Speciated Isotope Dilution for the Determination of Methylmercury in Tuna Fish by GC-MS

Application Note

Environmental

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Abstract

A GC-MS with electron impact ionization was used for the development of a speciation method for the determination of methylmercury in fish samples. The method is based on isotope dilution using a spike containing 201Hg-enriched methylmercury. The spike was applied to the determination of methylmercury in tuna samples with excellent results.

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Introduction

Among the various mercury species, methylmercury is the most hazardous because of its accumulative and persistent character in the environment. Sensitive, specific, and precise analytical methods are needed to perform studies at ambient levels. Already the first step in the analysis, the isolation of methylmercury from the sample matrix can be troublesome. Since recovery of the analyte from some matrices is not always quantitative, recovery factors during isolation must be determined. This is usually done by standard addition techniques or recently by using isotope dilution mass spectrometry (IDMS).

Isotope dilution (ID) methodologies provide superior accuracy and precision compared to more common calibration strategies. ID for trace element speciation has been widely applied using ICP-MS and, recently, using GC-MS, a routine technique in testing laboratories.

Experimental

Reagents

Monomethylmercury chloride (96%) was obtained from Aldrich (Steinheim, Germany). Stock solutions were prepared by dissolving the salt in a 3:1 mixture of acetic acid (Merck, Darmstadt, Germany) and methanol (Merck). All standard solutions were kept in the dark at –18 °C and diluted working solutions were prepared by weight daily before the analysis. Acetic acid (Merck) and methanol (Merck) were used for the extraction of the organotin compounds from the solid matrices.

Sodium tetraethyl borate (Galab, Geesthacht, Germany) solutions of 2% (w/v) were prepared daily in 0.1 M sodium hydroxide solution (Merck).

A buffer solution at pH 5.3 was prepared by mixing appropriate volumes of 0.2 M acetic acid (Merck) and 0.2 M sodium acetate (Merck) solutions.

The spike solution (^{201}Hg-enriched monomethylmercury) was obtained from ISC-Science (Oviedo, Spain), diluted by weight with a mixture of methanol and acetic acid (3:1), and stored in the dark at –18 °C. Table 1 shows the isotopic composition as well as the concentration of the butyltin species in the spike solution.

Table 1. Isotopic Composition (Content %) and Concentration of the ^{201}Hg-Enriched Monomethylmercury (Uncertainty Corresponds to 95% Confidence Interval)

Hg-196	**Hg-198**	**Hg-199**	**Hg-200**
<0.01	0.043 (2)	0.109 (5)	0.890 (10)
Hg-201	**Hg-202**	**Hg-204**	
96.495 (29)	2.372 (22)	0.091 (5)	
Concentration: 5.49 ± 0.02 µg g-1 as Hg			

Additional information on www.isc-science.com

Instrumentation

GC/MS: Chromatographic analysis was performed with an Agilent (Agilent Technologies, Santa Clara, CA) gas chromatograph, model 6890N, fitted with a split/splitless injector and an HP-5MS capillary column (cross-linked 5% phenyl methyl siloxane, 30 m × 0.25 mm id × 0.25 µm coating). The gas chromatograph was equipped with an Agilent (Agilent Technologies) mass spectrometric detector, model 5973, network MSD (quadrupole based).

Helium was employed as carrier gas with a constant flow of 1.2 mL min^{-1}. The column temperature was initially held at 60 °C for 1 minute, increased at 30 °C min^{-1} to a final temperature of 300 °C. Injection was performed using a split/splitless injector in splitless mode. The transfer line and ion source temperatures were at 280 and 230 °C, respectively. Electron impact ionization was performed at an electron energy of 70 eV. The measurement of isotope ratios for methylmercury was performed on the molecular ion using 10-ms dwell-time per mass.

The solid-phase microextraction (SPME) device used for manual extraction, a holder assembly and several replaceable divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS, 50 µm/30 µm) fibers were purchased from Supelco (Madrid, Spain).

Extraction and Derivatization of Methylmercury from Tuna Fish Samples

For extraction, approximately 0.4 g of sample was spiked with a solution of the ^{201}Hg-enriched methylmercury and mixed with 15 mL of a saturated sodium chloride solution and 100 µL of concentrated hydrochloric acid. The mixture was shaken mechanically at room temperature for 5 hours.

Three milliliters of extract was adjusted to pH 5.3 with 3 mL of acetic acid/sodium acetate buffer in SPME glass vials; 1 mL of sodium tetraethyl borate was added and the vial

was then immediately closed with a PTFE-coated silicon rubber septum. The SPME needle pierced the septum and the fiber was exposed to the solution headspace for 15 minutes at room temperature. The solution was intensively stirred with a PTFE-coated magnetic stirring bar with constant velocity. Finally, the fiber was withdrawn into the needle and transferred to the GC injector for thermal desorption for 1 minute at 260 °C. During headspace solid-phase microextraction (HS-SPME), the temperature was controlled by immersing the sample vials in a water bath.

Results and Discussion

Isotope Ratio Measurements by GC-MS

While elemental isotope ratios can be easily obtained with ICP-MS, in GC/MS the isotopic pattern in molecular ions is different from that of the naturally occurring elements due to the contributions from the organic groups attached to the metal because of the presence of ^{13}C. The contribution of ^{13}C to the observed *m+1* ions can be calculated in a fairly straightforward way, by applying equation 1:

$$I_{m+1} = I_m \cdot n x_{13C} \quad (1)$$

where x_{13C} is the relative abundance of ^{13}C with respect to ^{12}C (0.0111/0.9899), n is the number of C atoms in the molecular ion, and I is the intensities of the ions *m* and *m+1*, respectively. The measured signal intensities were corrected by monitoring five molecular clusters, corresponding to the ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, and ^{202}Hg isotopes, taking into account the ^{13}C contributions to *m+1*. The intensity (I) correction equations used were:

$$^{198}Hg = {}^{198}I \quad (2)$$
$$^{199}Hg = {}^{199}I - x(^{198}Hg) \quad (3)$$
$$^{200}Hg = {}^{200}I - x(^{199}Hg) \quad (4)$$
$$^{201}Hg = {}^{201}I - x(^{200}Hg) \quad (5)$$
$$^{202}Hg = {}^{202}I - x(^{201}Hg) \quad (6)$$

where x is the contribution factor *m+1*. The selected molecular clusters for the measurement of methylmercury by GC-MS and the contribution factor x are given in Table 2.

Table 2. Monitored Masses and Contribution Factors for Methylmercury

Corresponding Hg isotopes	*m/z* selected for SIM mode ($MeEtHg^+$)
198	242
199	243
200	244
201	245
202	246

X (*m* + *1*) = 0.034

Analysis of Reference Materials

Methylmercury was determined in the reference material BCR 464 (tuna fish) by the proposed ID procedure. Three independent spiking experiments were made on each certified reference material and each sample was injected three times in GC-MS systems. The overall results obtained for the reference material by GC-MS are given in Table 3.

Table 3. Determination of Methylmercury in BCR 464 Using the 202/201 Isotope Ratio for Quantitation (Data in µg g^{-1} as Hg)

Replicate	Methylmercury
1	5.09 ± 0.06
2	5.02 ± 0.09
3	5.04 ± 0.05
Average	**5.05 ± 0.04**
RSD (%)	0.71
Certified value	***5.12 ± 0.16***

The concentration values obtained for methylmercury in the certified reference material BCR 464 show an excellent agreement between the certified and found values.

Conclusions

A precise and accurate method for the determination of methylmercury in fish samples has been developed. A single injection allows the concentration of methylmercury in the samples to be computed quickly, without the need for time-consuming calibration, standard addition, or recovery correction procedures. The method corrects for all possible errors in the speciation of methylmercury, provides low detection limits, and is fast and simple to apply by untrained personnel.

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