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Paper

Optimization of pretreatment protocol for strontium-90 analysis in marine fish bone samples

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Abstract

For systematic monitoring of radioactive nuclides in marine products, this study aimed at streamlining and simplifying the analysis method for the prominent radioisotope, strontium-90 (^{90}Sr). The DGA chelate solid-phase extraction technique was employed for enhanced efficiency. The study focused on optimizing the necessary pretreatment procedures while minimizing the steps involving HNO_3 leaching. This protocol enabled the quantitative recovery of strontium, and it facilitated a rapid analysis without the need for a time-consuming evaporation step and without waiting for secular equilibrium between ^{90}Sr and its progeny to be reached. The method incorporating the optimized pretreatment protocol was applied to three diverse marine fish species and the accurate quantification of ^{90}Sr at background levels in surface seawater was achieved. The method obtained concentrations in bone samples from these species that ranged from 0.036 to 0.120 mBq per kg-dry, and chemical yield values were notably high, ranging from 87.7% to 92.5%.

Introduction

Strontium-90 (^{90}Sr) is widely acknowledged as a critical fission product in the field of radiation environmental monitoring due to its propensity to accumulate in the hard tissues of animals and the emission of high-energy beta rays by its progeny, yttrium-90 (^{90}Y). While various conventional methods for sample preparation have been established, including coprecipitation, ion exchange, and fuming HNO_3 precipitation, several pretreatment techniques also have been proposed, among which the chelating resin solid-phase extraction method [1, 2] is notable. After sample preparation, mass spectrometry methods such as Inductively Cupled Mass Spectrometry (ICP-MS) [3, 4], Thermal Ionization Mass Spectrometry (TIMS) [5–7], and Accelerator Mass Spectrometry (AMS) [8, 9] are now commonly applied for measurements. Despite its importance, the analysis of ^{90}Sr in marine products, such as marine fish, remains challenging due to the presence of abundant amounts of calcium (Ca) and stable Sr isotopes. Ca interferes with the chemical separation of Sr. In addition, the large amounts of stable Sr result in self-absorption of beta rays during radiation measurements or they lower the ^{90}Sr/Sr atom ratio in mass spectrometry, resulting in a quantitative limit below the detection limit.

Marine fish that are characterized by a low Sr enrichment factor (e.g. CR = 2.5) [10] require a substantial amount of bone samples for analyzing low concentrations of ^{90}Sr at global fallout levels. The minimum detectable activity by the solvent extraction method has been reported 0.10 Bq per kg in whole body ashed sample [11], which is almost the same as the global fallout level.

Stable Sr often surpasses the maximum adsorption capacity of Sr-specified resins. The solid phase extraction using DGA resin (DGA-SPE) for ^{90}Y separation represents a promising approach for the analysis of marine fish bone samples. DGA-SPE has been developed as a method for quantifying ^{90}Sr via ^{90}Y and it has

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been previously applied in soil and seawater matrices [12–14].

DGA-SPE effectively adsorbs Y under high concentration HNO_3 conditions and it is minimally affected by the sample matrix. Moreover, a subsequent rinse with various acids enables the removal of interfering radionuclides, making it particularly suitable for the analysis of low concentrations ^{90}Y [13, 15]. However, a drawback of DGA-SPE is its relatively slow flow rate. When handling large sample volumes, processing times are extended, which results in increased analytical uncertainties related to ^{90}Y decay correction. Typical operations for DGA resin prepacked in small cartridges (1 ml per 2 ml) are done at flow rates ranging from 1 to 2 ml per min, with the sample volume usually restricted to ~200 ml.

In this study, the focus was on optimizing the acid dissolution pretreatment process to efficiently separate ^{90}Y in bone samples from marine fish for DGA-SPE. Acid extraction data were acquired for different fish species and they were subsequently applied to the analysis of ^{90}Sr using actual bone samples.

Materials and methods

Samples

Marine fish samples were collected off the Pacific coast of Japan. Two bony fishes (class Osteichthyes) (Japanese Amberjack, *Seriola quinqueradiata* and Yellow Goosefish, *Lophius litulon*) and one cartilaginous fish (class Chondrichthyes) (Red Stingray, *Hemitrygon akajei*) were collected.

Raw fishes were cut with a knife, and the head, viscera, and muscles were removed. The muscles remaining on the vertebral bones were scraped off with a nylon brush as much as possible. However, bone marrow remained inside of the bones. Bones were dried at 110 °C for >2 days and combusted at 450 °C in a muffle oven to get ash bone samples.

The elemental compositions of fish bones (dried) are shown in Table 1. Ca concentration was the highest in the Japanese Amberjack (15.1%) and lowest in the yellow goosefish (13.2%). Sr concentration also showed approximately a two-fold difference among fish species. The Sr concentration in seawater was ~ 0.0079‰. The Sr concentration in dried bones was 175–340 times higher than that of seawater. Sr is excluded during shell formation because Sr interferes with the formation of the calcium skeleton. The trend may also vary depending on the trophic level within the food chain. Low Sr/Ca ratios in the bone compared with the ratio in seawater (0.0192; [16]) indicate that a process occurs during bone formation in which other elements are removed during the uptake of Ca.

Reagents

Ultrapure water (>18.2 MΩ•cm) was obtained from Milli-Q reference (Merck Millipore, USA). Preparations for all reagent and sample dilutions used the ultrapure water. HCl, HNO_3 and NH_4OH were available as Electro-industry Grade from Kanto Chemicals (Japan). Ultrapure Grade hydrofluoric acid (TAMAPURE AA-100, Tama Chemicals, Japan) was used to avoid contamination of Th and U series radionuclides. Purified Fe solution (1 mg/L) was prepared from $FeCl_3 \bullet 6H_2O$ (Wako Chemicals, Japan) by solvent extraction with diisopropyl ether. About 2 ml DGA resin prepacked cartridges (DN-R50-S, Eichrom Technology Inc., USA) were used for chemical separation.

Apparatus

The SPE-DGA method was carried out with a vacuum box system (Eichrom Technology Inc., USA) with a diaphragm vacuum pump (LABOPORT N96, KNF, USA). Elemental analysis for acid-leached samples and evaluation for Y chemical recovery during DGA-SPE were performed by an inductively coupled plasma optical emission spectrometer (ICP-OES; SPECTROBLUE TI, Ametek, USA). Beta counting was performed by an LB-4200 2 π gas flow proportional counter (Mirion Technology, USA) to determine ^{90}Y activity, which provided 3 mBq per sample of minimum detectable activity with a 40 h counting time.

Acid leaching process

For the acid leaching process, 40 g of an ash sample was processed. This is a sufficient amount for quantitative analysis of ^{90}Sr at the global fallout level, considering the stable Sr concentration in the bone samples and the specific activity of open ocean seawater [14, 17].

When a significant sample mass is introduced into the HNO_3 solution at one time, there is a potential risk of insoluble salts forming on the solid surface, potentially impeding the dissolution. Therefore, each ash bone sample was gradually added to 60 ml of 61% HNO_3 with continuous stirring to dissolve it.

The resulting solution was decanted into a 50 ml centrifuge tube, centrifuged at 3000 rpm for 10 min, and then filtered through a 0.45 μm pore mixed cellulose ester membrane filter (47 mm in diameter). Subsequently, the residue and centrifuge tube were rinsed three times with 30 ml of 8 M HNO_3, with each rinsing solution collected separately. These leachate samples were appropriately diluted and quantified using ICP-OES for the elements Ca, P, Na, K, Mg, and Sr.

Table 1. Chemical composition of fish bone samples (dry) and comparison with seawater.

	Ca %	P %	Na ‰	K ‰	Mg ‰	Sr ‰	Mg/Ca	Sr/Ca
Japanese amberjack	15.1	7.9	3.2	2.2	2.05	0.52	0.014	0.0034
Red stingray	17.9	10.3	11.2	4.0	3.69	0.70	0.021	0.0039
Yellow goosefish	13.2	7.4	17.7	5.9	2.27	1.14	0.017	0.0086
Seawater (Salinity =35)*	0.041		10.73	0.41	1.28	0.0079	0.31	0.0192

*from Millero et al. (2008) [16]

Strontium-90 analysis

Secular equilibrium between ^{90}Sr and ^{90}Y was established during 2 weeks following the acid leaching process. The leached samples were combined to determine ^{90}Sr activity concentration, resulting in HNO_3 concentration of ~6 M. The distribution coefficient of Y to DGA resin is known to be sufficiently high with > 1 M HNO_3 media [14]. This is attributed to the dissolution of calcium phosphate and carbonate component playing a role in neutralizing H^+.

The sample solution was introduced onto the DGA resin cartridge with vacuum box system after adding 200 μg of stable Y as a yield tracer. Tazoe *et al.* [12] previously reported the chromatogram of Y separation using the 1 ml cartridge. In this study, a cartridge with a 2 ml volume was employed to account for potential inhibition of Y adsorption due to the presence of abundant Ca, phosphate, and residual organic substances.

Following the sample introduction (Y retention), the DGA resin cartridge was rinsed with 20 ml of 8 M HNO_3. Interference elements and radionuclides such as Th and U were eluted using 20 ml of 8 M HCl, 40 ml of 3 M HNO_3 + 0.3 M HF, and 40 ml of 0.02 M HNO_3. Finally, ^{90}Y and stable Y were eluted using 30 ml of 0.1 M HCl.

To determine chemical yield of ^{90}Y, a 0.1 ml aliquot of the Y elution fraction was taken and diluted with 1% HNO_3 for Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Next, 1 mg of Fe was added to the remaining main portion of the Y elution fraction and Y was precipitated with Fe hydroxide by adding 1 ml of 28% NH_4OH. The precipitated Y included ^{90}Y. A mixed cellulose ester membrane filter (0.45 μm pore, 25 mm in diameter) was used to remove the Y and Fe hydroxide precipitates. After drying using an IR lamp, the filter and precipitates were encapsulated between an acrylic disc and a plastic film as a beta radiation source. A 60 h beta radiation measurement was conducted. This measurement consisted of 30 repetitions of 2 h measurements and that was sufficient to allow confirmation of ^{90}Y decay.

Results and discussion

Results for acid leaching process of bone samples

Figure 1 shows the changes in Sr, Ca, Na, and Mg recovery yields in the leachate during the HNO_3 leaching. The results of the leaching yielded over 80% recovery in the first extraction for all three fish species. Only yellow goosefish showed a slightly lower recovery rate, which may be attributed to a higher ash content. Incomplete combustion of organic substances and insoluble silicate could interfere with extraction of these elements from ash to HNO_3. In the fourth extraction, all recovery rates were <1%, indicating that quantitative Sr recovery could be achieved through three extraction steps. In this study, the chemical separation of ^{90}Y was conducted after allowing the necessary time for radiochemical equilibrium to be reached following acid dissolution. Notably, if secular equilibrium between ^{90}Sr and ^{90}Y is preserved post-extraction, this implies the feasibility of promptly carrying out DGA-SPE and successive beta counting. Eliminating the waiting period for secular equilibrium to be reached (2 weeks) is of paramount importance for urgent analyses in radiological emergency situations, as this time-consuming step, significantly restricts ^{90}Sr analysis.

Application to ^{90}Sr analysis in marine fish samples

Table 2 summarizes the results of the ^{90}Sr analysis method developed in this study when applied to marine fish bone samples. For Japanese Amberjack samples, 200 g of ashed bone sample was homogenized and divided into portions for duplicate analysis. The recovery rates for ^{90}Y ranged from 87.7% to 92.5% with an average of 89.5% (± 2.1% of standard deviation). There is no precedent for the successful single-stage extraction of Y from samples containing a large amount of matrix, making this achievement significant. Furthermore, all the ^{90}Sr concentration analysis values exceeded the minimum detectable activity concentration (MDC). The measurements for two samples of

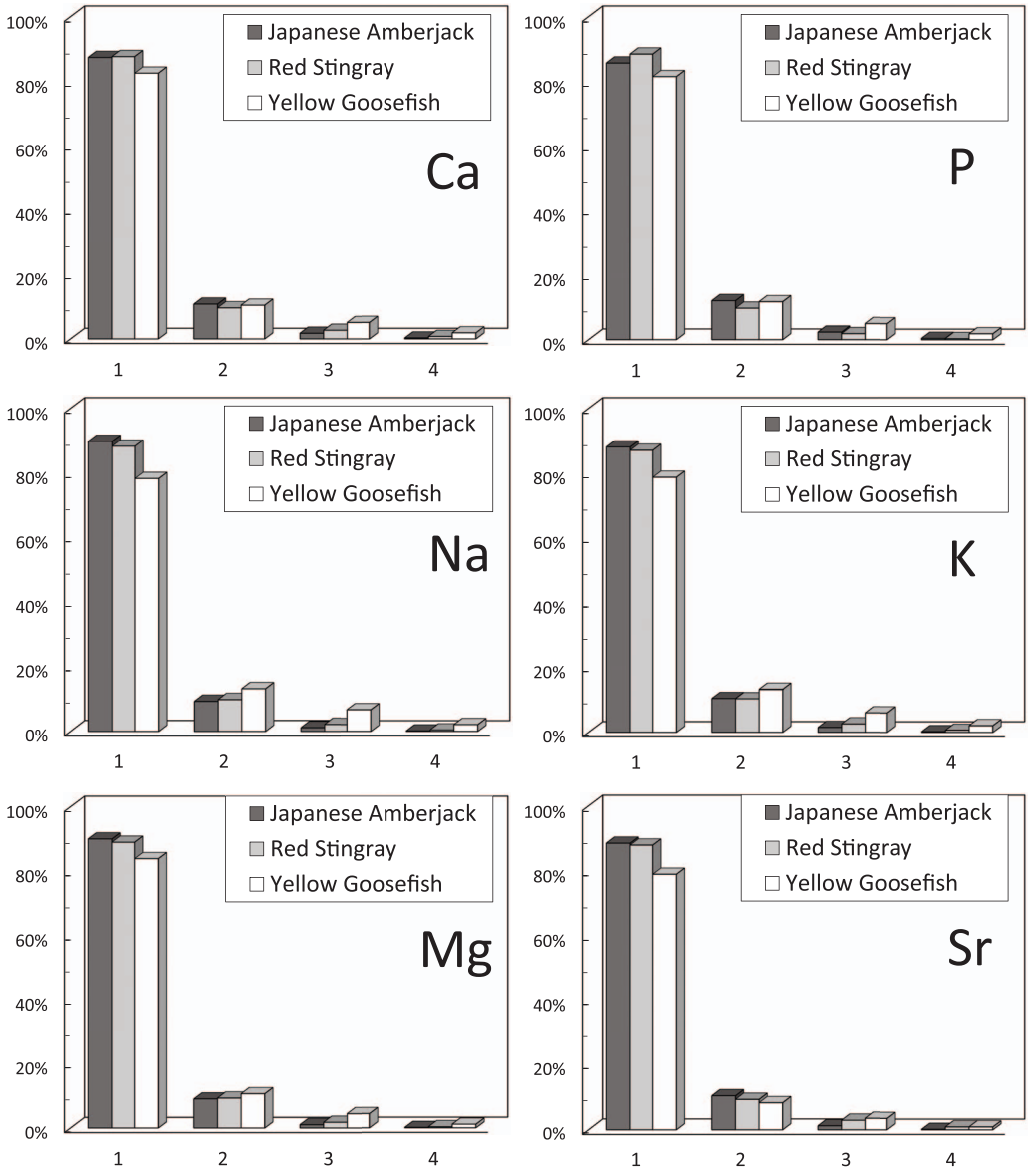

Figure 1. Element recovery rates in HNO_3 leaching experiments for solubilizing marine fish bone samples.

Japanese amberjack yielded results of 0.040 mBq per kg-dry (± 0.006 of combined standard uncertainty) and 0.036 (± 0.006) mBq per kg-dry, indicating a high degree of agreement. Variations were observed in other fish species, with yellow goosefish exhibiting the highest ^{90}Sr concentration, approximately three times that of Japanese amberjack. This trend mirrored stable strontium values, as shown in Table 1, and the fluctuations in the ^{90}Sr/stable Sr ratio were minimal. There was a possibility that Japanese amberjack might have a slightly smaller ^{90}Sr/stable Sr ratio within the analytical error. The seawater values near Jeju Island reported by Kim *et al*. from 2021 to 2023 were ~ 0.72–0.78 mBq per kg at 2 m depth and 0.79–0.91 mBq per kg at 100 m depth. Based on these data and the stable Sr concentration (0.0079 g per kg at salinity of 35 [16]) in seawater, the Sr-90/stable Sr ratio is in the range of 0.09 to 0.12. However, this does not account for variations in stable Sr concentration with salinity. Nevertheless, the Sr-90/stable Sr ratio of 0.07 to 0.12 obtained in this study is consistent with the seawater values, supporting the reliability of this analytical method.

Table 2. ^{90}Sr activity concentration in fish bone sample.

Sample ID	^{90}Y yield	^{90}Sr concentration (mBq/kg-dry)*			MDC (mBq/kg-dry)	^{90}Sr/stable Sr (Bq/g)*		
Japanese Amberjack								
1	92.5%	0.040	±	0.006	0.027	0.077	±	0.012
2	87.7%	0.036	±	04.006	0.027	0.069	±	0.012
Red Stingray	88.4%	0.086	±	0.008	0.032	0.122	±	0.011
Yellow Goosefish	89.6%	0.120	±	0.007	0.027	0.105	±	0.006

*± (combined standard uncertainty)

Conclusions

This study focused on the minimization of HNO_3 leaching as part of the pretreatment protocol to optimize the effective DGA-SPE method for the analysis of ^{90}Sr in bone samples from marine fish. The practical applicability of this method when applied to real samples was also assessed.

1. A three-step HNO_3 leaching process enabled the quantitative recovery of inorganic components, including strontium.
2. The leaching, centrifugation, and filtration processes could be completed in a single day, eliminating the need for subsequent concentration processes and allowing for direct DGA-SPE.
3. When applied to samples of three distinct marine fish species, the developed method exhibited high strontium recovery rates. The ^{90}Sr analysis results ranged from 0.036 to 0.12 mBq/kg.
4. Disparities in concentration among fish species could be attributed to differences in Sr uptake efficiency in their bones, and the levels observed were consistent with background levels.

These findings highlight the effectiveness and efficiency of this method for ^{90}Sr analysis in marine environments. From applications of the method valuable insights for future research in this field are expected.

Conflict of interest statement

The authors have no conflicts of interest directly relevant to the content of this article.

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