Preparation of organic standards containing both 14C and 3H in 2-mg organic samples demonstrated that this sample preparation methodology can also be applied to quantify both of these isotopes from a single sample.

Accelerator mass spectrometry (AMS) is a nuclear physics analytical technique with high sensitivity for quantitation of rare, long-lived isotopes. It was originally developed for carbon dating, but has found many applications in the biological and earth sciences.1 In biology, AMS facilitates radiotracer studies by allowing administration of low chemical or radiological doses of compounds, which are physiologically, pharmacologically, or environmentally relevant.2–5 In earth science, AMS is a valuable tool for radiocarbon dating and tracer investigations of complex natural systems.6

Biological AMS studies have been successfully conducted with 14C-labeled tracers. The sample preparation methods are well established and attomole (10−18 mol) quantitation of 14C is possible in milligram-sized samples.6,7 Tritium is another widely used radioisotope in biological tracer experiments, and AMS provides significant advantages for quantitation of 3H over traditional methods, such as liquid scintillation counting (LSC) and helium-3 in-growth mass spectrometry. LSC detects the energy released by 3H during radioactive decay, and helium-3 in-growth mass spectrometry measures subsequent accumulation of this decay product. The half-life of tritium is 12.3 y; hence, only ~0.01% of the 3H will decay during a 24-h period.8 Helium-3 in-growth mass spectrometry is a highly sensitive technique, but the sample size needed is large (e.g., >45 g for water) and analysis times are long (90 days).9 In contrast, AMS counts individual 3H atoms from each sample. This direct counting of 3H has resulted in a 100–1000-fold improvement in sensitivity for milligram-sized samples compared to the traditional methods, while reducing instrument analysis time to the order of minutes.10,11

The success of this technique depends on the development of a sample preparation method that is reproducible and that will allow accurate quantitation of 3H in different types of biological sample matrices, such as DNA, protein, tissue, and urine. The method we have developed for 3H AMS is based on well-established sample preparation procedures used for measurement of deuterium-to-hydrogen ratios (D/H).12–14 In stable-hydrogen isotope analysis, hydrogen gas is produced by reacting water on

[References]

zinc, and the gas is analyzed in a stable isotope mass spectrometer. However, for $^3$H AMS, the hydrogen must be in a solid form, and therefore, the D/H sample preparation methodology was expanded to capture the hydrogen in the form of a solid, titanium hydride. A cesium sputter ion source is used to ionize the sample, and the $^3$H/$^1$H ratio is measured.6,15 A description of the $^3$H AMS sample preparation procedure is presented, along with results from $^3$H analyses of water and organic samples. Also, preparation of samples containing both $^3$H and $^{14}$C is described for application in dual-isotope labeling studies.

**EXPERIMENTAL SECTION**

**Reagents.** Tributyrin (glycerol tributyrate) was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). Zinc alloy ("Indiana zinc") was purchased from the Biogeochemical Laboratories, Department of Geological Sciences, Indiana University. Copper(II) oxide wire, titanium powder, 99%–325 mesh, titanium hydride, bovine serum albumin (BSA), and deoxyribonucleic acid (DNA) were purchased from Sigma-Aldrich (St. Louis, MO). Solvable tissue solubilizer was from NEN Research Products (Boston, MA), and Hionic-Fluor NCSII scintillation cocktail (used with solubilized samples) was obtained from Packard Instrument Co. (Meriden, CT). Universol scintillation cocktail (for aqueous samples) and methanol (HPLC grade) were from Sigma-Aldrich.

Tritiated water ($^3$H$_2$O) was obtained from the Lawrence Livermore National Laboratory tritium facility. Unquenched LSC standards (Pharmacia, Peapack, NJ) for $^3$H, $^{14}$C, and blanks, calibrated against NBS reference standards, were used for LSC calibration. [ring-G-$^3$H]-2-Amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) used to spike organic samples was purchased from NCI Radiochemical Repository, ChemSyn Science Laboratories (Lenexa, KS). [ring-C]-$^{14}$CPhIP was purchased from Toronto Research Chemicals (Toronto, Canada). All radiolabeled chemicals were purified to >99% radiopurity and chemical purity prior to use, as previously described.16

**Caution:** PhIP is carcinogenic to rodents and should be handled with care. Finely powdered metals and hydrides can be flammable in air or reactive to water vapor.

**Preparation of Materials.** All quartz tubes were heated at 900 °C for 2 h to prevent potential contamination of samples with $^1$H and $^3$H from any organic material and surface-adsorbed water. Tubes were handled with gloves and stored under vacuum until ready for use. Copper(II) oxide wire was baked at 500 °C for 2 h to eliminate any organic material, titanium, and copper oxide. These were stored in a desiccator and opened in a glovebox filled with nitrogen gas.

**Description of Manifold.** A stainless steel manifold was built to prepare $^3$H samples for AMS analysis (Figure 1). The manifold has four sample ports each consisting of a high-vacuum B Series bellows sealed valve with a rotary handle (Nupro Co., Willoughby, OH) (valves 1–4), a modified valve to break the oxidation tube, and a 1/2-in. Ultra-Torr union fitting with a 3/8-in. reducer (Swagelok Co., M acedonia, OH) to attach the water reaction tubes.

![Figure 1. Diagram of the sample manifold for $^3$H AMS preparation.](image)

![Figure 2. Flowchart of the steps in the preparation of samples for $^3$H AMS analysis.](image)

The high-vacuum valves used for breaking the oxidation tubes were modified by drilling the core to 1/2-in. o.d. so that the valve stem cracked the tubes by rotating the handle. Three other valves (a–c) isolate sections of the manifold when samples require simultaneous collection of both water for $^3$H analysis and carbon dioxide for $^{14}$C analysis. The lower portion of the manifold is heated with standard strip heating elements controlled by Watlow Series 93 temperature controllers (Therm-X, Hayward, CA) and insulated to maintain a constant temperature of 120 ± 1 °C. The manifold is evacuated with a Minuteman Turbo-V70 LP dry pumping system (Varian, Inc., Lexington, MA), and the vacuum is monitored in three areas with Granville-Phillips Series 375 Convectron gauges (Helix Technology Corp., Santa Clara, CA).

**Tritiated Water Sample Preparation for AMS.** Steps to prepare samples for $^3$H AMS are illustrated in Figure 2. Sample preparation involves a single step (step 2) that reduces water to hydrogen gas using zinc, followed by reaction of the hydrogen gas with titanium powder.16 Water samples were prepared from a stock solution containing (54 ± 0.1) × 10^4 dpm $^3$H/mL with a

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corresponding $^{3}$H/$^{1}$H ratio of $(760 \pm 12) \times 10^{-13}$. This solution was serially diluted to prepare four working standards. Triplicate 50- or 500-μL aliquots of the standards were counted by LSC for 10 min each using a Pharmacia Wallac 1410 liquid scintillation counter (Gaithersburg, MD), and the resulting dpm were converted to a mole/mole $^{3}$H/$^{1}$H ratio (Table 1).

The samples were prepared in the glovebox under flowing nitrogen gas to minimize contamination and provide a noncondensable gas atmosphere. Hydrogen gas was formed by reaction of water standards on 200 mg of granular zinc in a 9-mm o.d. quartz tube. A 2μL capillary micropipet containing the sample was dropped into the quartz tube, and 10 mg of titanium in a 6 mm o.d. × 50 mm borosilicate glass tube was then added to the tube. A septum cap was placed on the quartz tube, and the tube was put into a Dewar flask containing liquid nitrogen to keep the water frozen. The tubes were kept in liquid nitrogen, attached to the ports of the manifold, evacuated, and flame-sealed using an oxygen–acetylene torch. The sealed tubes were placed in a programmable box furnace (Ney Dental Inc., Bloomfield, CT) heated at a rate of 10.0 °C/min, held at 530 °C for 4 h, cooled at a rate of 2.0 °C/min, and then held at 400 °C for 6 h. Once the heating program finished, the tubes were left undisturbed until the furnace returned to room temperature. Sample preparation blanks (water samples containing background levels of $^{3}$H) were prepared using 2 μL of Greenland Ice Sheet Precipitation (GISP) water from deep ice cores.

**Preparation of Organic Samples for AMS.** Sample preparation for organic samples first involves oxidizing the sample to water and carbon dioxide (step 1 in Figure 2). Tributyrin, an organic, nonvolatile liquid (C15H30O2, 1.032 g/mL, M W = 302.4, 99% purity), was spiked with $[3$H$]$PhIP and used as an organic standard. A volume of 2 μL of standard in a capillary micropipet was added to a 6-mm-o.d. quartz tube and inserted into a 9-mm-o.d. quartz oxidation tube containing 200 mg of copper(II) oxide. The tube was evacuated, flame sealed, and placed in a programmable box furnace at 900 °C for 2 h.

Water from the oxidized sample was transferred to a 9-mm-o.d. quartz tube containing zinc and titanium, referred to as the reaction tube, using the heated manifold (step 2 in Figure 2). The sealed tube containing the oxidized sample was inserted into the manifold directly above the Ultra-Torr union. The Ultra-Torr union contains a wire mesh to prevent broken quartz from falling into the tubes below. The reaction tube was attached to the bottom of the Ultra-Torr union, evacuated, and heated with the oxygen–acetylene torch for 30 s to eliminate any adsorbed moisture.

**Table 1. Tritium (dpm/mL) and Calculated $^{3}$H/$^{1}$H Ratios of Serially Diluted Water Standards by LSC**

<table>
<thead>
<tr>
<th>sample size (μL)</th>
<th>dpm/mL ± SD$^{a}$</th>
<th>calculated $^{3}$H/$^{1}$H ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>$(54 \pm 0.1) \times 10^{4}$</td>
<td>$(760 \pm 12) \times 10^{-13}$</td>
</tr>
<tr>
<td>50</td>
<td>$(590 \pm 3) \times 10^{4}$</td>
<td>$(830 \pm 4) \times 10^{-14}$</td>
</tr>
<tr>
<td>50</td>
<td>$(610 \pm 23) \times 10^{3}$</td>
<td>$(850 \pm 3) \times 10^{-15}$</td>
</tr>
<tr>
<td>500</td>
<td>685 ± 1</td>
<td>$(96 \pm 2) \times 10^{-15}$</td>
</tr>
<tr>
<td>500</td>
<td>69 ± 4</td>
<td>$(97 \pm 6) \times 10^{-16}$</td>
</tr>
<tr>
<td>500$^{b}$</td>
<td>10 ± 1</td>
<td>$(14 \pm 2) \times 10^{-16}$</td>
</tr>
</tbody>
</table>

$^{a}$ Average of three samples. $^{b}$ GISP blank.

**Table 2. Tritium (dpm/mL) and Calculated $^{3}$H/$^{1}$H Ratios of Serially Diluted $[3$H$]$PhIP Standards in Tributyrin by LSC**

<table>
<thead>
<tr>
<th>sample size (μL)</th>
<th>dpm/mL ± SD$^{a}$</th>
<th>calculated $^{3}$H/$^{1}$H ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>$(55 \pm 2) \times 10^{4}$</td>
<td>$(96 \pm 4) \times 10^{-12}$</td>
</tr>
<tr>
<td>50</td>
<td>$(53 \pm 3) \times 10^{4}$</td>
<td>$(94 \pm 5) \times 10^{-13}$</td>
</tr>
<tr>
<td>50</td>
<td>$(44 \pm 2) \times 10^{3}$</td>
<td>$(80 \pm 5) \times 10^{-14}$</td>
</tr>
<tr>
<td>500</td>
<td>496 ± 11</td>
<td>$(88 \pm 2) \times 10^{-15}$</td>
</tr>
<tr>
<td>500</td>
<td>47 ± 7</td>
<td>$(84 \pm 1) \times 10^{-15}$</td>
</tr>
<tr>
<td>1000</td>
<td>30 ± 3</td>
<td>$(54 \pm 6) \times 10^{-16}$</td>
</tr>
<tr>
<td>1000$^{b}$</td>
<td>7 ± 2</td>
<td>$(13 \pm 4) \times 10^{-16}$</td>
</tr>
</tbody>
</table>

$^{a}$ Average of three samples. $^{b}$ Blank tributyrin.

Samples were allowed to degas for 2 min, the valves were then closed, and a Dewar flask containing liquid nitrogen was placed around the reaction tube. The tube containing the oxidized sample was cracked using the tube-breaking valve, and the oxidation products were collected in the reaction tube. After 3 min, a Dewar flask containing a dry ice–isopropyl alcohol slurry was placed around the reaction tube and the temperature was allowed to equilibrate for 2 min. The reaction tube was evacuated to remove all the noncondensable gases while retaining the frozen water, then switched back to liquid nitrogen, and sealed. The reaction tubes were placed in a programmable box furnace and heated as described above for the preparation of water samples.

Organic samples were prepared from a stock solution containing $(55 \pm 2) \times 10^{4}$ dpm $[3$H$]$PhIP diluted in 1.0 mL of tributyrin with a corresponding $^{3}$H/$^{1}$H ratio of $(96 \pm 4) \times 10^{-12}$. Five serially diluted standards were then used to assess the accuracy, precision, and limit of detection of the sample preparation method. The $^{3}$H/$^{1}$H ratios were calculated by decay counting 50–1000-μL (51–1020 mg) aliquots in triplicate for 10 min each (Table 2) and $^{1}$H moles for tributyrin were calculated using 8.6% w/w hydrogen (Table 3). For $^{3}$H AMS, six replicates containing 2.0 μL equivalent to 2.0 mg of each diluted standard were converted to titanium hydride and the $^{3}$H/$^{1}$H ratios measured and compared to the $^{3}$H/$^{1}$H ratios calculated by LSC.

**Biological Sample Matrixes.** The method was validated for different types of biological samples by spiking homogenized rat liver tissue, DNA (2 mg/mL in water), protein (4 mg/mL BSA in water), plasma, and urine samples with $[3$H$]$PhIP, and then comparing AMS data to the results from LSC. All samples for LSC were counted for 10 min.

The size used for AMS sample preparation was an amount that provided between 2 and 10 μL of water following oxidation. Six replicate samples containing 10 mg of wet rat liver tissue or 4 mg of BSA were added to replicate 6-mm-o.d. quartz tubes and freeze-dried overnight to remove unbound water. Samples were then

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oxidized and processed as described for the organic samples. Six replicate samples of 2 μL of urine and plasma were added to replicate 6-mm-o.d. quartz tubes and oxidized without drying.

As it was not possible to dry down sufficient DNA in a quartz tube for AMS, 500 μg of DNA was added to replicate 6-mm-o.d. quartz tubes, freeze-dried overnight, and oxidized with the addition of 2 μL of tributyrin as hydrogen carrier.

Six replicate LSC samples were prepared by adding 4 mg of DNA, 10 mg of BSA, or 100 mg of wet liver tissue to glass scintillation vials and freeze-dried for 16 h to remove unbound water. The DNA and BSA were redissolved in water, 10 mL of aqueous scintillation cocktail was added, and the samples were counted by LSC. Two milliliters of tissue solubilizer was added to vials containing the dried liver tissue, and the samples were left in a shaking water bath at 30 °C for 12 h. After solubilization was complete, 10 mL of Hionic-Fluor NCSII scintillation cocktail was added and the samples were counted. For urine and plasma, aqueous scintillation cocktail was added to 100-μL aliquots and counted.

The resulting 3H dpm values were converted to 3H/1H ratios based upon the percent (w/w) hydrogen of the organic sample (Table 3).

Hydrogen Analysis of Organic Samples. The percent (w/w) hydrogen was measured by hydrogen elemental analysis using an Elementar vario EL (Hanau, Germany). Five replicates of 2–6 mg of dry liver tissue, BSA, or calf thymus DNA were placed in five 8 × 5 mm tin capsules (Elemental Micronova) and measured for hydrogen content. The percent (w/w) hydrogen of water was used for urine and plasma.18

Simultaneous Preparation of Organically Bound 3H and 14C Samples for AMS. Separation of [3H]PhIP and [14C]PhIP from the same sample was demonstrated by oxidizing replicate 2-μL tributyrin samples containing 184 ± 13 dpm 3H/mg (3H/1H = (36 ± 3) × 10^-12) and 0.33 ± 0.02 dpm 14C/mg by LSC and cryogenically separating H2O from 14CO2. The tube containing the oxidized sample was cracked as described earlier, and the oxidation products were collected in the titanium and zinc reaction tube in liquid nitrogen with valves 1 and 2 closed (Figure 1). The reaction tube was then transferred into the dry ice–isopropl slurry and equilibrated for 2 min. The manifold valves a and b were closed and valves 1 and 2 were opened simultaneously, and the 14CO2 was collected in a reaction tube containing cobalt powder, zinc, and titanium to make graphite, as previously described.17 The transfer of CO2 was monitored with the Convec- tron pressure gauges, and valve b was reopened before sealing both reaction tubes. The manifold is designed to prepare an additional dual-labeled sample with valves 3, 4, and c. The 14C samples were measured using standard AMS methods.17

AMS Measurement and Statistics. Tritium AMS samples were counted three to six times depending on the counting precision, which was typically less than 5%. Each counting lasted for a maximum of 10 500 3H counts (a few seconds for samples with 3H/1H ratios of 10^-10–10^-12) to at least 5 min for lower activity samples. The 3H/1H ratios of unknowns were normalized to four nearest in time measurements of standards whose 3H/1H ratios were determined from LSC.

The 3H/1H ratios obtained for the organic and water standards by AMS represent the mean ± SD of six samples. The 3H/1H ratios calculated by LSC represent the mean ± SD of three samples. The AMS limit of detection (LOD) of the organic samples was calculated as the sum of the mean 3H/1H ratio of 2.0±0.014C/mg tributyrin blank samples (n = 12) and three times the SD of the mean. AMS and LSC measurements were compared in the dual-isotope labeling experiment with a t test using a 95% confidence level. These analyses were performed using the statistics package provided with Microsoft Excel 98. The coefficient of variation (CV) is defined as the precision or closeness of individual measurements of replicate samples and was expressed as a percent. The CV was calculated by dividing the SD by the mean value. Accuracy describes the closeness of the measured values to the expected value from LSC.

RESULTS AND DISCUSSION

We report a method to prepare water and organic samples for 3H analysis by AMS. Applications of this methodology include high-sensitivity quantitation of 3H in biological samples from 3H tracer studies and environmental 3H monitoring where sample size or analysis time is limiting.

Previous studies on atomolde detection of 3H in biological samples showed that a modified method for 3C sample preparation could be used to make titanium hydrate for AMS analysis.10,16 However, the intersample precision (CV) of several replicates of biological tissue was poor. For example, the variability of replicate liver tissues was 21% (unpublished data; Lawrence Livermore National Laboratory, 2000) and was reduced to less than 5% with the current method. Changes to the method included using 9-mm-o.d. quartz tubes instead of Pyrex tubes for all the steps because quartz is best suited for water recovery and Pyrex cannot be used above 560 °C, which is close to the temperature used for the water reduction step.13 Another change to the method was to transfer water using a stainless steel heated manifold with a modified valve to break the oxidation tubes, rather than using Tygon tubing and a plastic Y-tube used for transferring CO2 by the 3C method.17 The heated manifold is similar to the sample preparation method used for stable-hydrogen isotope analyses.14

The sample preparation method was validated by comparing measured 3H/1H ratios of serially diluted tritiated water and organic standards by AMS and ratios calculated by LSC (Table 4). To do this, more than 500 μL of sample was required for LSC.

Table 4. Measured AMS 3H/1H Ratios and Calculated 3H/1H Ratios from LSC of Serially Diluted [3H]PhIP Standards in Tributyrin

<table>
<thead>
<tr>
<th>LSC sample size (μL)</th>
<th>LSC a 3H/1H ratio ± SD</th>
<th>AMS sample size (μL)</th>
<th>AMSS a 3H/1H ratio ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>(96 ± 4) × 10^-12</td>
<td>2</td>
<td>(91 ± 6) × 10^-12</td>
</tr>
<tr>
<td>50</td>
<td>(94± 2) × 10^-13</td>
<td>2</td>
<td>(83 ± 4) × 10^-13</td>
</tr>
<tr>
<td>500</td>
<td>(80 ± 5) × 10^-14</td>
<td>2</td>
<td>(80 ± 3) × 10^-14</td>
</tr>
<tr>
<td>500</td>
<td>(88± 2) × 10^-15</td>
<td>2</td>
<td>(88± 8) × 10^-15</td>
</tr>
<tr>
<td>500</td>
<td>(8± 1) × 10^-15</td>
<td>2</td>
<td>(9 ± 1) × 10^-15</td>
</tr>
<tr>
<td>1000</td>
<td>(54 ± 6) × 10^-16</td>
<td>2</td>
<td>(44 ± 5) × 10^-16</td>
</tr>
<tr>
<td>5000</td>
<td>(13 ± 4) × 10^-16</td>
<td>2</td>
<td>(30 ± 2) × 10^-16</td>
</tr>
</tbody>
</table>

a LSC values are averages of triplicate samples. b AMS values are averages of six samples. c Tributyrin blanks.

Figure 3. Mean ± SD $^{3}$H/$^{1}$H ratios of serially diluted $[^{3}$H]$\text{PhIP}$ tributyrin samples analyzed by AMS ($n = 6$) and LSC ($n = 3$). A ratio of $1 \times 10^{-10}$ corresponds to 570 dpm/2 mg of tributyrin. The dashed line shows the LOD.

Figure 4. AMS measured average $^{3}$H/$^{1}$H ratios ± SD of samples containing 0.25, 0.5, 1.0, 2.0, 5.0, and 10.0 mg $[^{3}$H]$\text{PhIP}$ tributyrin ($n = 6$). Dashed line represents the expected $^{3}$H/$^{1}$H ratio ($9.6 \times 10^{-11}$).


analytical methods

AMS for quantifying low levels of $^3$H in small samples. However, in this study, the LOD is $3.0 \times 10^{-10}$ and $2.8 \times 10^{-10}$ background of the scintillation counter was 15%. The sample counts at these low ratios are close to the line shows the LOD.

The dynamic range of $^{3}$H/$^{1}$H ratios measured by AMS spanned almost 5 orders of magnitude ($9.1 \times 10^{-11}$ and $4.4 \times 10^{-15}$). A range was reported in an earlier study to be between $1 \times 10^{-10}$ and $2.8 \times 10^{-15}$ with an LOD of 0.15 dpm or 2.4 amol of $^{1}$H/mg. However, in this study, the LOD is $3.0 \times 10^{-15}$ and is 6-fold lower. This ratio is equivalent to 0.02 dpm or 0.32 amol of $^{1}$H/mg. Similar backgrounds were measured for the GISP water samples. These lower backgrounds were achieved by modifying the method as described earlier and by careful handling of materials used for the sample preparation.

Sample memory effects in the manifold caused by carryover of $^{1}$H to subsequent samples and from port to port were tested by measuring the difference in $^{3}$H/$^{1}$H ratio of blanks prepared before and after samples with a high $^{3}$H/$^{1}$H ratio. The products of an oxidized tributyrin blank were transferred in each port. The oxidized tributyrin standard with a $^{3}$H/$^{1}$H ratio of 9.6 $\times 10^{-11}$ was then placed in ports 1 and 3 and blanks were placed in ports 2 and 4. Finally, two sets of oxidized tributyrin blank samples were transferred using each port. The percent carryover was calculated from the differences in the $^{3}$H/$^{1}$H ratios of these samples measured by AMS. Sample memory across the manifold ports was less than 0.1% and subsequent samples in the same port was between 0.1 and 0.2%.

The effect of sample size on the measured $^{3}$H/$^{1}$H ratios was investigated as part of the method development. Different weights of a $[^{3}$H]$\text{PhIP}$ tributyrin standard (mean $^{3}$H/$^{1}$H ratio of $(96 \pm 4) \times 10^{-12}$) were processed to determine how different sample sizes affect the $^{3}$H/$^{1}$H ratio measured by AMS. $^{3}$H/$^{1}$H ratios of samples ($n = 6$ per group) containing 0.25, 0.5, 1.0, 2.0, 5.0, and 10.0 mg of the standard were measured by AMS. All samples were prepared from a 40 mg/mL $[^{3}$H]$\text{PhIP}$ tributyrin stock solution in methanol, dried in a vacuum centrifuge for 2.0 h, oxidized, and processed for AMS, as described earlier for the organic samples.

Measured $^{3}$H/$^{1}$H ratios for organic samples ranging in size between 0.25 and 10.0 mg are shown in Figure 4. The expected $^{3}$H/$^{1}$H ratio was $9.6 \times 10^{-11}$. Sample sizes between 1.0 and 10.0 mg agree, within the uncertainty, with the expected ratio. However, the measured ratios of sample sizes less than 1.0 mg were lower than the expected ratio and 43% lower for the smallest sample size. The decrease in the measured $^{3}$H/$^{1}$H ratios suggests a dilution effect caused by an intrinsic contribution of $^{1}$H (0.017 ± 0.001 mmol) that is negligible when samples are larger than 1.0 mg. Smaller sample sizes also reduce the signal generated from the $^{1}$H ions. This signal, for samples that are smaller than 1 mg, can be improved by adding 2 mg of tributyrin as a carrier and is equivalent to 0.182 mmol of $^{1}$H. Adding carrier to the sample increases the $^{1}$H current to $\sim 50 \mu$A, which improves counting statistics. The $^{3}$H/$^{1}$H ratios of samples with carrier are then determined following subtraction of the $^{1}$H and $^{3}$H contribution from any added carrier.

Different biological samples were processed to investigate potential matrix effects with the AMS method. This is important because salts and other elements might interfere with the sample preparation process. The $^{3}$H/$^{1}$H ratios generated by AMS were compared to ratios calculated by LSC and are shown in Table 5. Ratios by LSC were calculated using the percentage (w/w) of hydrogen in the samples shown in Table 3, and the $^{3}$H and $^{1}$H component from added tributyrin carrier was subtracted from the DNA samples. As shown in the table, the measured AMS ratios for the biological matrices did not differ from the calculated LSC ratios by more than a few percent, except for plasma, where it differed by 11%. These differences are within the 15% limits for accuracy as established for bioanalytical method validation. Furthermore, the CV for these matrices is less than 3% except for DNA, which is larger. Less precision in the AMS analysis of
DNA may be due to the errors associated with the dilution of the sample with carrier prior to the oxidation step.

One major application of ^3H AMS is to use it in conjunction with ^14C AMS in dual-isotope labeling experiments, so that ^14CO₂ and ^3H₂O can be cryogenically separated from the same sample. Results from this study show that the AMS ^3H/^1H ratio (44 \pm 1 \times 10^{-12}) of samples containing both organically bound ^3H and ^14C was not significantly different (P = 0.23) from the LSC calculated ^3H/^1H ratio (39 \pm 6 \times 10^{-12}). Similarly, the [^14C]PhIP measured by AMS was 44 \pm 1 modern (1 modern = 13.6 dpm/g of C) and was not significantly different (P = 0.40) from LSC (41 \pm 3). The CV for triplicate ^3H AMS analyses was 1.6% while for ^14C AMS the CV was 0.7%

CONCLUSIONS

Tritium AMS is a methodology that can accurately quantify ^3H tracer levels in milligram-sized water and organic samples with the sensitivity and analysis times not possible by other techniques. It complements the established AMS technology for the analysis of ^14C in biological and environmental samples and is expected to have comparable applications. The high sensitivity should greatly facilitate tracer studies using ^3H-labeled compounds in natural systems at doses more relevant to human exposures. It will also facilitate absorption, distribution, metabolism, and elimination studies. Submilligram samples, such as DNA, can be analyzed after the addition of a tritium-free hydrogen carrier. The ability to separate and analyze both ^14C and ^3H from the same sample facilitates double-labeling experiments to concurrently learn the fate and distribution of separate compounds following low-level exposures or to trace separate parts of a single compound. Furthermore, due to the commercial availability of a large number of ^3H-labeled tracers, research using AMS will be possible using an expanded list of compounds with respect to the ^14C containing compounds.

ACKNOWLEDGMENT

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Table 5. Calculated LSC and Measured AMS ^3H/^1H Ratios of [^3H]PhIP in Biological Matrixesa

<table>
<thead>
<tr>
<th>sample matrix</th>
<th>LSC dpm ± SD</th>
<th>LSC ^3H/^1H ratio</th>
<th>AMS ^3H/^1H ratio</th>
<th>%diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver tissue</td>
<td>14 ± 5</td>
<td>85 ± 4</td>
<td>84 ± 3</td>
<td>2</td>
</tr>
<tr>
<td>protein</td>
<td>44 ± 1</td>
<td>91 ± 3</td>
<td>86 ± 2</td>
<td>6</td>
</tr>
<tr>
<td>DNA</td>
<td>27 ± 1</td>
<td>82 ± 2</td>
<td>88 ± 9</td>
<td>7</td>
</tr>
<tr>
<td>plasma</td>
<td>(53 ± 1) × 10³</td>
<td>75 ± 2</td>
<td>84 ± 1</td>
<td>11</td>
</tr>
<tr>
<td>urine</td>
<td>(53 ± 2) × 10³</td>
<td>75 ± 2</td>
<td>70 ± 2</td>
<td>8</td>
</tr>
</tbody>
</table>

a See text for details on sample size for each counting method; n = 6.