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On-line coupling of flow injection displacement sorption preconcentration to high-performance liquid chromatography for speciation analysis of mercury in seafood

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Abstract

A simple and cost-effective method for speciation analysis of trace mercury in seafood was developed by on-line coupling flow injection microcolumn displacement sorption preconcentration to high-performance liquid chromatography (HPLC) with UV detection. The methodology involved the presorption of the Cu-PDC (pyrrolidine dithiocarbamate) chelate onto a microcolumn packed with a cigarette filter sorbent, simultaneous preconcentration of Hg(II), methylmercury (MeHg), ethylmercury (EtHg), and phenylmercury (PhHg) onto the microcolumn through a displacement reaction with the presorbed Cu-PDC, and their subsequent elution from the microcolumn for on-line HPLC separation. Interferences from heavy metal ions with lower stability of their PDC chelates relative to Cu-PDC were minimized without the need of any masking agents. With the consumption of 4.0 ml of sample solution, the enrichment factors were about 80. The detection limits were 10–25 ng g^{-1} (as Hg) in fresh tissue. Precision (R.S.D. (%), $n = 5$) ranged from 2 to 3% at the 500 μg l^{-1} (as Hg) level. The developed technique was validated by analyzing a certified reference material (DORM-2, dogfish-muscle), and was shown to be useful for mercury speciation in real seafood samples.

Keywords: Food analysis; Speciation analysis; Preconcentration; Flow injection; Mercury

1. Introduction

Mercury is a highly dangerous element because of its accumulative and persistent character in the environment and biota [1,2]. It is well known that the toxicity, biogeochemical behavior and transportation of mercury in the environment are heavily dependent on its chemical form [3,4]. Methylmercury is the most commonly occurring organo-mercury compound in environmental and biological materials and the most toxic mercury species, which is of particular concern because of its accumulation as it passes through the food chain, whereas ethylmercury and phenylmercury are quite rarely present in the environment [1–4]. In the last two decades, the development of species-selective analytical methodologies has made possible the identification and quantification of such species. The most common methods of mercury speciation are gas chromatography (GC) [4,5] and high-performance liquid chromatography (HPLC) [4–6] coupled with a mercury-specific detector. The use of HPLC has the advantage of simplified sample preparation. It is essential to form volatile, thermally stable derivatives for GC, but not for HPLC. HPLC allows separation of mercury compounds at ambient temperature, and easy automation [7]. The detection methods coupled with GC or HPLC for mercury speciation include atomic absorption spectrometry (AAS) [4–6,8,9], atomic fluorescence spectrometry (AFS) [4–7,10–12], inductively coupled plasma-mass spectrometry (ICP-MS) [11,13,14], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [15,16] and microwave-induced plasma atomic emission spectrometry (MIP-AES) [4,5,17]. Although these detection methods are attractive for mercury speciation because of their excellent detection limits and selectivity, their relatively high instrumental and/or running costs as well as complicated instrument setup make it difficult to employ these hyphenated techniques for routine speciation analysis. In this aspect, HPLC with UV detection

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would provide a simple and cost-effective way for speciation analysis of mercury if the detection limits could be lowered sufficiently for the detection of mercury species in real samples.

Owing to matrix interferences and/or insufficient detection power, however, direct determination of mercury species in complicated matrices is difficult, and a preliminary preconcentration step is usually mandatory [18,19]. Flow injection (FI) on-line solid phase extraction preconcentration techniques based on the sorption of their pyrrolidine dithiocarbamate (PDC) [12,20], diethyldithiocarbamate (DDTC) [12], dithizone [18] and dithiophosphoric acid diacyl ester [19] chelates onto a RP-C_{18} packed microcolumn have been developed for mercury speciation analyses with HPLC. However, these preconcentration systems suffer undesirable interferences from coexisting transition metals owing to their competition for the complexing agent and/or active sites at the column packing.

Recently, an FI on-line displacement sorption preconcentration coupled with flame atomic absorption spectrometry (FAAS) was developed for interference-free determination of trace copper in complicated matrices using a PTFE knotted reactor (KR) as soprtion medium [21]. The methodology involved on-line formation of the Pb-DDTC chelate, presorption of the resultant Pb-DDTC onto the inner walls of the KR, retention of the analyte Cu(II) onto the inner walls of the KR through a displacement reaction between the Cu(II) and the sorbed Pb-DDTC, elution of the retained analyte with ethanol for FAAS detection. Interferences from coexisting ions with lower stability of their DDTC complexes relative to Pb-DDTC due to their competition for DDTC and the active sorption sites on the inner walls of the KR in conventional FI on-line sorption preconcentration systems were eliminated without need for any masking agents. However, work dealing with on-line coupling of the FI on-line displacement sorption preconcentration to HPLC for speciation analysis has not been reported before.

In the present paper, an FI on-line displacement sorption preconcentration using a microcolumn packed with cigarette filter material was coupled to HPLC with UV detection to develop a simple, selective, and sensitive method for speciation of mercury in seafood.

2. Experimental

2.1. Apparatus

The chromatographic system consisted of a Waters model 600 HPLC pump and a Waters 2996 Photodiode Array Detector (Milford, MA, USA). All separations were achieved on an analytical reversed-phase column (Maxsil ODS 5 μm, 4.6 mm i.d. × 25 cm length, Hertz Biotech, Zibo, China) at room temperature under isocratic conditions. The Empower Software was used to acquire and process spectral and chromatographic data from the Photodiode Array Detector 2996. The diode array detector was operated between 210 and 350 nm, setting 281 nm for peak area measurements.

A Model FIA-3100 flow injection system (Vital Instruments, Beijing, China) for the displacement sorption preconcentration. It consists of two peristaltic pumps and a standard rotary injection valve (eight ports on the rotor, and eight ports on the stator). The rotation speed of the two peristaltic pumps, their stop and go intervals, and the actuation of the injection valve were programmed (see Table 1). Tygon pump tubes were used to deliver the samples and reagents. Small-bore (0.5 mm i.d.) PTFE tubings were adapted for all connections, which were kept the shortest possible length to minimize the dead volume.

2.2. Reagents

All reagents were of the highest available purity and at least of analytical grade. Doubly deionized water (DDW, 18 MΩ cm) obtained from a WaterPro water system (Labconco Corporation, Kansas City, MO, USA) was used throughout.

The chelating agent solution was prepared by dissolving ammonium pyrrolidine dithiocarbamate (APDC) (Sigma) in DDW just prior to use. Copper solutions were prepared by stepwise dilution of the stock solution of 1000 mg l^{-1} Cu (National Research Center for Standard Materials, Beijing, China) immediately before use. Concentrated nitric acid and hydrochloric acid were from the Third Chemicals Co., Tianjin, China.

Table 1
Operational sequence of FI on-line displacement sorption preconcentration system coupled with HPLC for speciation analysis of mercury

Step	Function	Valve position		Duration (s)	Medium pump	Flow rate (ml min^{-1})	
		Injector	Electromagnatic			Pump 1	Pump 2
1 (Fig. 1a)	Presorption	Inject	Off	60	3 mg l^{-1} Cu, 0.02% APDC	Off	3.6
2 (Fig. 1b)	Column washing	Fill	Off	30	DDW	Off	3.0
3 (Fig. 1b)	Displacement sorption	Fill	On	60	Sample	4.0	Off
4 (Fig. 1b)	Remove residual solution	Fill	Off	20	Air	3.0	Off
5 (Fig. 1a)	Fill eluent loop	Inject	Off	10	Methanol	3.0	Off
6 (Fig. 1b)	Elution and eluate introduction into HPLC system	Fill	Off	15	Air	3.0	Off

The solvents used to prepare the mobile phase were methanol (Concord Technology, Tianjin, China) and acetonitrile (Tianjin Hongyan Chemicals, Tianjin, China). The mobile phase was filtered through 0.45 μm filter prior to use. High purity helium was used for degassing the mobile phase.

Inorganic mercury stock solution of 1000 mg l^{-1} was prepared by dissolving the chloride mercury (Beijing Chemical Co., Beijing, China) in DDW. The stock solutions of 1000 mg l^{-1} (as Hg) of methylmercury (MeHg), ethylmercury (EtHg), and phenylmercury (PhHg) were prepared by dissolving methylmercury, ethylmercury, and phenylmercury chloride (Alfa) in methanol, respectively. Working solutions were prepared from the stock solutions by stepwise dilution just before use.

2.3. Samples

A certified reference material DORM-2 (dogfish-muscle, NRCC) was analyzed to check the accuracy of the developed technique. Fresh seafood samples were collected from local markets. The tissue was homogenized in a blender for subsequent extraction.

2.4. Extraction procedure for mercury speciation

An acid leaching procedure as reported by Ortiz et al. [22] was employed to liberate mercury species from biological samples. Briefly, 5 ml of 5 mol l^{-1} hydrochloric acid was added to 0.5 g of the certified reference material DORM-2, or 5 g of the homogenized fish tissue in a 10 ml centrifuge tube. The mixture was then placed in an ultrasonic bath for 10 min. After extraction, the suspension was centrifuged at 3500 rpm for 10 min, and the supernatant was taken to a 50 ml flask. The residue was extracted again as described above. The two supernatant portions were combined, neutralized with 10 mol l^{-1} NaOH, adjusted to pH 4.3 with acetic acid–acetate buffer, and diluted to volume with DDW just before determination.

2.5. Preconcentration and separation procedure

The PTFE microcolumn (1.5 cm × 4 mm i.d.) used for the preconcentration was packed with the sorbent from a cigarette filter. The cigarette filter was directly obtained from the Baisha cigarettes (Changsha Cigarette Factory, Hunan, China). The main composition of the cigarette filter was characterized to be the cellulose acetate fiber by FT-IR. No special re-use and conditioning conditions for the sorbent other than those given in Table 1 were required. A freshly prepared microcolumn can be used for about 100 preconcentration cycles without significant loss of preconcentration efficiency and precision.

The developed FI manifold for the two different valve positions is shown in Fig. 1. Details of the duration and function of each step for on-line microcolumn displacement sorption preconcentration are given in Table 1. A complete cycle of the separation and preconcentration required 195 s including a sample loading time of 60 s.

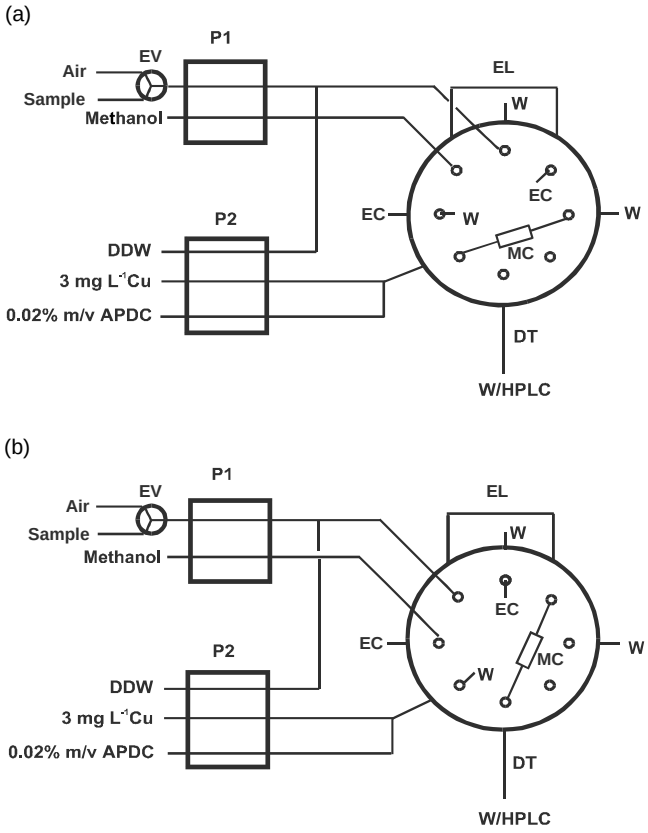

Fig. 1. The FI manifold for the on-line microcolumn displacement sorption preconcentration coupled with HPLC for mercury speciation. P1 and P2, peristaltic pumps; MC, microcolumn; DT, delivery tubing; EL, eluent loop; EC, eluent container; W, waste; EV, electromagnetic valve; 'off' for air flow, and 'on' for sample solution; HPLC, high-performance liquid chromatography. Valve position: (a) inject; (b) fill.

3. Results and discussion

3.1. Consideration of an FI on-line displacement sorption preconcentration procedure for HPLC speciation of mercury with UV detection

In previous work on FI on-line preconcentration systems for HPLC [12,18–20], the preconcentration of mercury species was always achieved by sorption of the chelates of mercury species formed by reacting with a chelating agent through direct merging sample solution with a chelating agent solution on an RP-C_{18} microcolumn. Such preconcentration systems suffered undesirable interferences from coexisting transition metals owing to their competition for the complexing agent and/or active sites at the column packing. Moreover, the preconcentrated chelates of other heavy metal with the chelating agent would interfere the subsequent HPLC separation and UV detection [23].

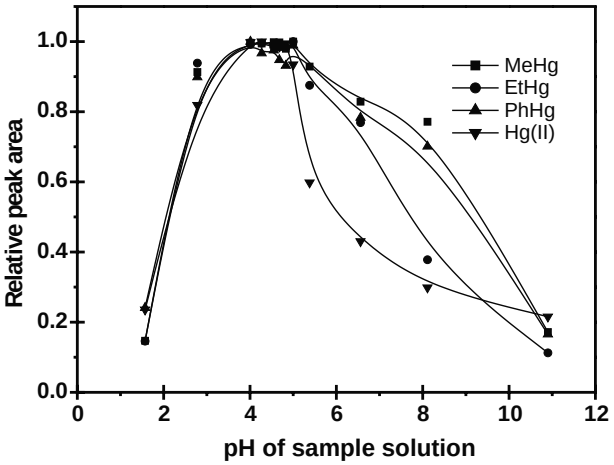

Fig. 2. Effect of pH in sample solution on the displacement sorption for 500 μg l^{-1} of each individual mercury species. All other conditions are as in Table 1 and Fig. 1.

In the present work the cigarette filter instead of RP-C_{18} was employed as the sorbent for simultaneous preconcentration of mercury species as PDC chelates. Compared with the RP-C_{18} sorbent, the cigarette filter sorbent is much cheaper and more easily available. It is well known that the cigarette filter can efficiently adsorb many poisonous organic compounds, and hence alleviate the poisonous effect on smokers. Our previous experiments demonstrated that the sorbent also favored the adsorption of the neutral hydrophobic DDTC chelate of each individual mercury species in a certain pH range [24]. However, the DDTC–cigarette filer sorbent system was found unsuitable for effectively simultaneous preconcentration of Hg(II), MeHg, EtHg, and PhHg in one pH range [24]. In the present work, the cigarette filter sorbent was found to be very effective for the simultaneous sorption of the PDC chelates of the four mercury species in the pH range of 4.0–5.0 (see Fig. 2).

To minimize the interferences from coexisting heavy metal ions, a newly developed FI sorption preconcentration and separation technique [21] was coupled to HPLC with UV detection for mercury speciation. To this end, Cu(II) and APDC solution merged on-line and passed through the microcolumn packed with the cigarette filter material, the formed Cu-PDC chelate was then presorbed onto the microcolumn. Subsequently, the sample solution was loaded onto the column, and the mercury species in the sample solution were preconcentrated onto the microcolumn as a result of the displacement reaction between the mercury species and the presorbed Cu-PDC because of the relatively higher stability of the PDC chelates of mercury species than Cu-PDC. However, those coexisting heavy metal ions with poorer stability of their PDC chelates than Cu-PDC could not displace Cu(II) from the presorbed Cu-PDC, and hence would be left in the solution and removed to the waste. In this way, the interferences from heavy metal ions with relatively lower stability of the PDC chelates of mercury species than Cu-PDC were eliminated/minimized without the need of any masking agents.

3.2. Optimization of HPLC conditions

There were the PDC chelates of the four mercury species, the chelating agent APDC that had been presorbed onto the preconcentration column, and the undisplaced Cu-PDC chelate in the eluate from the preconcentration column, which should subject to subsequent HPLC separation. To this end, an RP-HPLC mode with an RP-C_{18} separation column and a mobile phase of the methanol, acetonitrile and water mixture was employed. Potential factors influencing the HPLC separation were optimized, including the pH of the mobile phase, the content of methanol, acetonitrile, and water in the mobile phase, and the concentration of APDC included in the mobile phase.

The pH of the mobile phase used for HPLC was the main operational parameter. The PDC chelates of the four mercury species and APDC could realize baseline-separation in the pH range of 2.8–3.4 within 20 min. Accordingly, a mobile phase pH of 3.0 was chosen for baseline separation of four mercury species, Cu(II) and APDC.

The contents of methanol, acetonitrile, and water in the mobile phase are the most important factor for the separation. Inclusion of 33% (v/v) of acetonitrile in the mobile phase was found to be effective for eliminating the broadening of APDC peak, and separating APDC from its mercury chelates. Decreasing the methanol content in the mobile phase resulted in longer retention times of the four mercury chelates.

The sorption and decomposition of the chelates to the residual silanol groups on the separation column led to reduced peak heights and longer retention times, which could be avoided by addition of free ligand to the mobile phase [23]. In this work, the effect of the APDC concentration on the separation was investigated in the range of 0.005–0.05% APDC. Adding 0.01% APDC to the mobile phase was found to prevent the sorption and decomposition of the mercury-PDC chelates. Methanol:acetonitrile:water (45:33:22) containing 0.01% APDC (pH 3.0) was found to be a suitable mobile phase for baseline separation of the four mercury species, Cu(II) and APDC on an RP-C_{18} column.

3.3. Factors affecting the presorption of Cu-PDC onto the preconcentration column

The displacement adsorption for mercury species related to the on-line chelating and presorption of Cu-PDC, and consequently was subject to such factors as the concentration of copper solution, the concentration of chelating agent APDC, and the acidity for the Cu-PDC formation and presorption.

Studies on the effect of the Cu concentration on the integrated absorbance of 500 μg l^{-1} (as Hg) of each individual mercury species showed that the integrated absorbance increased significantly with the Cu concentration until

2 mg l^{-1} Cu, and then remained constant in the range of 2–5 mg l^{-1} Cu, but decreased with further increase of Cu concentration (over 5 mg l^{-1} Cu). For further experiments, a concentration of 3 mg l^{-1} Cu was selected for presorption.

In this work, nitric acid was selected to adjust the acidity of Cu solution. The effect of nitric acid concentration on the formation of Cu-PDC and its presorbed onto the preconcentration microcolumn was tested at an APDC concentration of 0.02%. The optimal sample acidity ranged from 0.0001 to 1% (v/v) HNO_3. Lower (<0.1% (v/v)) or higher (>0.5% (v/v)) HNO_3 concentrations were not favorable for the on-line formation of Cu-PDC chelate and/or its subsequent sorption. Therefore, a HNO_3 concentration of 0.2% (v/v) was used for further experiments.

The effect of APDC concentration on the integrated absorbance of 500 μg l^{-1} (as Hg) of mercury species was examined at a Cu concentration of 0.3 mg l^{-1}. It was found that the optimal APDC concentration ranged from 0.001 to 0.05%. Therefore, a concentration of 0.02% APDC was selected for the rest of this work.

3.4. Factors affecting the on-line displacement sorption preconcentration of mercury species

Sample acidity plays an important role in the displacement sorption preconcentration because it would affect the stability of the presorbed Cu-PDC, the retention of mercury species chelates, and/or the displacement reaction. Fig. 2 shows the effect of sample acidity on the integrated absorbance of 500 μg l^{-1} mercury species. No preconcentration of mercury species was observed below pH 1.5 and over 10. The absorbance increased rapidly as the pH of sample solution increased from 1.8 to 4.0, then leveled off in the pH range of 4.0–5.0. Further increase in pH led to a significant decrease in the absorbance. For efficient simultaneous preconcentration of the four mercury species, a pH 4.3 of sample solution was selected.

The influence of sample loading flow rate on the displacement sorption preconcentration of mercury species were investigated with 500 μg l^{-1} mercury species for 60 s preconcentration. It was found that the integrated absorbance increased linearly with increasing a sample loading flow rate up to at least 6.0 ml min^{-1}. Studies on the effect of sample loading time on the absorbance of 500 μg l^{-1} mercury species at a sample flow rate of 4.0 ml min^{-1} demonstrated that the absorbance increased almost linearly as the sample loading time increased up to at least 150 s.

3.5. Desorption of the analytes from the preconcentration column

In this work, methanol was selected as the eluent for the desorption of the mercury species chelates from the preconcentration column because it is also a major component of the mobile phase and it is a strong solvent for the elution. No detrimental effect of the methanol on the separation of mercury species was observed. Methanol (200 μl) at a flow rate of 3.0 ml min^{-1} sufficed to completely elute the sorbed mercury chelates from the microcolumn.

3.6. Evaluation of potential interferences

In order to demonstrate the selectivity of the developed FI on-line microcolumn displacement sorption preconcentration system for the determination of mercury species, the effect of Cu(II), Fe(III), Ni(II), Co(II), Zn(II), Cd(II), Cr(III), and Pb(II) on the measurement of 500 μg l^{-1} of each individual mercury species was investigated. The results showed that up to 10 mg l^{-1} of these metal ions had no significant interferences with the determination of the four mercury species. For comparison, the effect of these heavy metal ions was also examined for a conventional FI on-line sorption preconcentration procedure (i.e. the PDC chelates of mercury species were formed through direct merging of sample solution with APDC solution, and were subsequently sorbed onto the microcolumn packed with the cigarette filter sorbent) for HPLC speciation of 500 μg l^{-1} of each individual mercury species under the same conditions for FI and chemical variables as in the FI displacement sorption preconcentration. It was found that even the presence of 300 μg l^{-1} of these metal ions led to some unwanted peaks, impaired the peaks of mercury species and sensitivity. These results clearly demonstrated the high selectivity of the present FI on-line displacement sorption preconcentration and separation for HPLC speciation of mercury species.

3.7. Analytical figures of merit

The analytical characteristic data of the present FI on-line displacement sorption preconcentration and separation coupled with HPLC for speciation of the four mercury species were summarized in Table 2. With the consumption of 4.0 ml sample solution, the enrichment factors ranged from 75 to 85 in comparison with direct injection of 200 μl sample solution. Calibration graphs were linear in the ranges of 10–2000 μg l^{-1} for PhHg and Hg, 10–3000 μg l^{-1} for MeHg and EtHg. The precision (R.S.D.) of the peak area for five replicate determinations of a mixture of 500 μg l^{-1} (as Hg) of each individual mercury species were in the range of 2–3%. The detection limits defined as the three times

Table 2
Characteristic data of optimized FI on-line microcolumn displacement sorption preconcentration for HPLC speciation analysis of mercury

Enrichment factor[a]	75–85
Detection limit in fresh tissue (ng g^{-1})[b]	10–25
Precision ($n = 5$) (%, R.S.D.)[c]	2–3

[a] Values for preconcentration of 4.0 ml solution in comparison with direct injection of 200 μl sample solution.
[b] For 50 ml extract of 5 g fresh tissue.
[c] Using a mixture of 500 μg l^{-1} of each mercury species.

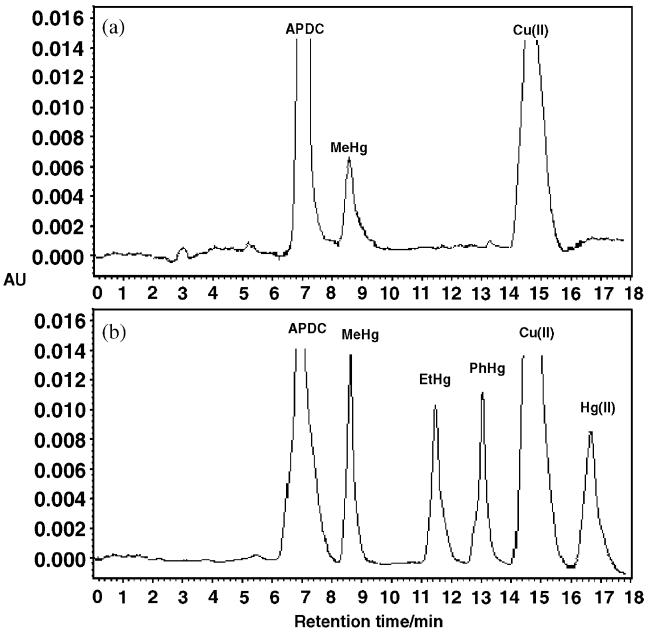

Fig. 3. Chromatograms of (a) the extract of DORM-2 and (b) a mixture standard solution of 50 μg l^{-1} (as Hg) of each individual mercury species obtained by the developed FI on-line microcolumn displacement sorption preconcentration and separation coupled with HPLC monitored at 281 nm for mercury speciation. HPLC separation column: Maxsil ODS 5 μm, 4.6 mm i.d. × 25 cm length; mobile phase: a mixture of methanol, acetonitrile, and water (45:33:22) containing 0.01% APDC (pH 3.0); flow rate of the mobile phase: 1 ml min^{-1}. Other conditions are as in Fig. 1 and Table 1.

of the baseline noise were 10–25 ng g^{-1} (as Hg) in fresh tissue.

To evaluate the accuracy of the developed method, a certified reference material DORM-2 (dogfish-muscle, NRCC) was analyzed. The chromatograms for the extract of DORM-2 and a mixture standard solution of 50 μg l^{-1} (as Hg) of each individual mercury species obtained by the developed FI on-line microcolumn displacement sorption preconcentration and separation coupled with HPLC monitored at 281 nm are shown in Fig. 3. The concentration of methylmercury (as Hg) in DORM-2 was found to be 4.4 ± 0.2 μg g^{-1} by the proposed method with simple external calibration, in good agreement with the certified value (4.47 ± 0.32 μg g^{-1}) for methylmercury content. No other three mercury species were detected.

To evaluate the usefulness of the developed method for the speciation analysis of mercury at the levels usually found in seafood products, seven real seafood samples collected from local markets were analyzed. The recoveries of mercury species (50 μg l^{-1}) spiked in these seafood samples ranged from 85 to 110%. No EtHg and PhHg were detected in any of these samples. Inorganic mercury Hg(II) was found in only two samples, ranging from 290 to 560 ng g^{-1} (wet mass). The concentration of MeHg was determined to be 105–285 ng g^{-1} (wet mass) in five out of seven samples studied. The concentration of MeHg in the other two samples was below the detection limit (19 ng g^{-1}, wet mass). The concentration levels of mercury species in the seafood samples studied are generally below the maximum permissible mercury concentration in fish (usually in the range of 0.4–1.0 μg g^{-1}, wet mass) used for human consumption established by many countries [25,26].

4. Conclusions

The present results show the feasibility of the developed FI on-line microcolumn displacement sorption preconcentration coupled with HPLC with UV detection for mercury speciation in seafood. Compared with HPLC hyphenated with an element-specific detector (e.g. ICP-MS, ICP-OES, CV-AAS, and CV-AFS), the present method provides simple, low instrumental and running cost, and easy operation for mercury speciation in seafood.

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