Determination of Mercury Species in Foodstuffs using LC-ICP-MS: the Applicability and Limitations of the Method

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Abstract

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Reversed-phase liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (LC-ICP-MS) was used for mercury speciation analysis in food samples. A short chromatographic column (Purospher® RP-8e, 75 × 4 mm, 3 µm) and a mobile phase containing 0.02 mol/l CH_3COONH_4 + 0.2% (v/v) 2-mercaptoethanol (2-ME) + 1% (v/v) CH_3OH were applied. A repeated extraction of samples with hydrochloric acid/2-ME solution (1 mol/l HCl + 0.2% (v/v) 2-ME) was applied as the isolation step. The results were satisfactory for most food matrices (fish, shellfish, plant materials). Conversely, to analyse high-protein animal matrices, which contain mostly the inorganic form of mercury, a procedure including partial hydrolysis using hydrochloric acid should be used. For methylmercury and inorganic divalent mercury, the LOQ values of 0.3 and 2 ng/g, respectively, can be achieved if precautionary measures against contamination are fulfilled. The method was applied for the determination of methylmercury and inorganic divalent mercury in fish, vegetables, herbs and cereal products.

Keywords: mercury speciation; liquid chromatography; inductively coupled plasma-mass spectrometry

As a global contaminant, mercury enters the food chain in both inorganic and organic chemical forms. In aquatic ecosystems, the concentrations of mercury in biota – compared to the concentration in water – are substantially increased as a result of bioaccumulation (Selin 2009). The determination of individual chemical species of mercury in food and biological samples is significant because various mercury compounds show somewhat different toxic effects (Berlin *et al.* 2007). Speciation analysis can provide valuable data for the assessment of risk associated with the specific food groups as well as for studying the environmental fate of the species (Koplík *et al.* 1997). A number of circumstances might affect the accuracy of mercury speciation analysis. In the present paper, we focused on some crucial points of the analytical procedure of mercury speciation by liquid chromatography-inductively coupled plasma mass spectrometry.

A wide range of analytical methods are applied for mercury speciation (Leermakers *et al.* 2005). Since the 1960ies, gas chromatography (GC) has been used for the determination of methylmercury and other mercury species in biological samples (Westöö 1966, 1967, 1968; Jiang *et al.* 1989; Cai *et al.* 1996; He *et al.* 1998; Chen *et al.* 2004; Voegborlo *et al.* 2011). To detect the separated mercury species, various approaches have been applied; they have included the use of common GC detectors, such as an electron-capture detector (Westöö 1966, 1967, 1968; Voegborlo *et al.* 2011) or mass spectrometric detectors (Chen *et al.* 2004) or element-selective detection methods based on atomic absorption (Jiang *et al.* 1989; He *et al.* 1998), atomic fluorescence (Cai *et al.* 1996, 1997) or atomic emission spectrometry (Tu *et al.* 2000; Kuballa *et al.* 2009). When inductively coupled plasma mass spectrometry (ICP-MS) was

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established as a routine method of trace element analysis, it became an important element-selective detection technique for element speciation after separation methods. Because ICP-MS can be easily hyphenated with liquid chromatography (LC), LC-ICP-MS became a powerful tool for element speciation analysis (Michalke 2002a,b), applied especially to biological samples (Szpunar 2000).

In recent years, both gas and liquid chromatographic methods have been used in mercury speciation analysis. Although gas chromatography gives a high resolution capability and enables the fast determination of more chemical species (inorganic divalent mercury and various alkyl- and aryl-mercurials), this approach needs a more complex sample preparation. The procedure normally includes a derivatisation step to volatilise the mercury species; it is usually accomplished by alkylation using sodium tetraethylborate (Rapsomanikis *et al*. 1986; Tu *et al*. 2000; Kuballa *et al*. 2009; Rodrigues *et al*. 2011) or tetrapropylborate (De Smaele *et al*. 1998; Yang *et al*. 2003; Carrasco *et al*. 2007; Kuballa *et al*. 2009; Rodrigues *et al*. 2011). The resulting alkylated species are then extracted with a non-polar solvent or using a solid phase micro-extraction (SPME) technique (He *et al*. 1998; Yang *et al*. 2003; Carrasco *et al*. 2007). A complex sample preparation prior to gas chromatographic determination is time consuming; moreover, some steps can lead to sample contamination, loss of the species or partial transformation of the species. Some of these errors can be checked using isotope techniques or even compensated via isotope dilution quantification.

Conversely, the LC methods (Harrington 2000), which are sufficient for the determination of the two main naturally occurring species (i.e. methylmercury and inorganic divalent mercury), manage without any derivatisation. Mobile phases usually contain some thiol compounds, most commonly 2-mercaptoethanol (Huang & Jiang 1993; Ramalhosa *et al*. 2001; Vidler *et al*. 2007; Lin *et al*. 2008; Weiyue *et al*. 2011), cysteine (Wan *et al*. 1997; Hight & Cheng 2006; Percy *et al*. 2007; Vallant *et al*. 2007; Sannac *et al*. 2009) or both these compounds (Chiou 2001; de Souza 2010; Rodrigues *et al*. 2010). The thiol compounds are also used as components of extractants to isolate mercury species from solid samples (Chiou *et al*. 2001; Hight & Cheng 2006; Wang *et al*. 2007; Sannac *et al*. 2009; Rodrigues *et al*. 2010; de Souza *et al*. 2010; Weiyue *et al*. 2011). The addition of a thiol compound results in a formation of thiolates or chelates of mercury species with an Hg-S bond. Therefore, the solubility of the mercury species during extraction is increased and the chromatographic behaviour is modified.

In this paper, we describe a simple analytical procedure of mercury speciation analysis in some foodstuffs by LC-ICP-MS hyphenation. Furthermore, we emphasise some problems (e.g. calibration or sample extraction) that could be a pitfall for a less experienced analyst and consequently deteriorate the quality of analytical results.

MATERIAL AND METHODS

Chemicals and standards. Hydrochloric acid (30%) used for the preparation of extractants was of Suprapur® grade (Merck, Darmstadt, Germany). The further common chemicals were methanol (99.9%), Lichrosolv® grade (Merck), 2-mercaptoethanol (2-ME) ≥ 99% and ammonium acetate puriss. p.a. (both Fluka, Buchs, Germany).

Methylmercury chloride, 99.2% (Fluka) and ethylmercury chloride, 98% (Dr. Ehrenstorfer, Augsburg, Germany) were used as primary standards of organomercury species. The stock solutions of methylmercury (MeHg) and ethylmercury (EtHg) (200 μg/ml Hg) were prepared by dissolution of the corresponding compound mass in 30 ml of distilled deionised water (DDW) acidified with 5 ml of 30% HCl. For the dissolution of ethylmercury chloride, an addition of 30 ml of ethanol was necessary. The solutions were then transferred into 100-ml glass calibrated flasks and made up to the mark with DDW. The solutions were stored in dark in a fridge. The corresponding intermediate-concentration solutions of MeHg and EtHg (10 μg/ml Hg) were prepared by proper dilution of the stock solutions in diluted hydrochloric acid (the resulting HCl concentration was 0.01 mol/l). The working solutions were stored in dark and were used for a week.

The stock solution of inorganic divalent mercury (1000 μg/ml) in the matrix of diluted nitric acid was prepared from mercury(II) chloride (p.a. grade, Merck).

An aqueous solution containing traces of elemental mercury was prepared to perform a qualitative analytical test. A drop of metallic mercury (approx. 2 μl) was mixed with 100 ml of DDW in a glass flask by shaking for three hours. Then the aqueous phase was 50 fold diluted with DDW.

Calibration solutions of MeHg (conc. 2, 4, and 10 ng/ml Hg) or mixed calibration solutions of the three species or only two species (MeHg and inor-

ganic Hg^{II}) were prepared by proper dilution of the intermediate-concentration solutions of the individual species (10 µg/ml Hg) in the mobile phase on a daily basis.

Food samples and reference materials. Samples of fish muscle (both marine and freshwater fish) and of plant materials (vegetables, herbs, flour, bread) were purchased in the market. Before extraction, the samples of fish tissues and vegetables were mixed using an UltraTurrax T25 homogeniser (IKA-Werke, Staufen, Germany) to achieve a mushy state. Bread samples were diced into cubes. Reference materials with biological matrix and certified content of total mercury or both methylmercury and total mercury were used to test the accuracy of analytical results. They included SRM 1570a Trace Elements in Spinach Leaves, SRM 2976 Mussel Tissue – Trace Elements & Methylmercury (both NIST, Gaithersburg, USA), CRM 185 Bovine Liver, CRM 422 Trace Elements in Cod Muscle (both BCR, Belgium) and DORM-2 – Dogfish Muscle Certified Reference Material for Trace Metals (NRC, Ottawa, Canada).

Sample preparation. *Procedure A* (mercaptoethanol extraction): the mercury species were extracted from samples by an acidic solution of 2-ME. A sample portion (2 g of a food sample or 300 mg of reference material) was placed in a 30-ml screw-capped fluoroplastic centrifuge tube and treated with 10 ml of the extractant (1 mol/l HCl + 0.2% (v/v) 2-ME). The extraction was accomplished by mechanical agitation for 2 h in a laboratory shaker (oscillation frequency 800 min^{-1}). In the course of shaking, the tube was in horizontal position. Then the mixture was centrifuged (20 000 *g*, 15°C, 20 min) and the supernatant was poured into a 50-ml glass calibrated flask. The solid residue in the tube was mixed with another 10-ml portion of the extractant by vigorous hand shaking for 2 min followed by further 5 min on the shaker. The mixture was centrifuged again and the supernatant was added to the first portion in the calibrated flask. Then the solid residue was thoroughly mixed with 10 ml of DDW; after centrifugation, the supernatant was added to the calibrated flask. Just before injection of the extract into the column, the acidic extract was buffered with ammonium acetate. A 10-ml portion of 2 mol/l CH_3COONH_4 was added and the flask was made up to the mark with DDW.

Procedure B (hydrolysis): 1 ml of 30% hydrochloric acid and 1 ml of DDW were added to a sample portion (2 g of a food sample or up to 500 mg of lyophilised CRM) in the centrifuge tube. The closed tube was immersed in a 90°C water bath for one hour. Starting with the addition of the mercaptoethanol solution, the procedure A was then followed. At the end, the addition of more ammonium acetate was necessary (15 ml of 2 mol/l solution).

LC-ICP-MS analysis. We used a short reversed-phase column Purospher® RP8e (75 × 4 mm, 3 µm) with a guard column (4 × 4 mm, 5 µm) and the mobile phase containing 0.02 mol/l CH_3COONH_4 + 0.2% (v/v) 2-ME + 1% (v/v) methanol. The mobile phase flow rate was 0.8 ml/min. The chromatographic apparatus consisted of a high-pressure pump with a degasser (Perkin-Elmer Series 200), the first Rheodyne 9010 injector (Idex Health & Science, Oak Harbor, USA) with a 100-µl or 210-µl PEEK sample loop, the guard column, the analytical column and the second Rheodyne 9010 injector equipped with a 211-µl PEEK sample loop. The second injector was used for the optional calibration by post-column injec-

Table 1. Parameters of LC-ICP-MS analysis of mercury species

Chromatography	
Column	Purospher® STAR RP-8e, 75 × 4 mm, 3 µm
Guard column	Purospher® STAR RP-8e, 4 × 4 mm, 5 µm
Mobile phase	0.02 mol/l ammonium acetate + 0.2% (v/v) 2-mercaptoethanol + 1% (v/v) methanol
Column temperature	ambient (18–22°C)
Mobile phase flow rate	0.8 ml/min
Sample injection volume	100 µl or 210 µl
ICP-MS detection	
Power	1100 W
Nebulizer Ar flow	0.68-0.74 l/min (optimised)
Spray chamber cooling	off
AutoLens	on
Mode of measurement	standard, peak hopping
PSV	800 V
QRO	–7 V
CRO	–18 V
CPV	–16 V
Rpa	0
RPq	0.25
Nuclides (dwell times)	^{202}Hg (90 ms), ^{103}Rh (24 ms)
Sweeps per replicate	10
Replicates per sample	800
Replicate time	1.2 s
Total time per sample	16 min

tion of standards. The outlet PTFE capillary behind the second injector was joined by a T-piece with the container of the internal standard solution (50 ng/ml Rh in 0.15 mol/l HNO_3). The mixed flow (1.2 ml/min) was delivered by a peristaltic pump to the nebuliser of Elan DRC-e inductively coupled plasma mass spectrometer (Perkin-Elmer, Norwalk, USA).

Sample extracts and blanks were injected into the column through a syringe disc filter (0.45 μm) that was previously rinsed by 0.15 mol/l HNO_3 and by a portion of mobile phase. Prior to the sample analysis, the calibration was performed either using three mixed calibration solutions (concentration of both species corresponded to 2.0, 4.0 and 10.0 ng/ml Hg) or optionally by post column injection of three solutions of methylmercury chloride (2.0, 4.0, and 10.0 ng/ml Hg). The instrumental conditions of the LC-ICP-MS analysis are summarised in Table 1.

RESULTS AND DISCUSSION

Chromatographic separation of mercury species. To separate inorganic divalent mercury, methylmercury and ethylmercury species, we applied reversed-phase chromatography on the octylated stationary phase and various mobile phases containing 2-ME. This choice, in contrast with a more common octadecylated phase, could enable to use a mobile phase with a low content of an organic solvent, which is advantageous for ICP-MS detection. We tested the influence of methanol content and the presence of buffering component on the species separation. Figure 1 shows the corresponding chromatograms. The order of elution was: MeHg, inorganic Hg^{II} and EtHg, which was consistent with reported data (Lin *et al.* 2008).

Table 2 summarises retention data of the three species and the achieved resolution values for the pair of peaks of methylmercury and inorganic divalent mercury. The resolution was made worse by the addition of ammonium acetate to the mobile phase, but it was conversely improved by lowering the methanol content. As expected, the capacity factors were the highest and the resolution was the best using the mobile phase containing 1% (v/v) of methanol only. The corresponding mobile phase (0.02 mol/l CH_3COONH_4 + 0.2% (v/v) 2-ME + 1% (v/v) MeOH) was selected for further analyses of methylmercury and inorganic divalent mercury. The disadvantage was that the retention time of ethylmercury was extremely long. This made us finish the analysis after the elution of methylmercury; this did not matter because ethylmercury is not normally present in mercury-contaminated foodstuffs (Lin *et al.* 2008; Park *et al.* 2011). In contrast to methylmercury, ethylmercury is not practically created by an alkylation process from inorganic mercury in the environment. On the other hand, Thimerosal, a synthetic compound containing an ethylmercury moiety, is used in pharmacy as a preservative for vaccines. Thimerosal is a potential source of ethyl-

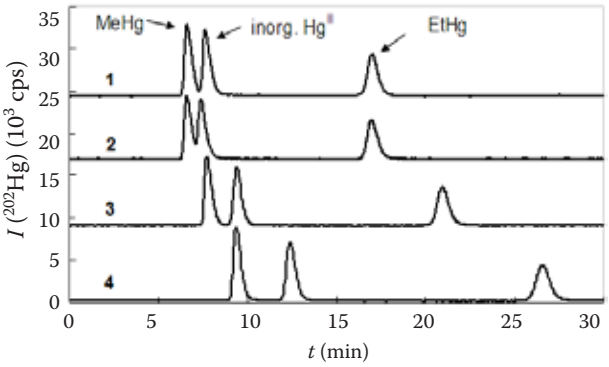

Figure 1. Effect of mobile phase composition on the separation of mercury species

flow rate 0.8 ml/min; injection volume 100 μl; standards of $HgCl_2$, MeHgCl and EtHgCl (conc. of 10 ng/ml Hg of each species); 1 – 0.2% (v/v) 2-ME, 5% MeOH; 2 – 0.02 mol/l CH_3COONH_4, 0.2% (v/v) 2-ME, 5% MeOH; 3 – 0.02 mol/l CH_3COONH_4, 0.2% (v/v) 2-ME, 3% MeOH; 4 – 0.02 mol/l CH_3COONH_4, 0.2% (v/v) 2-ME, 1% MeOH

Table 2. Effect of mobile phase on the mercury species retention and the resolution of inorganic divalent mercury and methylmercury peaks

Mobile phase[a]	MeHg		Inorganic Hg^{II}		EtHg		R
	t_R (min)	k'	t_R (min)	k'	t_R (min)	k'	
1	6.6	3.0	7.7	3.6	17.0	9.3	0.98
2	6.5	3.0	7.4	3.5	17.0	9.3	0.90
3	7.7	3.7	9.4	4.6	21.0	11.7	1.40
4	9.2	4.5	12.4	6.5	26.6	15.1	2.32

[a]according to Fig. 1; t_R – retention time; k' – capacity factor;, R – resolution of the MeHg and inorganic Hg^{II} peaks

mercury contamination in food samples, because it is metabolised to ethylmercury and 2-mercaptobenzoic acid. Meat originating from tissues of the animals that had been vaccinated might contain traces of ethylmercury, even though this appears rather unlikely. Ethylmercury is less toxic and less stable as an organometallic species than methylmercury (Dórea *et al*. 2013). Therefore, ethylmercury is a less persistent species and during the animal life it can be decomposed to inorganic mercury.

Using the above-mentioned mobile phase, the isocratic chromatographic analysis of methylmercury and inorganic divalent mercury takes 16 minutes. Although the retention time of ethylmercury was ca. 27 min, we would have noticed the presence of ethylmercury in the samples because each sample analysis was followed by blank analysis; therefore, the lately eluted ethylmercury peak would necessarily appear in the record. We found no such signal in any case. Therefore we can conclude that the analysed samples did not contain ethylmercury. This is consistent with the results of Park *et al* (2011), who found no ethylmercury in the survey of 177 fish samples.

As far as the chromatographic resolution is concerned (Table 2), one could consider that a value higher than 2.0 is unnecessary. Nevertheless, it became evident that the higher resolution was useful to determine minor quantities of one species (usually inorganic divalent mercury) in the large excess of the other species (methylmercury). An example is shown in Figure 2.

Standards and calibration. Limited stability of calibration solutions was the main difficulty of calibration. Calibration solutions of mercury species for chromatographic analysis were prepared in the mobile phase on a daily basis. An example of the record of standards is shown in Figure 3. The corresponding

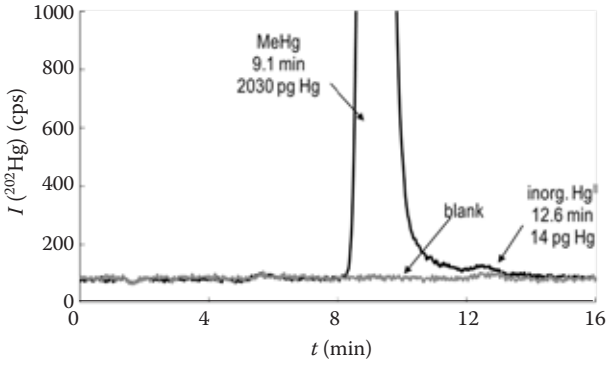

Figure 2. Mercury species analysis in a swordfish sample: a detail view

Mobile phase 4; injection volume 210 µl; other conditions as in Figure 1

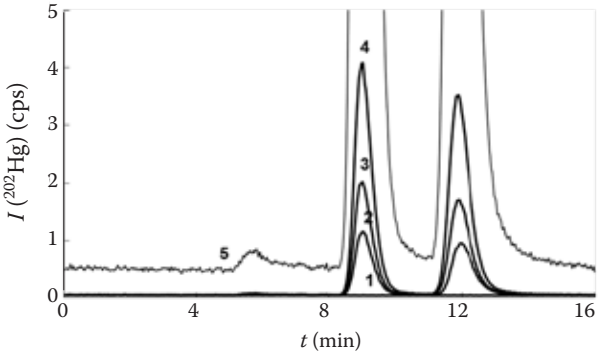

Figure 3. Analyses of calibration standards of methylmercury and inorganic divalent mercury

1 – calibration blank; 2 – 2.0 ng/ml Hg in the form of MeHg + 2.0 ng/ml Hg in the form of Hg^{II}; 3 – 4.0 ng/ml Hg in the form of MeHg + 4.0 ng/ml in the form of Hg^{II}; 4 – 10.0 ng/ml Hg in the form of MeHg + 10.0 ng/ml in the form of Hg^{II}; 5 – ten times expanded curve of 10.0 ng/ml

peak areas were equal for both species. As shown in Figure 3, curve 5, a trace amount of another mercury species was detected in the standard solutions at the retention time of ca. 5.8 minutes. At the beginning of experiments, the peak of this new species seemed to be negligible. But during the period of three weeks, when the same intermediate solutions of mercury species (10 µg/ml Hg) were used to prepare working calibration solutions, we observed an increasing amount of this species in calibration solutions that gradually attained an appreciable level. We presumed that the "new" mercury species might be elemental mercury formed from inorganic divalent mercury via a slow spontaneous reduction caused by trace organic impurities from DDW or organic compounds leached from the plastic container, in which the intermediate solution was stored. As this presumption was proved lately, we excluded all plastic bottles as storage containers for standards.

We compared the chromatograms of a freshly prepared standard solution and a one-week-old standard solution of divalent inorganic mercury (Figure 4). Two peaks were detected in the old solution, while only inorganic divalent mercury was found in the fresh solution. The area of the new peak (at t_R = 5.8 min) represents approx. 40% of the area of the inorganic Hg^{II} peak. Compared with the fresh solution, the area of the inorganic Hg^{II} peak in the old solution was not decreased significantly. It would seemingly be logical to expect a decrease of the inorganic Hg^{II} peak, when another appreciable mercury peak appeared. But actually, the almost equal areas of inorganic Hg^{II} peaks in both solutions supported our presumption that

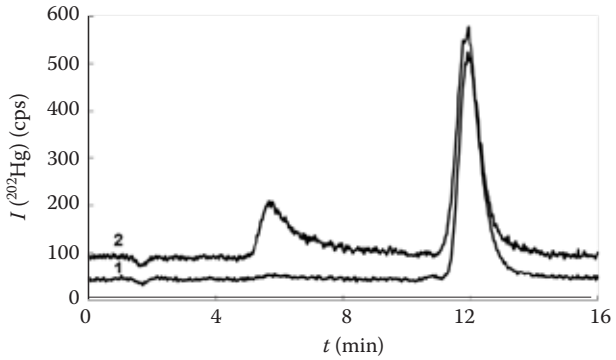

Figure 4. Analyses of a freshly prepared standard solution and a week-old standard solution of inorganic divalent mercury (1 ng/ml Hg)

1 – the new solution; 2 – the old solution; curve 2 is shifted upward; curve 1 shows the real background

the new mercury species was elemental mercury. The behaviour of elemental mercury in the nebuliser of ICP-MS must be totally different from the behaviour of non-volatile mercury compounds. Elemental mercury is much more effectively transported into the spectrometer because it is volatilised easily during nebulisation and enters the spectrometer both in the gaseous phase and in the liquid phase of sample aerosol, while the non-volatile species remain only in the liquid phase of aerosol formed with a low efficiency (Thompson & Reynolds 1971; Hawley & Ingle 1975). Thus when a minor amount of inorganic Hg^{II} (1–2%) is transformed into elemental mercury, an appreciable signal of elemental mercury will appear, while the decrease of the inorganic Hg^{II} peak is not easily noticeable. An analogous experiment with a fresh solution and an old solution of methylmercury showed that no elemental mercury was formed from methylmercury.

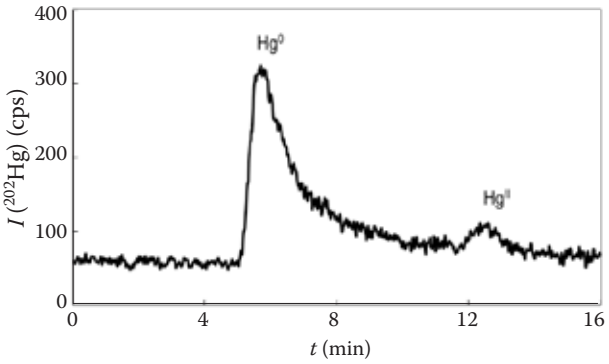

Figure 5. Analysis of diluted aqueous solution of metallic mercury

Using the chromatographic analysis of an aqueous solution of elemental mercury, we definitely proved that the new mercury species was elemental mercury (Figure 5). The freshly prepared diluted aqueous solution of elemental mercury was immediately analysed by LC-ICP-MS. Behind the tailing peak of elemental mercury some inorganic divalent mercury was also detected. When the elemental mercury was present in the analysed solution, a higher noise of the elution curve was observed.

To quantify mercury species the peak areas were calculated by integrating intensities ratios (i.e. intensity of ^{202}Hg/intensity of ^{103}Rh). We verified the linearity of calibrations in a wider working range (0, 20, 40, 60, 80, and 100 ng/ml). The data were statistically treated using Mandel's test (Funk *et al.* 2007). The test proved that a linear calibration model fits the data well. For practical reasons, we used only three-point calibrations in routine analyses.

The actual values of the sensitivity of mercury species detection (slopes of calibration lines) varied day to day and ranged from 0.2184 ml/ng to 0.3148 ml/ng according to the current ICP-MS detection power. On the other hand, within a day the sensitivities for both species were the same. Therefore, the simpler calibration of both species determination can be accomplished using only one stable species, i.e. methylmercury. It is advantageous to apply the technique of flow injection analysis (FIA) by injection of methylmercury standards into the flow of the pure mobile phase using the second injector placed behind the analytical column (Figure 6). To calculate the quantity of injected analyte, the exact volumes of PEEK loops of the first and the second injector were determined by an accurate weighing of loops filled with water. The volumes were almost equal

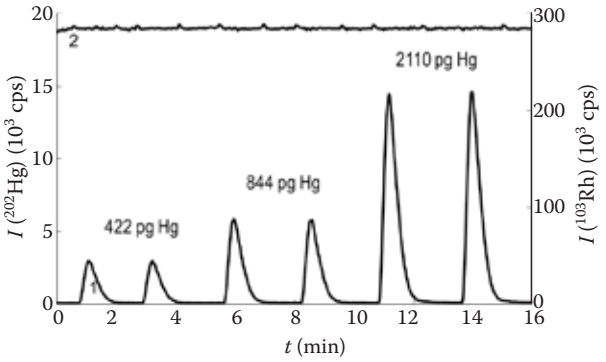

Figure 6. Calibration of Hg species analysis by post-column injection of methylmercury standards (2.0, 4.0 and 10.0 ng/ml Hg)

1 – intensity of ^{202}Hg, 2 – intensity of ^{103}Rh (internal standard)

(210 and 211 µl). Although FIA peaks have different height and shape compared to the chromatographic peaks of the corresponding quantity of MeHg, the areas of peaks are the same. Taking advantage of the FIA-ICP-MS technique the whole calibration can be done during a 16-min run, which saves much time in analyses of large sample series.

Sample preparation and analysis of food samples. In order to liberate the mercury species from the biological matrices investigated in this work, an extraction with hydrochloric acid-2-ME solution (procedure A) was applied. The selection of the extractant came from the preceding analogous application of diluted acids (Westöö 1966; Kratzer *et al*. 1994; Margentínová *et al*. 2008; Reyes *et al*. 2009) or solutions of thiol compounds (Hight & Cheng 2006; Wang *et al*. 2007). A strongly acidic medium enables to liberate mercury species from a solid matrix. High concentration of halogenide ions, as ligands of mercury, or the presence of sulphur-containing compounds helps to increase extraction efficiency. The carbon-mercury bond in methylmercury is not attacked by mineral acids (Hintelmann 2003).

Several reference materials with certified total mercury content or both methylmercury and total mercury content were used to verify the accuracy of mercury species determination using procedure A. The reference materials included various matrices such as fish, shellfish and mammalian tissues and plant leaves and the certified contents of mercury ranged from tenths of ng/g to µg/g levels (Table 3). Whereas methylmercury was a dominating species in both fish materials (dogfish and cod), quite comparable amounts of methylmercury and inorganic divalent mercury were present in mussel. On the other hand, only inorganic divalent mercury was found in bovine liver and spinach reference materials. The recovery of mercury species, which was summed up and compared with the certified total mercury content, attained practically 100% in almost all reference materials. The only exception was the case of bovine liver (CRM 185); only 65% of total mercury was isolated from this matrix. This is probably caused by the more stable binding of inorganic mercury which is fixed tightly to the proteinaceous matrix. In contrast with bovine liver, inorganic divalent mercury was quantitatively extracted from spinach matrix. Only two reference materials used had certified contents of both methylmercury and total mercury. The measured

Table 3. Analyses of reference materials (using sample preparation procedure A)

Reference material	Determined[a] (ng/g)	Certified (ng/g)
DORM-2 Dogfish Muscle (NRC)		
MeHg (as Hg)	4280 ± 50	4470 ± 320
Inorganic Hg^{II}	290 ± 30	–
Total[b]	4570 ± 50	4640 ± 260
CRM 422 Cod Muscle (BCR)		
MeHg (as Hg)	500 ± 15	–
Inorganic Hg^{II}	75 ± 5	–
Total	575 ± 17	559 ± 16
SRM 2976 Mussel Tissue (NIST)		
MeHg (as Hg)	27.4 ± 1.6	28.1 ± 0.3
Inorganic Hg^{II}	35.1 ± 6.5	–
Total	62.5 ± 7.3	61.0 ± 3.6
SRM 1570a Spinach Leaves (NIST)		
MeHg (as Hg)	not detected	–
Inorganic Hg^{II}	30.6 ± 2	–
Total	30.6 ± 2	30 ± 3
CRM 185 Bovine Liver (BCR)		
MeHg (as Hg)	< 1	–
Inorganic Hg^{II}	28.2 ± 1.7	–
Total	ca. 28	44 ± 3

[a]expressed as an average and expanded uncertainty (n = 4); [b]individual values of total mercury were calculated as a sum of species

methylmercury concentrations agreed satisfactorily with the certified values.

The LC-ICP-MS analysis based on sample preparation procedure A was applied to analyse various food matrices including sea and freshwater fish (tuna, swordfish, sardine, cod, flatfish, salmon, trout, carp, pike), cereal products (bread and flours), vegetables and herbs (spinach, carrot, watercress, rose hip). As expected, methylmercury was the main species in fish samples representing 78–98% of the total mercury content which ranged from 6 ng/g to 250 ng/g. The total mercury content in cereals and vegetables was generally lower than 15 ng/g. Although inorganic divalent mercury was the dominating mercury species in foods of plant origin, trace amounts of methylmercury were just detected in spinach samples.

To overcome the low recovery of mercury from the bovine liver sample, much stronger solubilisation conditions have to be used. Vallant *et al*. (2007) suggested ultrasonic extraction with 5 mol/l hydrochloric acid as a sample treatment for LC-ICP-MS analysis. Therefore, we applied partial hydrolysis using 5 mol/l HCl at an elevated temperature followed by 2-mercaptoethanol extraction (procedure B). The solubilisation of most solid matter was apparent as a result of hydrolysis. Although the digests appeared as brownish suspensions of very fine particles, clear brown solutions were obtained after centrifugation. Using this procedure, we found 48 ± 6 ng/g of inorganic divalent mercury and no methylmercury in CRM 185, which was acceptable compared to the certified value (44 ± 3 ng/g of total mercury). Unfortunately, this procedure resulted in somewhat higher blanks.

Sample contamination and blanks. If trace quantities of mercury species are to be determined, any sample contamination during the whole analytical procedure can introduce analytical errors. Mercury impurities can originate from chemicals, laboratory glassware or plastic ware, disposable filters, syringes and finally from sample handling. As a result, the limit of quantification increases and the accuracy might become worse. We selected high purity chemicals and checked mercury impurities of all chemicals in each bottle we used. The main source of mercury impurities among the chemicals was 2-ME. Practically, the mercury level in 2-ME determined the chromatographic baseline background.

To minimise other sources of contamination, we kept the sample preparation step as simple as possible. The procedure involved an extraction step taking place in a centrifuge tube and a transfer of the extract into a calibrated flask completed by buffering of the sample just before chromatographic analysis. Fluoroplastic centrifuge tubes can be used repeatedly, if thorough cleaning is provided. We found that the tubes filled with the extractant and heated at 90°C overnight were efficiently decontaminated.

The last moment when some contamination can occur is the sample injection. We checked the injection system via inserting an "injection blank" in each sequence of chromatographic analyses just after the last calibration solution; it means we injected the portion of the mobile phase through a pre-cleaned disc filter and recorded the injection blank curve. If this record is completely clean (no peak appeared), the injections of procedure blanks follow using the same pre-cleaned filter. For the injection of a sample extract, another "injection blank" was inserted. The order of injections is illustrated in Figure 7 using the sample of very low mercury content.

In procedure blanks, we found traces of mercury exclusively in the form of inorganic divalent mercury. When all precautionary measures were taken, the amount of mercury in blanks ranged typically from 3 pg to 5 pg corresponding to a concentration of 0.015–0.025 ng/ml. A "dirty" blank might attain a quantity of 10 or even 20 pg Hg (0.05–0.1 ng/ml). The limit of quantification (LOQ) of methylmercury is determined only by the signal to noise ratio, which is basically dependent on the actual instrument setting. Using analyses of diluted standards of methylmercury (0.01–0.05 ng/ml Hg), we estimated under optimum conditions the LOQ of MeHg to be 0.012 ng/ml (as Hg); at the sample mass of 2 g (wet tissue), this value corresponds to 0.3 ng/g. The LOQ of inorganic divalent mercury depends much more

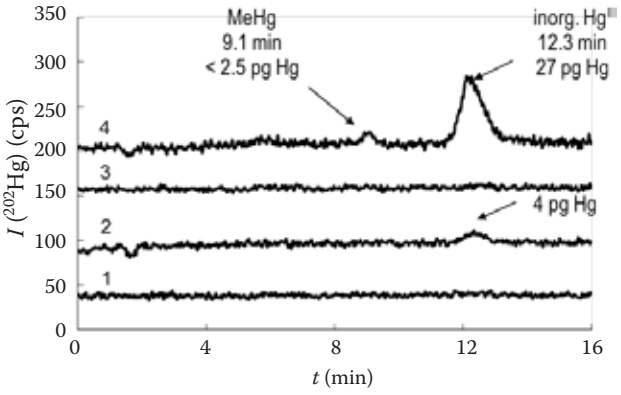

Figure 7. Sequence of blanks and sample analyses

1 – injection blank (a portion of mobile phase through the pre-cleaned filter); 2 – procedure blank; 3 – injection blank; 4 – sample of spinach extract; curves 2–4 are shifted upward; curve 1 shows the real background

on the actual blank levels. Nevertheless, the value of 0.08 ng/ml (or 2 ng/g) can realistically be attained.

CONCLUSIONS

The proposed extraction procedure (procedure A) is effective in isolating the mercury species from fish muscle and vegetable tissues. In contrast, less than 70% of total mercury could be recovered from meat and offal of terrestrial animals. It is likely that in these tissues the majority of mercury is represented by inorganic divalent mercury which is more tightly bound to the insoluble matrix compared with methylmercury, which is the main species in fish. To analyse mammalian tissues accurately, the longer procedure B is to be used.

The chromatographic resolution of methylmercury and inorganic divalent mercury peaks is sufficient even if a trace amount of one species is present in a large excess of the other. The ICP-MS detection is specific and sensitive enough to quantify mercury species at lower ng/g levels. To achieve reliable results, a proper calibration is necessary; fresh calibration solutions should be prepared on a daily basis. Moreover, any source of sample contamination should be eliminated or minimised. Therefore, we kept the way of sample preparation very simple. As a ubiquitous element in chemical laboratories, mercury is prone to contaminate the samples, especially as inorganic divalent mercury. All chemicals should be checked for impurities of mercury. The precautions against contamination include namely a thorough cleaning of disposables (filters and syringes) and centrifuge tubes, which are used for sample extraction, and the insertion of an injection blank before each sample injection. In addition, several procedure blanks have to be included in each sample series.

All these precautions make the analysis more time consuming. To shorten the analysis time, the simpler calibration can be done using a post-column injection of calibration solutions of methylmercury as a single species, because methylmercury is stable and shows the same sensitivity as inorganic divalent mercury.

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