Talanta 85 (2011) 49–55

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Measurement of methyl mercury (I) and mercury (II) in fish tissues and sediments by HPLC-ICPMS and HPLC-HGAAS

Rajani Jagtap[a], Frank Krikowa[a], William Maher[a,*], Simon Foster[a], Michael Ellwood[b]

[a] Ecochemistry Laboratory, Institute for Applied Ecology, University of Canberra, Bruce, ACT 2601, Australia
[b] Research School of Earth Sciences, The Australian National University, Canberra, ACT 0200, Australia

ARTICLE INFO

Article history:
Received 2 January 2011
Received in revised form 4 March 2011
Accepted 8 March 2011
Available online 1 April 2011

Keywords:
Mercury
Mecaptoethanol extraction
HPLC
ICPMS

ABSTRACT

A procedure for the extraction and determination of methyl mercury and mercury (II) in fish muscle tissues and sediment samples is presented. The procedure involves extraction with 5% (v/v) 2-mercaptoethanol, separation and determination of mercury species by HPLC-ICPMS using a Perkin-Elmer 3 μm C8 (33 mm × 3 mm) column and a mobile phase 3 containing 0.5% (v/v) 2-mercaptoethanol and 5% (v/v) CH_3OH (pH 5.5) at a flow rate 1.5 ml min^{-1} and a temperature of 25 °C. Calibration curves for methyl mercury (I) and mercury (II) standards were linear in the range of 0–100 μg l^{-1} (r^2 = 0.9990 and r^2 = 0.9995 respectively). The lowest measurable mercury was 0.4 μg l^{-1} which corresponds to 0.01 μg g^{-1} in fish tissues and sediments. Methyl mercury concentrations measured in biological certified reference materials, NRCC DORM – 2 Dogfish muscle (4.4 ± 0.8 μg g^{-1}), NRCC Dolt – 3 Dogfish liver (1.55 ± 0.09 μg g^{-1}), NIST RM 50 Albacore Tuna (0.89 ± 0.08 μg g^{-1}) and IRMM IMEP-20 Tuna fish (3.6 ± 0.6 μg g^{-1}) were in agreement with the certified value (4.47 ± 0.32 μg g^{-1}, 1.59 ± 0.12 μg g^{-1}, 0.87 ± 0.03 μg g^{-1}, 4.24 ± 0.27 μg g^{-1} respectively). For the sediment reference material ERM CC 580, a methyl mercury concentration of 0.070 ± 0.002 μg g^{-1} was measured which corresponds to an extraction efficiency of 92 ± 3% of certified values (0.076 ± 0.04 μg g^{-1}) but within the range of published values (0.040–0.084 μg g^{-1}; mean ± s.d.: 0.073 ± 0.05 μg g^{-1}, n = 40) for this material. The extraction procedure for the fish tissues was also compared against an enzymatic extraction using Protease type XIV that has been previously published and similar results were obtained. The use of HPLC-HGAAS with a Phenomenox 5 μm Luna C18 (250 mm × 4.6 mm) column and a mobile phase containing 0.06 mol l^{-1} ammonium acetate (Merck Pty Limited, Australia) in 5% (v/v) methanol and 0.1% (w/v) L-cysteine at 25 °C was evaluated as a complementary alternative to HPLC-ICPMS for the measurement of mercury species in fish tissues. The lowest measurable mercury concentration was 2 μg l^{-1} and this corresponds to 0.1 μg g^{-1} in fish tissues. Analysis of enzymatic extracts analysed by HPLC-HGAAS and HPLC-ICPMS gave equivalent results.

1. Introduction

Mercury is considered to be a major environmental pollutant on a global scale [1]. Environmental and health risks for mercury are caused by methyl mercury that is formed by biomethylation of mercury (II) which is carried out by microorganisms in sediment and soil [1]. Methyl mercury concentrations, however, usually does not exceed 1.5% of the total mercury content in sediments [2]. Methyl mercury binds strongly with biological ligands containing sulfhydryl groups affecting the function of enzymes and interfering with protein synthesis [3]. It is a neurotoxin that readily crosses biological membranes and accumulates to harmful concentrations in organisms and biomagnifies in aquatic food webs to concentrations of toxicological concern [4].

Developing a sensitive, reliable and cost effective method to measure mercury species in environmental samples is important for monitoring mercury concentrations in biota and sediments to avoid ecotoxicological risks and understand the biogeochemical cycling of mercury species in the environment [5]. High Pressure Liquid Chromatography (HPLC) coupled to Inductively Coupled Plasma Spectrometry (ICPMS), Atomic Fluorescence Spectroscopy or Atomic Absorption Spectroscopy (AAS) with or without hydride generation can be used to separate and quantify mercury species [5–7].

One of the main problems in measuring methyl mercury concentrations in sediments is the extraction of this species from a

* Corresponding author. Tel.: +61 6262012531.
E-mail address: bill.maher@canberra.edu.au (W. Maher).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.03.022

Table 1
Operating conditions for ICP-MS.

Plasma conditions	
RF forward power	1200 W
Plasma argon flow rate	15 l min^{-1}
Auxiliary argon flow rate	1.2 l min^{-1}
Nebulizer gas flow	0.92 l min^{-1}
Mass spectrometer settings	
Acquisition mode	Peak hopping
Isotopes monitored	^{202}Hg, ^{201}Hg, ^{200}Hg, ^{198}Hg, ^{181}Ta, ^{184}W, ^{103}Rh, ^{115} In, ^{159}Tb, ^{165}Ho
Dwell time	50 ms
Sweep time	13.5 s
Sweeps per reading	15
Replicates	Total: 3
Integration time	750 ms per element

Table 2
Operating conditions for HPLC-ICP-MS.

Chromatography	
HPLC column	PE C8, 3 μm (33 mm × 3 mm)
Mobile phase	0.5% (v/v) 2-mercaptoethanol in 5% (v/v) CH_3OH, pH 5.3, flow rate, 1.5 ml min^{-1}; temp, 25 °C
Sample volume	100 μl
Plasma conditions	
RF forward power	1200 W
Plasma argon flow rate	15 l min^{-1}
Auxiliary argon flow rate	1.2 l min^{-1}
Nebulizer gas flow	0.84 l min^{-1}
Mass spectrometer settings	
Acquisition mode	Peak hopping
Isotopes monitored	^{202}Hg, ^{201}Hg, ^{200}Hg, ^{198}Hg, ^{181}Ta and ^{184}W
Dwell time	100 ms
Sweeps per reading	1
Replicates	1
Readings	500 (6.52 min)

Table 3
Operating conditions for HPLC-HG-AAS.

Chromatography	
HPLC column	Phenomenex Luna 5 μm C18 (250 mm × 4.6 mm) Mobile phase 5% (v/v) CH_3OH, 0.06 mol l^{-1} CH_3COONH_4 and 0.1% (w/v) L-cysteine, pH 6.8, flow rate, 1.0 ml min^{-1}; temp, 25 °C.
Sample volume	100 μl
Hydride generation	
HCl concentration	2% (v/v)
HCl flow rate	1 ml min^{-1}
$NaBH_4$	0.75% (w/v) in 0.05% (w/v) NaOH
$NaBH_4$ flow rate	1 ml min^{-1}
Gas flow rate	70 ml min^{-1}
Atomic absorption spectroscopy	
Wavelength	253.7 nm
Slit width	0.7 nm
Cell temperature	350 °C

complex matrix. The main requirement of an extraction method is complete separation of the analyte from the interfering matrix without analyte loss, no sample contamination or changes in speciation [2]. The most commonly used procedures for extraction of methyl mercury from sediment samples are the use of microwave heating [2,5–11], sonication [10], distillation [12–15] with acid [6,8,11,15–18], alkali [15] or thiol containing reagents such as 2-mercaptoethanol [19], thiourea [20], or cysteine [21] as extraction reagents. Most of these procedures, however, are incompatible with HPLC-ICPMS [10,14,15,18,22–25] as they use strong acids or bases that even after neutralisation result in poor chromatography of mercury species.

In our laboratory we routinely use HPLC-ICPMS to analyse mercury species in 100–200 fish samples per year and wished to develop a complementary alternative procedure using hydride generation-atomic absorption spectrometry (HGAAS) to reduce costs and free up our ICPMS. We have previously reported the successful use of Protease type XIV to extract mercury (II) and methyl mercury (I) from fish muscle tissues and the separation and quantitation of mercury species by HPLC-ICPMS [26].

This work reports the use of 2-mercaptoethanol for the extraction of mercury species from fish tissues and sediments and analysis by HPLC-ICPMS. The development of a complementary alternative HPLC-HGAAS system for the cold vapour measurement of mercury species in fish tissues is described. The 2-mercaptoethanol extraction procedure for the fish tissues was also compared with the enzymatic extraction using Protease type XIV.

2. Methods

2.1. Equipment

Total mercury concentrations in digests were determined using a Perkin Elmer SCIEX Elan DRC-e ICPMS (Table 1). Mercury species were determined using either a Perkin Elmer series 200 HPLC pump with a Perkin-Elmer 3 μm C8 (33 mm × 3 mm) column coupled to a Perkin Elmer Sciex Elan 6000 ICPMS (Table 2) or a GBC L1100 HPLC pump with a Phenomenox 5 μm Luna C18 (250 mm × 4.6 mm) column coupled to a Perkin Elmer 3100 atomic absorption spectrometer fitted with a Perkin Elmer electrically heated atomisation cell via a Perkin Elmer FIAS 400 flow injection analyser system (Table 3).

2.2. Standards

A stock solution (1000 mg l^{-1}) of mercury (II) was prepared by dissolving the appropriate amount of mercuric chloride (BDH Chemicals Ltd., England) in deionised water (Sartorius arium 611, Australia). The methyl mercury stock solution (1000 mg l^{-1}) was prepared by dissolving the appropriate amount of methyl mercury (I) chloride (Aldrich Chemical Company Inc., USA) in methanol and diluting to volume with deionised water. Stock solutions were stored in airtight bottles and refrigerated. Working standards (3, 6, 12, 25, 50,100 μg l^{-1}) were prepared daily from the stock solution by serial dilution using extracting solutions.

Table 4
Mercury measurements in the certified reference materials and fish tissues using 2-mercaptoethanol extraction and HPLC-ICPMS. Mean ± standard deviation, $n = 3$, where ± reported as 0.0 is <±0.005 μg g^{-1}.

Tissue	Total (μg g^{-1})	Extracted (μg g^{-1})	$MeHg^+$ (μg g^{-1})	Hg^{2+} (μg g^{-1})
Dogfish muscle – NRCC Dorm 2	4.64 ± 0.026	4.40 ± 0.00	4.4 ± 0.8	< 0.01
Dogfish liver – NRCC Dolt 3	3.37 ± 0.14	3.2 ± 0.3	1.55 ± 0.09	1.6 ± 0.2
Albacore Tuna – NIST RM 50	0.95 ± 0.03	1.1 ± 0.1	0.89 ± 0.08	0.18 ± 0.00
Tuna Fish – IRMM IMEP-20	4.32 ± 0.16	3.8 ± 0.6	3.6 ± 0.6	0.38 ± 0.00
Yellow fin Tuna 1 *Thunnus albacares*	1.39 ± 0.09	1.3 ± 0.3	1.14 ± 0.05	0.18 ± 0.00
Yellow fin Tuna 2 *Thunnus albacares*	1.3 ± 0.2	1.2 ± 0.1	0.97 ± 0.05	0.20 ± 0.00
Orange roughy *Hoplostethus atlanticus*	2.5 ± 0.2	2.4 ± 0.1	2.04 ± 0.05	0.34 ± 0.00

2.3. Reagents

All reagents were analytical reagent grade and used without further purification. Enzyme Protease type XIV was obtained from Sigma, Australia. A phosphate buffer (0.06 mol l^{-1}) was prepared by dissolving ammonium di-hydrogen phosphate (Suprapur, Merck, Germany) and (0.1%, w/w) L-cysteine (Sigma–Aldrich, Japan) in high purity water (Sartorius arium 611, Australia) with the pH adjusted to 7.5 with ammonia (Pronalys, May and Becker, Australia). A 2-mercaptoethanol solution was prepared containing 0.5% (v/v) 2-mercaptoethanol (Calbiochem, USA) in 5% (v/v) methanol (Scharlau Chemie S.A., Spain) and used as the extracting reagent and mobile phase for HPLC-ICPMS work. For the optimisation of HPLC-HGAAS system a mobile phase containing 0.06 mol l^{-1} ammonium acetate (Merck Pty Limited, Australia) in 5% (v/v) methanol and 0.1% (w/v) L-cysteine was used.

2.4. Measurement of total mercury concentration

All samples were lyophilised for approximately 24 h (Labconco, Australia) and ground to a homogenous powder using a IKA A11 basic analytical mill (Germany). Total mercury concentrations were determined after nitric acid digestion [27]. Freeze dried tissue or sediment samples (0.07 g) were weighed into 7 ml polytetrafluroacetate digestion vessels (A.I. Scientific, Australia) and 1.0 ml of concentrated nitric acid (Suprapur, Merck KGaA, Germany) added. Samples were digested in a microwave oven (CEM MDS 2000, USA) at 600 W for 2 min, 0 W for 2 min and 450 W for 45 min. After cooling, digests were diluted to 10 ml with internal standard (Ho, In, Tb, Rh: 10 μg l^{-1} in 1% HNO_3) in 10 ml polyethylene vials (Sarstedt, Australia). Total mercury concentrations in digests were measured by ELAN DRC-e ICPMS (standard mode) (Table 1). ^{181}Ta and ^{184}W isotopes were also measured to monitor the production of polyatomic oxides that can interfere with the quantification of mercury.

2.5. Measurement of mercury species concentrations

2.5.1. Fish muscle tissues and sediments by 2-mercaptoethanol extraction

Freeze-dried samples (0.2 g) were weighed into 55 ml polytetrafluroacetate (PTFE) digestion vessels (CEM, USA) with 5 ml and 2.5 ml of 0.5% (v/v) 2-mercaptoethanol for fish tissues and sediment samples respectively. The vessels were heated in a microwave oven (MARS, CEM, USA) at 120 °C for 15 min. The extracts were transferred to acid washed 10 ml polypropylene centrifuge tubes (Sarstedt, Australia) and centrifuged in an Eppendorf centrifuge 5804 for 20 min at 3000 rpm. For sediment samples, after the supernatant was separated, the extraction was repeated and extracts combined.

2.5.2. Fish muscle tissues by enzymatic extraction

Freeze-dried samples (0.2 g) were weighed into 50 ml FEP tubes (Oakridge, Selby Scientific, Australia) with 10 mg of Protease type XIV and 4 ml of phosphate buffer (pH = 7.5) containing 0.05% cysteine [1,26]. The tubes were incubated for 2 h in a hybridisation oven (XTRON HI 200, Bartlett Instruments, USA) at 37 °C with rotation of samples at 20 rpm. Extracts were transferred to acid washed 10 ml polypropylene centrifuge tubes (Sarstedt, Australia), made up to a final volume of 5 ml with buffer and centrifuged in an Eppendorf centrifuge 5804 for 20 min at 3000 rpm.

2.5.3. HPLC-ICPMS and HPLC-HGAAS

All supernatants were filtered through Acrodisc LC 13-mm Syringe filters with 0.2 μm PVDF membranes (Gelman, USA) before

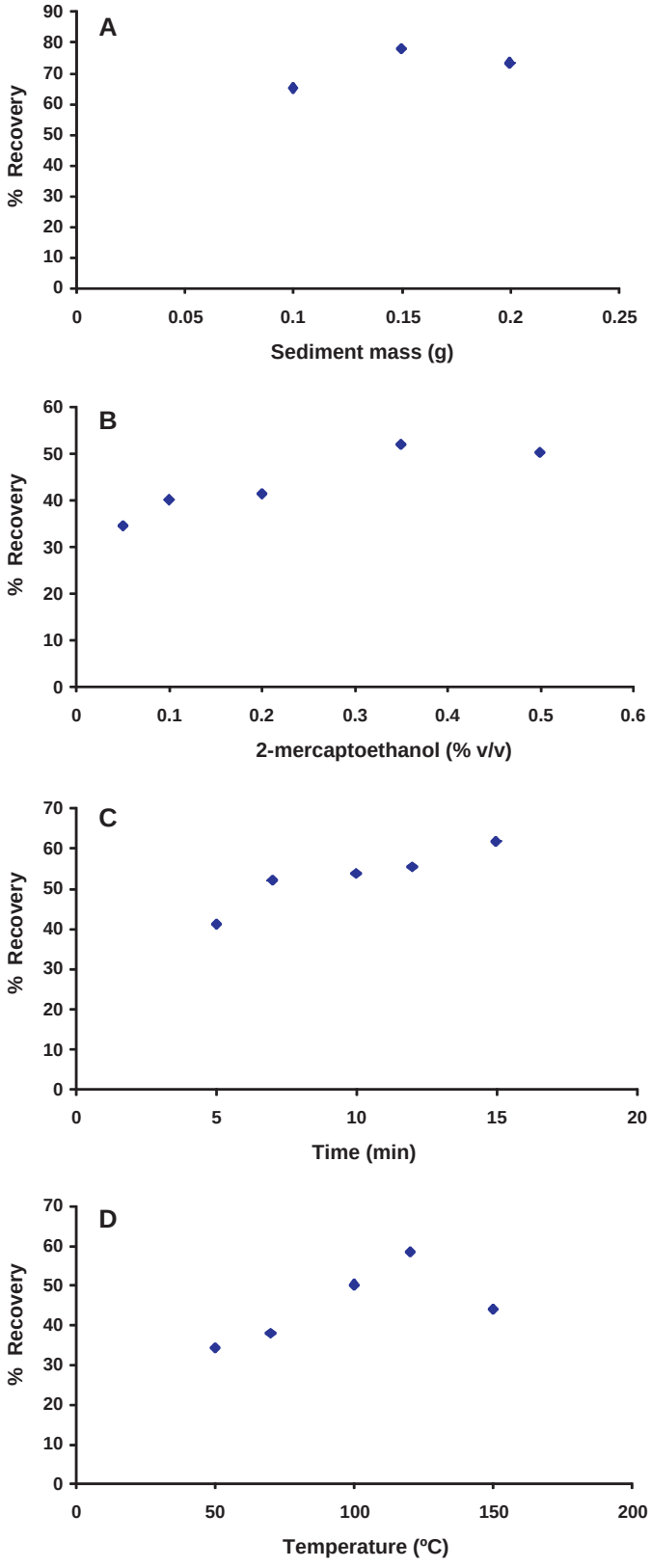

Fig. 1. The effect of sample mass, 2-mercaptoethanol concentration, extraction time and extraction temperature on the recovery of mercury from sediment reference material ERM CC 580.

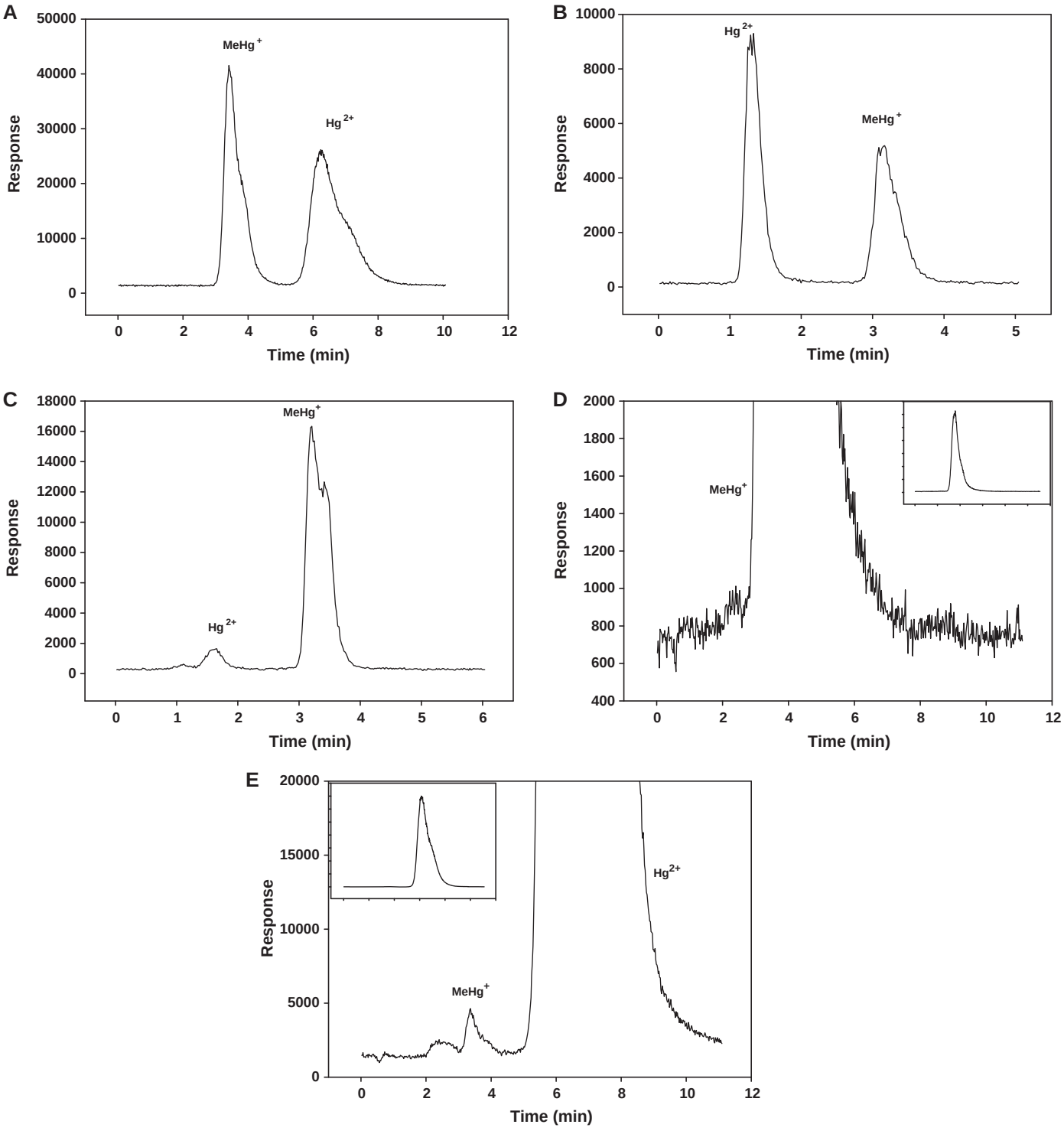

Fig. 2. HPLC-ICPMS chromatogram of mercury standards and reference materials, using A Perkin-Elmer 3 μm C8 (33 mm × 3 mm) column and mobile phase containing 0.5% 2-mercaptoethanol and 5% (v/v) CH_3OH (pH 5.3) at a flow rate, 1.5 ml/min and a temperature of 25 °C. (A) Mercury standards (100 μg/l) in 2-mercaptoethanol. (B) Mercury standards (100 μg/l) in 1% (w/w) cysteine. (C) NRCC DORM – 2 extracted with protease. (D) NRCC DORM – 2 extracted with 2-mercaptoethanol. (E) Sediment reference material ERM CC 580 extracted with 2-mercaptoethanol.

analysis. Aliquots of extracts (100 μl) were injected onto either the HPLC–ICPMS (Table 2) or HPLC-HGAAS system (Table 3). External calibration using the standards 0–100 $\mu g\,l^{-1}$ was used. The chromatography package Turbochrom Navigator (Perkin Elmer, Australia) was used to quantify mercury species by peak area.

3. Results and discussion

3.1. Extraction of mercury species from fish tissues using 2-mercaptoethanol and Protease type XIV

The use of the 2-mercaptoethanol solution to extract the certified fish tissue reference materials NRCC DORM – 2 Dogfish muscle,

Table 5
Mercury measurements in certified reference materials and fish tissues using by HPLC-ICPMS and HPLC-HGAAS using enzymatic extraction. Mean ± standard deviation, $n = 3$, where ± reported as 0.00 is <±0.005 μg g^{-1}.

Tissue	Total (μg g^{-1})	Extracted (μg g^{-1})	HPLC-ICPMS (μg g^{-1})		HPLC-HGAAS (μg g^{-1})	
			$MeHg^+$	Hg^{2+}	$MeHg^+$	Hg^{2+}
Dogfish muscle – NRCC Dorm 2	4.64 ± 0.026	4.7 ± 0.1	4.47 ± 0.11	0.25 ± 0.00	4.6 ± 0.6	0.15 ± 0.05
Dogfish liver – NRCC Dolt 3	3.37 ± 0.14	2.50 ± 0.00	1.40 ± 0.05	1.08 ± 0.00	1.5 ± 0.1	0.9 ± 0.1
Albacore Tuna – NIST RM 50	0.95 ± 0.03	0.97 ± 0.00	0.85 ± 0.05	0.12 ± 0.00	1.20 ± 0.00	<0.1
Tuna fish – IRMM IMEP-20	4.32 ± 0.16	4.5 ± 1	4.24 ± 0.10	0.3 ± 0.1	4.0 ± 0.9	<0.1
Yellow fin Tuna 1 *Thunnus albacares*	1.39 ± 0.09	1.5 ± 0.5	1.3 ± 0.5	0.11 ± 0.00	1.7 ± 0.1	<0.1
Yellow fin Tuna 2 *Thunnus albacares*	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.1	0.12 ± 0.00	0.9 ± 0.4	<0.1
Orange Roughy *Hoplostethus atlanticus*	2.5 ± 0.2	2.60 ± 0.00	2.44 ± 0.00	0.16 ± 0.00	2.90 ± 0.00	<0.1

NRCC Dolt – 3 Dogfish liver, NIST RM 50 Albacore Tuna and IRMM IMEP-20 Tuna fish gave methyl mercury values (4.4 ± 0.8 μg g^{-1}, 1.55 ± 0.09 μg g^{-1}, 0.89 ± 0.08 μg g^{-1} and 3.6 ± 0.6 μg g^{-1} respectively) in agreement with the certified values (4.47 ± 0.32 μg g^{-1}, 1.59 ± 0.12 μg g^{-1}, 0.87 ± 0.03 μg g^{-1}, 4.24 ± 0.27 μg g^{-1} respectively) and with those obtained using the enzymatic extraction procedure (4.47 ± 0.10 μg g^{-1}, 1.4 ± 0.0 μg g^{-1}, 0.85 ± 0.0 μg g^{-1}, 4.24 ± 0.10 μg g^{-1} Table 4). When a range of fish tissues were extracted, complete recovery of mercury was also obtained (Tables 4 and 5).

3.2. Extraction of mercury species from sediments using 2-mercaptoethanol

Initially the extraction of sediments using 5 ml of 0.5% (w/w) cysteine, thiourea and 2-mercaptoethanol solutions was compared (28 ± 3%, <0.1%, and 42 ± 4% respectively). As the best recoveries were obtained using 2-mercaptoethanol, the use of this extractant was further investigated. The effect of sample mass, 2-mercaptoethanol concentration, extraction time and extraction temperature on the recovery of methyl mercury from the sediment certified reference material ERM CC580 were studied (Fig. 1). Initially, 0.2 g of sample using a single extraction was used to determine the effects of mercaptoethanol concentration extraction temperature and time on recoveries of mercury. The use of 0.4–0.5% (w/w) mercaptoethanol solutions, an extraction temperature of 120 °C and an extraction time of 10–15 min gave the best recoveries. Similar recoveries from 0.1 to 0.2 g were obtained. Approximately 70 ± 5% of the methyl mercury was recovered using optimised conditions and a single extraction. When two 2.5 ml aliquots of the 2-mercaptoethanol solution were used, recoveries were increased to 92 ± 3% (0.070 ± 0.002 μg g^{-1} compared to certified value 0.075 ± 0.0037 μg g^{-1}). This is within the range of published values (0.040–0.084 μg g^{-1}; mean ± s.d. 0.073 ± 0.05 μg g^{-1}, $n = 40$; Supplementary Table 1) for this material. Spiking of an in-house reference sediment material with 5, 10, 20 and 40 μg g^{-1} of methyl mercury gave recoveries of 82 ± 12, 92 ± 3, 96 ± 1 and 89 ± 2% respectively. No change in mercury species occurred during extraction and analysis as reported for other extraction techniques.

3.3. Separation of mercury species by HPLC-ICPMS

The use of a 0.5% (v/v) 2-mercaptoethanol in 5% (v/v) methanol gave baseline resolution of methyl mercury and inorganic mercury species (Fig. 2A). Varying the 2-mercaptoethanol concentration had little effect on the retention time of the methyl mercury species but increasing 2-mercaptoethanol concentration decreased the retention time of inorganic mercury. Methanol was added to reduce the retention times of the mercury species. Retention times were not affected in the pH range 5–7. When 2-mercaptoethanol was used as an extractant and present in mobile phase, methyl mercury elutes before inorganic mercury (Fig. 2A). However, when cysteine was used in the extracting solution the order of elution was reversed (Fig. 2B). The retention time of the methyl mercury complexes are the same while the retention time of inorganic mercury is drastically reduced (6.2–1.3 min) in the presence of cysteine. We calculated the pK_a's of mercaptoethanol complexes

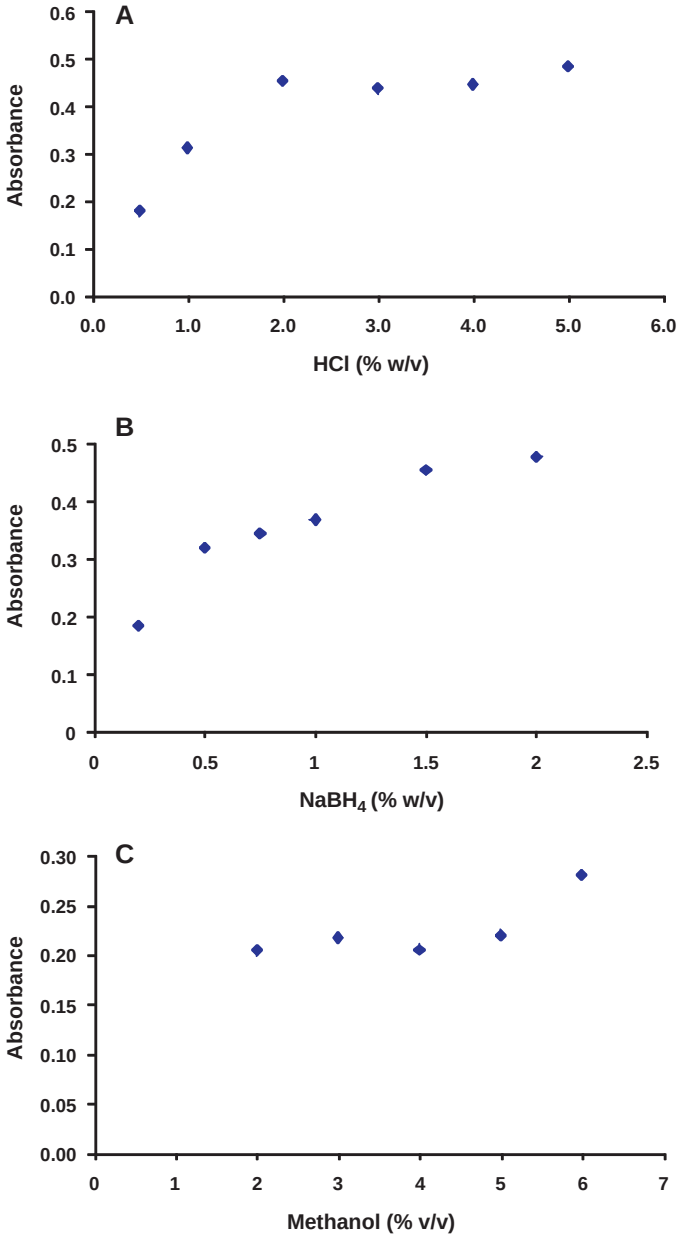

Fig. 3. The effect of HCl and $NaBH_4$ concentration and mobile phase methanol concentration on methyl mercury (100 μg/l) absorbance signals.

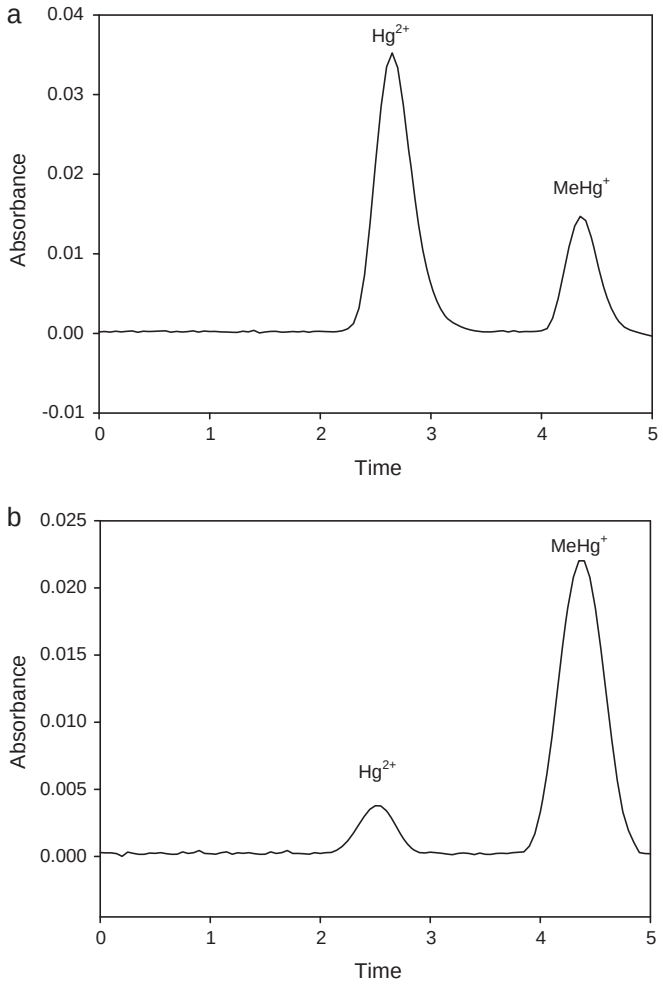

Fig. 4. HPLC-HGAAS chromatogram of mercury standards and a fish tissue sample C18 column and a mobile phase containing 0.06 mol/l ammonium acetate (Merck Pty Limited, Australia) in 5% (v/v) methanol and 0.1% (w/v) L-cysteine at 25 °C. (A) Mercury standards (10 μg/l) in 1% (w/w) cysteine. (B) NRCC DORM – 2 extracted with Protease XVII in ammonium di-hydrogen phosphate buffer pH 7.5 and 0.1% (w/w) L-cysteine.

of inorganic mercury and methyl mercury to be 15.13 and 15.83 respectively and the pK_a's of cysteine complexes of inorganic mercury and methyl mercury to be 7.32 and 7.09 respectively. Thus the mercaptoethanol complexes are charge neutral while the cysteine complexes have a slight positive charge at pH 5.3. It appears that the interaction of the methyl mercury complexes with the C8 column is via the methyl group as both the mercaptoethanol and cysteine complexes have the same retention time. The inorganic mercury mercaptoethanol complex is charge neutral and strongly interacts with the C8 column (longer retention time) while the charged cysteine complex has less affinity for the column and has a much shorter retention time. The elution of the methyl mercury species first is advantageous when analysing sediment extracts. Biological extracts normally contain relatively small amounts of mercury (II) (Fig. 2C and D) while sediment extracts normally contain relatively large amounts on mercury (II) (Fig. 2E). Elution of the mercury (II) peak first leads to severe peak tailing and memory effects in the ICPMS. The use of 2-mercaptoethanol and elution of the methyl mercury peak first allows accurate quantitation of this peak and diversion of the mercury (II) away from the ICPMS thus avoiding memory effects. Similarly, the use of cysteine in the extraction solution and the elution of mercury (II) first allowed the quantitation of small amounts of mercury (II) in the presence of methyl mercury (I). The calibration curves for methyl mercury and mercury (II) standards were linear in the range of 0 -100 μg l^{-1} (r^2 = 0.9990 and r^2 = 0.9995 respectively). The lowest quantifiable mercury concentration was 0.4 μg l^{-1} and this corresponds to 0.01 μg g^{-1} in fish tissues and sediments.

3.4. Optimisation of HPLC-HG-AAS system

The effect of HCl and $NaBH_4$ concentration and mobile phase methanol concentration on mercury absorbance signal were investigated (Fig. 3) and the analytical parameters selected are shown in Table 3. The largest and relatively stable absorbance signals were obtained when 2–5% (v/v) HCl, 1.5–2% (m/v) $NaBH_4$ and 2–5% (v/v) methanol were used. The transfer tubes from the hydride generation cell to the AAS was wound with resistance wire (28B&S Nichrome, Australia) and heated to 100 °C to reduce moisture condensation. Baseline separation of mercury (II) and methyl mercury (I) species was achieved within 6 min for standards and samples (Fig. 4A and B). A C18 column was used to give greater base line separation to allow for sample dispersion that occurs in the FIAS system during hydride generation. The calibration curves for mercury (II) and methyl mercury standards were linear in the range of 0–100 μg l^{-1} (r^2 = 0.9992; r^2 = 0.9831 respectively). The lowest quantifiable mercury concentration was 2 μg l^{-1} and this corresponds to 0.1 μg g^{-1} in fish tissues.

Measurement of enzymatic digests of a range of fish tissues by HPLC-ICPMS and HPLC-HGAAS gave similar results (Table 5). The low concentrations of inorganic mercury in fish, however, could not be measured.

4. Conclusions

Using 0.5% (v/v) 2-mercaptoethanol, mercury is quantitatively extracted from fish tissues while good recoveries of mercury from sediments are obtained. Using extracting reagents containing either 2-mercaptoethanol or cysteine changes the elution order of inorganic mercury and methyl mercury species from the C8 column. This has the advantage that small amounts of either species can be determined in the presence of relatively large amounts of the other species by using the extraction reagent that results in elution of the lowest concentration component first avoiding problems caused by peak tailing.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.03.022.

References

[1] S. Tutschku, M.M. Schantz, S.A. Wise, Analytical Chemistry 74 (2002) 4694–4701.
[2] E. Ramalhosa, et al., Journal of Analytical Atomic Spectrometry 16 (2001) 643–647.
[3] C. Maggi, et al., Analytica Chimica Acta 641 (2009) 32–36.
[4] S.N. Luoma, P.S. Rainbow, Science and Lateral Management (2008) 354–392.
[5] C.M. Tseng, et al., Journal of Analytical Atomic Spectrometry 12 (1997) 743–750.
[6] C.M. Tseng, et al., Chemosphere 39 (7) (1999) 1119–1136.
[7] R.A. Lorenzo, et al., Trends in Analytical Chemistry 18 (6) (1999) 410–416.
[8] M.J. Vazquez, et al., Analytical Chemistry 69 (1997) 221–225.
[9] P. Rivaro, et al., Microchimica Acta 158 (2007) 345–352.
[10] G.M.M. Rahman, H.M.S. Kingston, Analytical Chemistry 76 (13) (2004) 3548–3555.
[11] C. Dietz, Y. madrid, C. Camara, Journal of Analytical Atomic Spectrometry 16 (2001) 1397–1402.
[12] K.C. Bowles, S.C. Apte, Analytica Chimica Acta 419 (2000) 145–151.
[13] R. Falter, G. Ilgen, Fresenius Journal of Analytical Chemistry 358 (1997) 407–410.
[14] R.-D. Wilken, R. Falter, Applied Organometallic Chemistry 12 (1998) 551–557.

[15] H. Hintelmann, et al., Fresenius Journal of Analytical Chemistry 358 (1997) 363–370.
[16] J.S. Landaluze, et al., Analytica Chimica Acta 508 (2004) 107–117.
[17] M. Leermakers, et al., Analytical Bioanalytical Chemistry 377 (2003) 327–333.
[18] Y. Han, et al., Analytical Bioanalytical Chemistry 375 (2003) 428–436.
[19] J. Margetınova, P. Houserova-Pelcova, V. Kuban, Analytica Chimica Acta 615 (2008) 115–123.
[20] C.W. Shade, R.J.M. Hudson, Environmental Science and Technology 39 (2005) 4974–4982.
[21] G. Hu, X. Wang, Analytical Letters 31 (1998) 1445–1459.
[22] I. Cattani, et al., Talanta 74 (2008) 1520–1526.
[23] R. Falter, Chemoshere 39 (1999) 1051–1073.
[24] R. Falter, Chemosphere 39 (1999) 1075–1091.
[25] P. Quevauviller, et al., Applied Organometallic Chemistry 12 (1998) 531–539.
[26] R. Rai, W. Maher, F. Kirkowa, Journal of Analytical Atomic Spectrometry 12 (2002) 1560–1563.
[27] S. Baldwin, M. Deaker, W. Maher, Analyst 119 (1994) 1701–1704.