Radiostrontium accumulation in animal bones: development of a radiochemical method by ultra low-level liquid scintillation counting for its quantification

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Keywords
Animal bone, Beta emitters, Liquid scintillation, Radionuclides, Radiostrontium, Validation.

Summary
Strontium-90 ($^{90}$Sr) is a fission product, resulting from the use of uranium and plutonium in nuclear reactors and weapons. Consequently, it may be found in the environment as a consequence of nuclear fallout, nuclear weapon testing, and not correct waste management. When present in the environment, strontium-90 may be taken into animal body by drinking water, eating food, or breathing air. The primary health effects are bone tumors and tumors of the blood-cell forming organs, due to beta particles emitted by both $^{90}$Sr and yttrium-90 ($^{90}$Y). Moreover, another health concern is represented by inhibition of calcification and bone deformities in animals. Actually, radiometric methods for the determination of $^{90}$Sr in animal bones are lacking. This article describes a radiochemical method for the determination of $^{90}$Sr in animal bones, by ultra low-level liquid scintillation counting. The method precision and trueness have been demonstrated through validation tests (CV% = 12.4%; mean recovery = 98.4%). Detection limit and decision threshold corresponding to 8 and 3 mBecquerel (Bq) kg$^{-1}$, respectively, represent another strong point of this analytical procedure. This new radiochemical method permits the selective extraction of $^{90}$Sr, without interferences, and it is suitable for radioccontamination surveillance programs, and it is also an improvement with respect to food safety controls.

Parole chiave
Ossa animali, Beta emettitori, Scintillazione liquida, Radionuclidi, Radiostronzio, Validazione.

Riassunto
Lo stronzio-90 ($^{90}$Sr) è un radionucleide artificiale che decade in $^{90}$Y emettendo particelle beta. È un prodotto di fissione nucleare dell’uranio e del plutonio, pertanto può essere presente nell’ambiente soltanto a seguito di incidenti nucleari o di smaltimento non corretto dei rifiuti radioattivi. In questi casi lo $^{90}$Sr può essere assorbito dagli animali attraverso la respirazione o l’alimentazione e, possedendo una spiccata affinità chimica al calcio, può accumularsi nelle ossa e nel midollo osseo dove può provocare osteosarcomi, leucemie e tumori, oltre che fenomeni di decalcificazione e deformazione. Inoltre, questo radionucleide può passare dall’ambiente ai mangimi fino ai prodotti di origine animale particolarmente ricchi in calcio come latte e derivati. In questo studio è stato sviluppato e validato un metodo radiochimico per la determinazione di $^{90}$Sr nelle ossa animali, mediante conteggio in scintillazione liquida ad ultra basso fondo, dopo opportuno trattamento del campione e raggiungimento dell’equilibrio secolare $^{90}$Sr/$^{90}$Y. L’affidabilità e l’accuratezza di questa procedura analitica sono state verificate mediante una procedura di validazione implementata in-house, che ha consentito di valutare i più importanti parametri analitici, in accordo con gli attuali riferimenti legislativi, ovvero: selettività, linearità, detection limit e decision threshold, precisione ed esattezza, robustezza e incertezza di misura.
**Radiostrontium accumulation in animal bones**

Iammarino

**Introduction**

Strontium is a silvery metal that may be found naturally in the environment as a non-radioactive element. Sixteen isotopes of strontium are known, 4 of them are natural (strontium-84, strontium-86, strontium-87, strontium-88), the remaining 12 isotopes are radioactive. With respect to environmental pollution, strontium-90 ($^{90}$Sr) is considered the most important radioactive isotope. Strontium-89 and strontium-85 are also important, due to large use in nuclear reactors, industry, and medicine.

Strontium-90 decays, emitting moderate energy beta particles, forming yttrium-90 ($^{90}$Y), which is also a beta emitter that decays forming stable zirconium. The half-life of $^{90}$Sr and $^{90}$Y is equal to 29.1 years and 64 hours, respectively (Stamoulis et al. 2007, United States Environmental Protection Agency 2015, Wilken and Joshi 1991).

When present in the environment, $^{90}$Sr may be taken into human and animal body by drinking water, eating food, or breathing air. About 30-40% of ingested $^{90}$Sr is absorbed into the bloodstream, and then about 15% of what enters is deposited in bone, since it is characterized by high chemical affinity with calcium (the remaining goes to soft tissues as kidney or it is excreted in urine). The primary health effects are bone tumors and tumors of the blood-cell forming organs, due to beta particles emitted by both $^{90}$Sr and $^{90}$Y. Another health concern is represented by inhibition of calcification and bone deformities in animals. A reference maximum dose, which assures non-cancer effects in animals, was established to be 0.6 milligrams per kilogram body weight per day (mg/kg-day) (Peterson et al. 2007).

An interesting and complete study, concerning $^{90}$Sr accumulation in farm animals and relative animal source products, was published in 2001 (Annenkov and Averin 2001). The study, conducted in different contaminated areas, ascertained that sandy fields show the best $^{90}$Sr accumulation in comparison to clay-siliceous. Consequently, the $^{90}$Sr contamination in several cultivations was investigated, demonstrating not-negligible levels, also in raw materials usually used for animal feeding, namely: oat [1.2 Becquerel (Bq) kg$^{-1}$], rye (0.9 Bq kg$^{-1}$), wheat (1.3 Bq kg$^{-1}$), barley (1.6 Bq kg$^{-1}$). Other studies have also reported this type of contamination of animal feeds (Knizhnikov et al. 1991, Sapeika 1974, Shandala 1993). To the best of our knowledge, a specific radiometric method for the determination of $^{90}$Sr in animal bones is not available, and this is an important gap. Indeed, few studies concerning $^{90}$Sr accumulation in animal bones are actually available (Acar et al. 1989, Altitzoglou et al. 1998, Mietelski 2001, Pilviö et al. 1999, Staricenko 2011, Zanetti and Cutrufelli 1963).

In this work, an accurate, sensible, and rugged radiochemical method for the determination of $^{90}$Sr in animal bones, by ultra low-level liquid scintillation counting, after achievement of $^{90}$Sr/$^{90}$Y secular equilibrium, is described.

According to the reference European Regulations$^1$,$^2$, the method was submitted to an in-house validation procedure, in order to assess the most important validation parameters$^1$.

**Materials and methods**

The following chemicals were used: oxalic acid dehydrate (100.6%), sodium acetate (100%), hydrochloric acid (37% w/v), and toluene (99.8%) (VWR, Fontenay-sous-Bois, France); ammonium hydroxide (30% w/v) and hydrogen peroxide (33% w/v) (Panreac Quimica S.A.U., Castellar del Vallés, Barcelona, Spain); nitric acid (≥ 65% w/v), oxalic acid (99%), and ethanol (~ 96%) (Sigma-Aldrich Steinheim, Germany); hydrofluoric acid (48%) and bis(2-ethylhexyl)phosphate (HDEHP) (97%) (Merck Schuchardt OHG, Hohenbrunn, Germany); sodium sulphide nonahydrate (98%) (Carlo Erba Reagents, Rodano, Milan, Italy); Ultima Gold AB scintillation cocktail (Perkin-Elmer, Waltham, MA, USA); strontium, yttrium, lead and bismuth certified standard solutions in 4% HNO$_3$ (10,000 mg L$^{-1}$) (CPI International, Santa Rosa, CA, USA); $^{90}$Sr standardized solution at concentration of 7.441 kBq g$^{-1}$ (Eckert&Ziegler Isotope Products, Valencia, California, USA). All solutions were prepared by using ultrapure water (specific resistance: 18.2 MΩ·cm), supplied by a Milli-Q RG unit (Millipore, Bedford, MA, USA).

In order to obtain a correct quantification of $^{90}$Sr in animal bones, the radiochemical separation relating to $^{90}$Sr determination in solid foodstuffs as described by lammarino and colleagues (lammarino et al. 2016)
was slightly modified (sample ashing, as described in Figure 1).

Briefly, 30 g of bone sample, previously deprived of edible parts, were ashed at 1,000 °C according to the temperature ramp described in Figure 1. The ashes were dissolved in 8M HNO₃ + 0.5 mL of 50% HF and the mixture was then treated by leaching at 320 °C for 10 minutes and filtered. Oxalic acid (~ 20 g) and sodium acetate (~ 7 g) were added and the radionuclide present in the sample was precipitated as oxalate at pH 4.5 (by adding drop-by-drop, (30% w/v) ammonium hydroxide). The precipitate was filtered and dissolved in 35% H₂O₂ and 8M HNO₃ and then dried. The residue was dissolved in 0.1 M HCl; then the most significant interferences for this type of radiochemical determination, such as 210Pb and 210Bi (United States Environmental Protection Agency 2011), were removed by precipitation as sulphides, and the solution was placed in a separatory funnel with 200 mL of 20% HDEHP in toluene and mixed vigorously. This step is necessary to allow a correct achievement of 90Sr/90Y secular equilibrium, by removing from the sample 90Y. The acid phase (200 mL) was maintained at room temperature for at least 2 weeks, until 90Sr/90Y secular equilibrium was achieved. The solution (at a checked pH 1.0) was placed in a separatory funnel with 200 mL of 5% HDEHP in toluene and mixed vigorously. Yttrium forms strong complexes with HDEHP, so it can be selectively precipitated as oxalate at pH of 2.5 (by addition of 15% w/v ammonium hydroxide). Yttrium-oxalate was then dissolved in HCl and 12 mL of scintillation cocktail were added for counting by ultra low-level liquid scintillation counter (Quantulus Wallac 1200, Perkin-Elmer, Waltham, MA, USA).

Liquid scintillation counting (LSC) is a technique characterized by the incorporation of radiolabeled analyte into uniform distribution with a scintillation cocktail capable of converting the kinetic energy of nuclear emissions into light energy (University of Wisconsin 2015). Liquid scintillation counter settings are specified in Table I.

In order to assure the repeatability of an analytical method composed of a series of radiochemical separations, it is necessary to verify an acceptable recovery of Sr and Y from samples, for each determination. This verification, named “chemical yield”, was carried out by spectrometric quantifications of these stable elements (carriers), previously added to the samples. The quantifications were obtained by an inductively coupled mass spectrometer (ELAN DRC II; Perkin-Elmer, Waltham, MA, USA) and then used to calculate the final 90Sr activity. All Inductively Coupled Mass Spectrometer settings are specified in Table II.

In order to obtain the final quantification of 90Sr activity (A) the following equation was adopted:

\[
A = \frac{(R_c - R_f)\lambda_y \cdot \Delta T}{(1 - e^{-\lambda_y \Delta T}) \cdot W \cdot \varepsilon \cdot Y_{90Sr} / Y_y \cdot e^{\lambda_y \Delta T_y}} - \frac{e^{\lambda_y \Delta T}}{e^{\lambda_y \Delta T_y}}
\]

Where:
- \(Y_{90Sr}\) and \(Y_y\) are the strontium and yttrium chemical yields, respectively, obtained by ICP/MS;
- \(W\) is weight (kg) of analysed sample;
- \(\varepsilon\) is the counting efficiency;
- \(R_c\) is the count rate expressed in cps (counting for second);
- \(R_f\) is the background counting rate expressed in cps;
- \(\lambda_y\) and \(\lambda_{90Sr}\) are the 90Y and 90Sr decaying constants, respectively;
- \(\Delta T\) is the time counting (seconds);
- \(\Delta T_y\) is the time range between 90Y separation and counting start;
- \(\Delta T_{90Sr}\) is the time range between 90Sr separation and 90Sr solution reference data.

Methods performances such as counting efficiency, detection limit, decision threshold, linearity, specificity, and measurement uncertainty were carefully determined by adopting an intra-laboratory validation scheme, which followed the most important reference European Regulations and Guidelines (EURACHEM 2014). Decision threshold and detection limit were also calculated in compliance with ISO 11929:2010 (International Organization for Standardization 2010); whereas counting efficiency (\(\varepsilon\)) was achieved by analysing 3 90Sr solutions with an activity of 1.0 Bq, previously treated in order to separate the Y as Y-oxalate.

![Figure 1](image-url) Figure 1. Temperature ramp (from 25 °C to 1,000 °C) for obtaining the complete ashing of a bone sample (30 g).
according to the described procedure. The validation was integrated by analysing cow, pork, and chicken bone samples fortified with known activities of $^{90}$Sr (10 Bq kg$^{-1}$). 2 replicates of each species were carried out. Due to not negligible $^{90}$Sr activity concentration in animal bones, the samples were also analysed before fortification and the detected amount was subtracted to those related to fortified sample.

**Results and discussion**

**Validation parameters**

The characteristic LSC signals related to low-levels $^{90}$Sr sources and to instrumental noise are shown in Figure 2. During validation tests, the absence of β-interferences was verified, demonstrating method selectivity. Indeed, the β-spectra shape showed the characteristic maximum peak corresponding to the interesting radionuclide ($^{90}$Y) and the total counting decreases according to its decayment.

Counting efficiency obtained a value equal to 0.89 ± 0.06.

Decision threshold and detection limit resulted corresponding to 0.003 Bq kg$^{-1}$ and 0.008 Bq kg$^{-1}$ ($\alpha = \beta = 0.05$), respectively. These 2 parameters may be considered a strength of this radiochemical method, since these high sensibilities permit to adopt it for different types of environmental and food safety monitoring, not correlated to nuclear accidents.

The instrumental linearity was evaluated and assured by analysing 3 $^{90}$Sr sources at activity levels equal to 1.5, 95.48, and 494.40 Bq, and then by evaluating the calibration curve parameters, such as determination coefficient ($r^2$) (resulted higher than 0.9999) and the intercept (which differed not significantly from 0).

**Chemical yields**

The determination of Sr and Y chemical yields was carried out by adding 1 mL of 10,000 mg L$^{-1}$ strontium and yttrium standard solution (carriers) into samples and, then, by repeating 12 times entire radiochemical procedure described above. The mean recovery percentages resulted equal to 64% and 56% for Sr and Y, respectively. These 2 parameters are taken into consideration at each analysis, since if these minimal recoveries are not satisfied, it is necessary to repeat the entire procedure.

**Application to animal bone samples**

Six animal bone samples (2 cow, 2 pork, and 2 chicken) were collected from animal farmed in the province of Foggia, Italy (41° 27’ 43.914” N -
As a preliminary result, it is interesting to comment the data obtained by the analysis of the non-spiked bone samples. Indeed, the mean activity concentration detected in cow bone samples (4.00 Bq kg\(^{-1}\)) is notably higher than of those related to pork (0.86 Bq kg\(^{-1}\)) and chicken (1.40 Bq kg\(^{-1}\)) (Figure 5). This result is fully understandable when considering both the average farm life of these animals and a new aspect, recently underlined (Iammarino et al. 2015), related to animal feeding. Iammarino and colleagues verified that mean \(^{90}\)Sr activity concentration detected in raw materials used as animal feed (hay, silage, etc.) is higher than those related to processed animal feeds (2.50 Bq kg\(^{-1}\), Figure 3), and then analysed both before and after fortification with a known activity concentration of \(^{90}\)Sr (10 Bq kg\(^{-1}\)) (Table IV).

The 3 types of animal bone sample showed comparable values related to recovery percentage (trueness, in the range 87%-107%) and to CV% (precision, in the range 9%-11%). With respect to mean values, these parameters corresponding to 98.4% and 12.4% for recovery and precision, respectively, resulted analogous to those related to solid foodstuffs (92% and 14%) (Iammarino et al. 2016). Moreover, the homoscedasticity of these 6 data and 6 related to solid foodstuffs fortified at the same level was verified by one-way ANOVA test, confirming method ruggedness.

In Figure 4, LSC spectra related to the 3 types of animal bone samples are shown. By considering this figure, it is possible to appreciate that the spectra related to pork and chicken bone samples, characterized by similar \(^{90}\)Sr concentration activities equal to 1.19 and 1.23 Bq kg\(^{-1}\), respectively, are perfectly superimposable.

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Table V. Results obtained by analysing 10 cow bone samples.

<table>
<thead>
<tr>
<th>Cow bone sample</th>
<th>90Sr activity (Bq kg⁻¹) ± measurement uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample n.1</td>
<td>6.79 ± 1.09</td>
</tr>
<tr>
<td>Sample n.2</td>
<td>5.87 ± 0.94</td>
</tr>
<tr>
<td>Sample n.3</td>
<td>5.44 ± 0.87</td>
</tr>
<tr>
<td>Sample n.4</td>
<td>8.59 ± 1.97</td>
</tr>
<tr>
<td>Sample n.5</td>
<td>5.22 ± 0.84</td>
</tr>
<tr>
<td>Sample n.6</td>
<td>20.61 ± 3.30</td>
</tr>
<tr>
<td>Sample n.7</td>
<td>8.11 ± 1.30</td>
</tr>
<tr>
<td>Sample n.8</td>
<td>10.19 ± 1.63</td>
</tr>
<tr>
<td>Sample n.9</td>
<td>5.73 ± 0.92</td>
</tr>
<tr>
<td>Sample n.10</td>
<td>6.39 ± 1.02</td>
</tr>
</tbody>
</table>

Bq = Becquerel

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