CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Mercury speciation analysis in marine samples by HPLC-ICPMS

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A simple method for determination of mercury species in marine origin has been developed and in-house validated. It is based on acid extraction by sonification, HPLC separation of inorganic mercury and methylmercury by cation-exchange column using isocratic elution and detection by ICPMS.

Introduction

Mercury (Hg) exists as elemental mercury (metallic), inorganic mercury and organic mercury (primarily methylmercury). Methylmercury is probably by far the most toxic form of mercury in food. It is highly toxic, particularly to the nervous system, and the developing brain is thought to be the most sensitive target organ for methylmercury toxicity. It bioaccumulates and biomagnifies along the food chain and is the most common mercury species in fish and seafood. Human exposure to methylmercury is mainly from fish and other seafood consumption. Inorganic mercury is probably the predominant form of mercury in foods other than fish and seafood. The methylmercury species in feed and food is currently not regulated by the European Union. Only for total mercury maximum levels have been laid down. There is a current need for fully standardized methods for determination of methylmercury.

Extraction of methylmercury

The applied method was inspired by Vallent et al (2007). Samples were extracted twice with 5 M hydrochloric acid by sonication. Hereby the protein-bound mercury species are released. Before re-extraction the samples were centrifuged (10 min at 3170 x g) and the supernatant decanted. The combined sample extracts were added 10 M sodium hydroxide to increase pH, diluted in the mobile phase and filtered.

Detection of methylmercury

Analysis of methylmercury were performed using HPLC-ICPMS equipped with a MicroMist nebuliser. Typical plasma conditions were 1500 W RF power, 15 l/min plasma, 0.97 l/min carrier and 0.17 l/min makeup gas. Analysis was performed in the time resolved analysis mode monitoring the ^{202}Hg, ^{198}Hg, ^{35}Cl (m/z) with 1 s (Hg) and 0.01 s (Cl) integration time per data point.

Separation of mercury species

Since the ICP-MS detector is not selective towards specific mercury species, the HPLC separation is crucial for selective determination. Separation of inorganic mercury and methylmercury was obtained on a polymer-based cation-exchange column (Hamilton PRP-X200,150 x 2.1 mm id, 10 µm) using isocratic elution (0.2 ml/min at 40º C). The mobile fase (pH~3) consisted of L-cysteine (0.5% w/w), pyridine (50 mmol/L), methanol (5% v/w) and formic acid (0.8% v/w) dissolved in water. Separation of the inorganic mercury and methyl-mercury can be seen in Figure 1.
Methylmercury was quantified using peak height (m/z 202) and external calibration standards (0-10 µg/l) which were run before and after the samples.

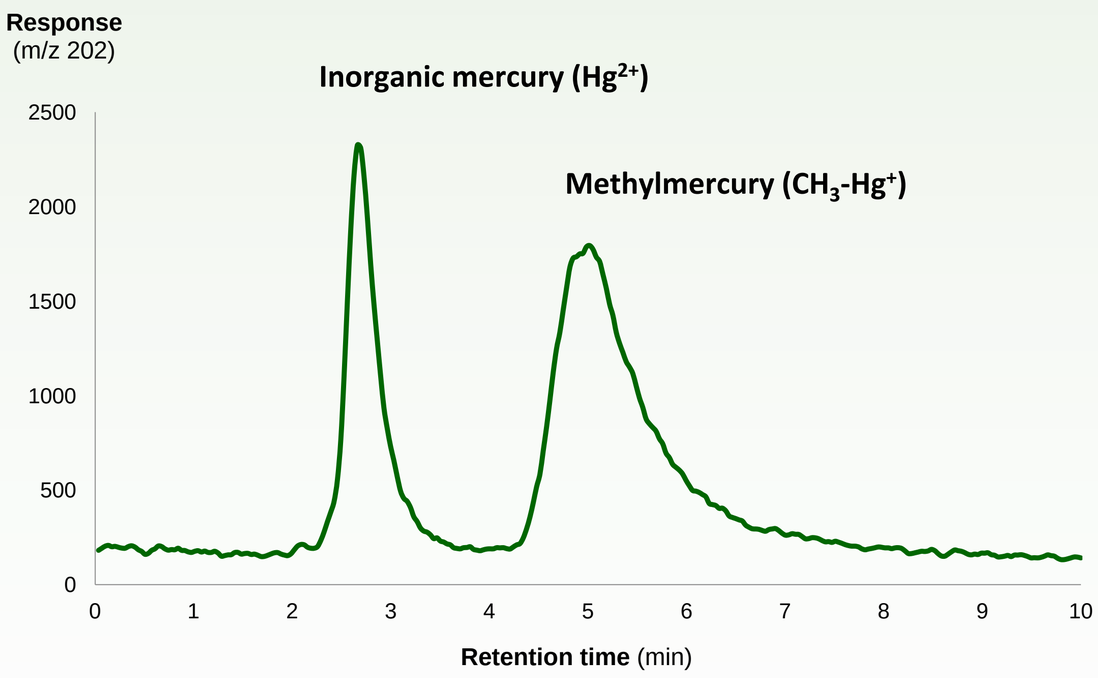

Figure 1. HPLC-ICP-MS chromatogram from extract of a TORT-2 sample (0.15 mg/kg methylmercury). The two mercury species inorganic mercury and methylmercury have different retention times.

Method performance

The method has been in-house validated using three different certified reference materials (DORM-2, TORT-2 and DORM-3) and four other fish and feed samples of marine origin (Table 1). All samples were analysed in triplicate and repeated on three different days. Limit of detection (LOD) and quantification (LOQ) were calculated as three and six times the standard deviation at intra laboratory reproducibility conditions (SD_{IR}) divided by average recovery for certified reference material (R_{rec}), respectively. LOD was 0.027 mg/kg and LOQ 0.054 mg/kg.
The in-house reproducibility standard deviations (RSD_{IR}) were less than ≤12% for samples containing 0.15 to 0.47 mg/kg methylmercury and less than ≤ 20% for samples with 0.06 mg/kg.

Table 1. Performance of the HPLC-ICPMS method for determination of methylmercury.

	DORM-2	TORT-2	DORM-3	Fish feed spiked	Codfish	Salmon	Fish feed
Ref. level (mg/kg)	4.47	0.15	0.36	0.21	0.17	0.06	0.06
Observations (N)	9	15	9	9	9	9	9
Mean recovery (%)	94	102	96	-	-	-	-
Repeatability RSD_r (%)	3	4	3	11	5	13	13
Reproducibility RSD_{IR} (%)	8	12	8	11	12	20	15

Fish feed

The method was applied to fish feed in use in Denmark. In total 23 samples (4 complete fish feed products, 5 fish silage samples and 14 fish meal samples) were collected by the Danish Plant Directorate. All samples were kept at -18°C. Samples (except fish silage) were homogenised before analysis. Certified reference material was used for quality control. The results can be seen in Table 2. For all samples the concentrations were below the EU maximum levels for total mercury in fish feed at 0.2 mg/kg and marine feed materials at 0.5 mg/kg (EU dir 2010/6/EC).

Table 2. Analysis of feed samples.

Feed sample type	CH_3-Hg^+ (µg/kg)	Feed sample type	CH_3-Hg^+ (µg/kg)
Fish Silage	<0.027	Fish meal	<0.027
	<0.027		<0.027
	<0.027		<0.027
	<0.027		<0.027
	<0.027		<0.027
Complete feed	<0.027		0.030
	<0.027		0.032
	<0.027		0.045
	0.032		0.053
LOD = 0.027 mg/kg, LOQ = 0.054 mg/kg			0.053
			0.055
			0.079
			0.125

Reference. Vallant B, Kadnar R and Goessler W (2007). Journal of Analytical Atomic Spectrometry 22, 322–325.

Future use and impact

The method present a simple approach for quantification of methylmercury in food and feed of marine origin.

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