Speciation analysis of mercury in sediments, zoobenthos and river water samples by high-performance liquid chromatography hyphenated to atomic fluorescence spectrometry following preconcentration by solid phase extraction

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A B S T R A C T

A high-pressure microwave digestion was applied for microwave-assisted extraction (MAE) of mercury species from sediments and zoobenthos samples. A mixture containing 3 mol L⁻¹ HCl, 50% aqueous methanol and 0.2 mol L⁻¹ citric acid (for masking co-extracted Fe³⁺) was selected as the most suitable extraction agent. The efficiency of proposed extraction method was better than 95% with R.S.D. below 6%. A preconcentration method utilizing a "home-made" C18 solid phase extraction (SPE) microcolumns was developed to enhance sensitivity of the mercury species determination using on-column complex formation of mercury-2-mercaptophenol complexes. Methanol was chosen for counter-current elution of the retained mercury complexes achieving a preconcentration factor as much as 1000. The preconcentration method was applied for the speciation analysis of mercury in river water samples. The high-performance liquid chromatography-cold vapour atomic fluorescence spectrometric (HPLC/CV-AFS) method was used for the speciation analysis of mercury. The complete separation of four mercury species was achieved by an isocratic elution of aqueous methanol (65%/35%) on a Zorbax SB-C18 column (4.6 mm x 150 mm, 5 μm) using the same complexation reagent (2-mercaptophenol). The limits of detection were 4.3 μg L⁻¹ for methylmercury (MeHg⁺), 1.4 μg L⁻¹ for ethylmercury (EtHg⁺), 0.8 μg L⁻¹ for inorganic mercury (Hg²⁺), 0.8 μg L⁻¹ for phenylmercury (PhHg⁺).

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1. Introduction

Mercury and mercury containing compounds are ubiquitous global pollutants that have been found at various concentration levels in all environmental compartments. Generally, atmospheric deposits (dry and/or wet), sorption/desorption deposition to sediments, re-emission to the atmosphere as elemental mercury and/or volatile mercury species are typical attributes of an aquatic mercury cycle. Some portion of the total mercury is accumulated by biota, with biomagnification of mercury species in higher trophic levels of food chains. The mercury cycle in aquatic ecosystems is complicated by numerous possible mercury species that are present, methylation and demethylation transformation processes within the lake

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or watershed and exchanges through tributaries and groundwater, and by many variables affecting speciation [1]. The speciation analysis of mercury in water samples of aquatic ecosystems does not convey well the real long-term pollution of a monitored site. Mercury species are quickly incorporated into bio-geochemical cycles. For this reason, it is necessary to pay attention to all components of aquatic ecosystem as well [2].

The speciation analysis of mercury in any compartment of aquatic ecosystems is very complicated from analytical aspect. Not only sediment matrix and very low contents of mercury species in these materials complicate the mercury species determination. Also the preservation of original mercury species distribution, their stability during sampling, storage and analysis belongs to indispensable analysis steps [3–8]. Production of artifact methylmercury is a well-known aspect. Not only sediment matrix and very low contents of aquatic ecosystems is very complicated from analytical view [2].

Production of artifact methylmercury is a well-known problem mainly associated to the measurement of mercury species. Also the preservation of original mercury species distribution, their stability during sampling, storage and analysis belongs to indispensable analysis steps [3–8]. Production of artifact methylmercury is a well-known problem mainly associated to the measurement of mercury species. Also the preservation of original mercury species distribution, their stability during sampling, storage and analysis belongs to indispensable analysis steps [3–8].

In addition to the most commonly used acid digestion, such as the Westbø method [9] and its modifications [8,10–18] or alkaline digestion [15,19–22] most often performed in a microwave or ultrasound extractor, supercritical fluid extraction (SFE) with carbon dioxide [23–25] or acid vapour distillation [8,11,26,27] are used for isolation of mercury species from sediment samples.

Usually, a preconcentration step is included in analytical procedures because of the quite low concentration of mercury species in unpolluted environmental samples. Different approaches have been proposed for preconcentration of mercury compounds from water including liquid–liquid, liquid–solid or more recently solid phase extraction (SPE) and solid phase microextraction (SPME) processes. In most applications, SPME is hyphenated to GC, but recent studies have reported the hyphenation of SPME to HPLC [28]. A diversity of complexing agents (mostly sulphur containing reagents) such as N,N-disubstituted dithiocarbamates (ammonium pyrrolidinedithiocarbamate – APDC [27–35], diethylidithiocarbamate [27,31,36], sulphhydril [37], 2-mercaptoethanol [37,38] and dithizone [39,40] have been used for sorption of mercury species on C18 solid phase cartridges. Although complexes of mercury species with 2-mercaptophenol are very easily retained on C18 solid phase cartridges owing to its chemical structure, the reagent was not till now used for preconcentration of mercury species.

Among numerous procedures described in the literature, high-performance liquid chromatography hyphenated to cold vapour atomic fluorescence spectrometry (HPLC/CV-AFS) [4,5,17,20,27,29,41,42–45] or to inductively coupled plasma mass spectrometry (ICP-MS) [4,36,46–51] is widely accepted for the speciation analysis of mercury. CV-AFS detection offers primarily simplicity, high sensitivity and low purchase price. ICP-MS detection offers particularly the excellent detection limits, specificity of the signal intensity of the determined element and availability of the isotope ratio information. Because of the HPLC separation, the presence of a complexing agent (pyrrolidinedithiocarbamate, mercaptoethanol, dithizone, etc.) forming highly stable complexes with mercury species is needed in the mobile phase and/or in the sample solution. Their presence is necessary for highly effective separation of the chemically and structurally very different compound such as mercury species. With regards to the complexation of mercury species already during of the preconcentration step further addition of any “modifier” into the mobile phase is not necessary.

The aim of this study was to develop sensitive and robust analytical method that would enable the speciation analysis of mercury both in sediments, zoobenthos and in waters after their preconcentration. The connection of a highly efficient chromatographic separation with a simple, universal and very fast microwave-assisted extraction of sediments and zoobenthos or with a preconcentration method of water samples was of the primary interest. The application of 2-mercaptophenol as a modifier for chromatographic separation and also its utilization for mercury species preconcentration has been found as the very advantageous idea.

2. Experimental

2.1. Chemical and reagents

A methanolic solution of 2-mercaptophenol (for synthesis, Merck, Darmstadt, Germany) was prepared by dissolving the appropriate amount of 2-mercaptophenol. Tetramethylammonium hydroxide (1 mol L⁻¹ in water) and HPLC Chromasolv® methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). HCl, HNO₃, H₂SO₄, NaCl, KBr, KBrO₃, CuCl₂, KOH, SnCl₂, acetic acid, citric acid and formic acid of analytical grade purity were purchased from Penta (Chrudim, Czech Republic).

Inorganic mercury calibration standard (1.000 ± 0.002 g L⁻¹ Hg₂⁺ in 2% (v/v) HNO₃) was obtained from the Czech Metrological Institute (Prague, Czech Republic). Methylmercury chloride (MeHg⁺) and phenylmercury chloride (PhHg⁺) were purchased from Sigma–Aldrich. Ethylmercury chloride (EtHg⁺) was purchased from Supelco (Munch, Germany). Standard solutions of MeHg⁺, EtHg⁺ and PhHg⁺ (c = 1 g L⁻¹) were prepared in methanol. Working standards (10 mg L⁻¹) were prepared from the stock solutions by diluting with aqueous methanol (50%/50%) or with DI water (AquaDem-02, AquaOsmotic, Tišnov, Czech Republic) further purified in a Millipore Milli Q system (Bedford, MA, USA). Final working solutions of mercury species were prepared in 5 mM 2-mercaptophenol and in methanol or in DI water for separation or preconcentration, respectively.

2.2. Instrumental

2.2.1. High-performance liquid chromatography-cold vapour atomic fluorescence spectrometry (HPLC/CV-AFS)

A liquid chromatograph LC-200 (bio-version, Perkin Elmer, Norwalk, CT, USA) was equipped with Series 200 high-pressure pumps, an AS-60 auto-sampler, an UV/Vis programmable absorbance detector (model 785A) and a PSA Millenium Merlin atomic fluorescence spectrometric (AFS) detector. TurboChrom chromatographic software controlled
the chromatographic system through 600 Series and 900 Series links (PE Nelson, Norwalk, USA). Effluent from Zorbax SB-C18 column (5 µm particle size, 4.6 mm × 150 mm, Agilent Technologies, Santa Clara, CA, USA) was merged with a stream of acidified bromide/bromate mixed solution (0.2 mol L⁻¹ KBr + 0.04 mol L⁻¹ KBrO₃ in 5% HCl). An excess of Br₂ was eliminated by 0.004% (m/v) hydroxylamine hydrochloride (flow rate 2.5 mL min⁻¹). The effluent then passed through a UV cracking reactor (PTFE tube 0.5 mm × 10 m, UV lamp power 12 W). All mercury species in individual chromatographic zones were converted to inorganic mercury. The inorganic mercury was reduced by reaction with SnCl₂ (2% (m/v) SnCl₂ in 10% HCl, flow rate 2.5 mL min⁻¹). Elemental mercury cold vapours were purged with argon stream, dried in a PermaPure® membrane unit and detected at 253.65 nm by the PSA Millenium Merlin AFS detector controlled by Avion software (P.S. Analytical Ltd., Orpington, UK). A Clarity data acquisition program (version 2.1, DataApex, Prague, Czech Republic) was used for the final AFS chromatograms processing.

An isocratic elution profile of aqueous methanol (65%/35%, v/v) was used for chromatographic separation of mercury species at a flow rate 0.8 mL min⁻¹. The column was washed with methanol for 3 min after each separation and equilibrated with 65% methanol (v/v) for 5 min prior to injection of a sample. Aliquots of the sample (100 µL) were injected into the flow of the mobile phase.

2.2.2. Determination of total mercury
An AMA 254 Advanced Mercury Analyzer controlled by WinAMA software (both Altec, Prague, Czech Republic) was used for determination of total mercury concentration by direct analysis of the environmental samples. The sample aliquots (100 mg or 100 µL) were introduced into the AMA 254 analyzer on pre-cleaned combustion boats. During the analysis the sample was dried at 120 °C for 90 s and thermally decomposed at 550 °C for 180 s under oxygen flow. Selectively trapped mercury was released from the gold amalgamator by a brief heat-up and finally quantified (measuring cycle, 60 s) as Hg⁰ by the cold vapour AAS technique at 253.65 nm.

2.3. Study area and samples
Mercury contamination was evaluated in three Moravian rivers (Jihlava, Loučka, Bečva). Sediment, water and zoobenthos samples were collected in selected sampling sites in July and in August 2006. The sampling site near town Hrubšice was selected in Jihlava basin. Jihlava river flows through an industrial region (boot, paper and engineering industry, nuclear power station – Dukovany) but also through an agricultural region in nearness Hrubšice. The water ecosystem of Loučka – processing of cruel benzole and tar) prevails in the sampling site.

Sediment samples were taken from a surface layer (maximum depth 15 cm); zoobenthos samples were collected manually from river stones; water samples were filtered and acidified to pH 3 (with HCl). All samples were placed in plastic sampling bottles, stored in iceboxes (frozen by dry ice) and transported into laboratory. The stability of the samples was ensured by freeze-drying (sediments and zoobenthos) and by deep-freezing.

2.4. Procedures

2.4.1. Sample preparation
Lyophilized (at −51 ± 1 °C, 48 h, Christ Alpha 1-2 lyophiliser, B. Braun Biotech International, Melsungen, Germany) zoobenthos samples were homogenized using a Grindomix GM 200 (Retsch, Haan, Germany) laboratory mill and stored at 4 °C until analysis. Sediment samples were manually crushed and sieved at 1 mm (the fraction higher than 1 mm was discarded). River water samples were preconcentrated (see Section 2.4.2).

The isolation of mercury species was performed in a microwave extractor Ethos SEL (Milestone, Sorisole, Italy). The extraction agent containing 3 mol L⁻¹ HCl + 0.2 mol L⁻¹ citric acid + 50% methanol (10 mL) was added to 0.5–4 g of a sample and extracted in the high-pressure microwave digestion unit. An optimal extraction time was 10 and 7 min for zoobenthos and sediment samples, respectively. Pulsed microwave treatment (power of microwave energy – 500 W) using a computer program regulated the temperature inside of the extraction vessels at 45 °C.

Acidity of the filtrated extracts (filter paper no. 389, disc diameter 12.5 cm) was adjusted to a pH value 3 by NaOH and finally 2-mercaptophenol was added. The total concentration of 2-mercaptophenol in extract of sediments and zoobenthos was 30 mmol L⁻¹. The treated extracts were injected into the HPLC/CV-AFS system.

2.4.2. Preconcentration
The Spe-ed C18 SPE stationary phase (Applied Separations, Allenton, PA, USA) was used for preparation of SPE microcolumns that enabled also countercurrent-flow elution. Approximately 100–300 mg of the Spe-ed C18 sorbent (dry weight) was mixed with methanol to form slurry and transferred to the microcolumn via a syringe. A Watrex PTFE tubing (3 mm i.d., Watrex International, San Francisco, CA, USA) was used for construction of SPE columns. The excess of methanol was drained through a porous PTFE plug and kept in methanol. The disposable columns were pre-treated with methanol (5 mL) and DI water (5 mL), followed by passing 20 mL of 14 mmol L⁻¹ 2-mercaptophenol in water and finally washed with 2 mL DI water before use.

A peristaltic pump Labeco PCR 01 (Villa-Labeco, Spišská Nová Ves, Slovakia) was used for delivery of all solutions to C18 columns. The conditioning solutions (DI water, methanol, 2-mercaptophenol) and sample solution were delivered at a constant flow rate of 5 mL min⁻¹. The elution solution (methanol) was pumped at a flow rate of 0.1 mL min⁻¹. The counter-current flow of elution solution was used for elution of mercury species.
Fig. 1 – (a) Extraction efficiencies of mixed extraction agents on the basis of 3 M HCl. Columns identification: (1) 3 M HCl + 10% MeOH, (2) 3 M HCl + 25% MeOH, (3) 3 M HCl + 50% MeOH, (4) 3 M HCl + 0.05 M NaCl, (5) 3 M HCl + 0.1 M NaCl, (6) 3 M HCl + 0.5 M NaCl, (7) 3 M HCl + 0.1 M NaCl + 10% MeOH, (8) 3 M HCl + 0.1 M NaCl + 25% MeOH, (9) 3 M HCl + 0.1 M NaCl + 50% MeOH, (10) 3 M HCl + 0.1 M KBr, (11) 3 M HCl + 0.1 M KBr + 50% MeOH, (12) 3 M HCl + 1 M KBr, (13) 3 M HCl + 0.1 M CuCl₂, (14) 3 M HCl + 0.1 M CuCl₂ + 50% MeOH, (15) 3 M HCl + 1 M CuCl₂, (16) 3 M HCl + 0.1 M citric acid, (17) 3 M HCl + 0.5 M citric acid, (18) 3 M HCl + 0.1 M citric acid + 50% MeOH, (19) 3 M HCl + 0.1 M citric acid + 50% MeOH, (20) 3 M HCl + 0.2 M citric acid, (21) 3 M HCl + 0.2 M citric acid + 50% MeOH, (22) 3 M HCl + 0.3 M citric acid, (23) 3 M HCl + 0.3 M citric acid + 50% MeOH. (b) Extraction efficiencies of next tested extraction agents. Columns identification: (1) 1 M citric acid, (2) 1 M citric acid + 50% MeOH, (3) 3 M citric acid, (4) 3 M citric acid, (5) 3 M formic acid, (6) 6 M HNO₃, (7) 3 M formic acid, (8) 3 M formic acid, (9) 6 M HNO₃, (10) 6 M H₂SO₄, (11) 50% methanol, (12) 3 M KOH, (13) 3 M KOH + 25% MeOH, (14) 3 M KOH + 50% MeOH, (15) 1 M TMAH in water. (c) Dependence of the extraction recovery of mercury (%) on concentration of HCl in extraction agent [mol L⁻¹]. Conditions of microwave extraction: t = 10 min, T = 45 °C, 500 W.
3. Results and discussion

3.1. Stability of mercury species during storage and analysis

Neither loss nor transformation of the mercury species was observed in the zoobenthos samples during storage (6 months), if the samples were kept in freeze-drying or deep-freezing. A transformation of the mercury species (methylation of Hg²⁺) was observed in the sediment samples. A slow increase in the methylmercury concentration was observed, but the total mercury contents were constant, if the sediment samples were not stored deep-frozen. 4% enhancement of methylmercury concentration during 2 months was observed. Stability of the tested sediments was extended by freeze-drying and by deep-freezing storage.

A transformation of the mercury species was not observed during mercury species isolation. The extract stability was 3 days at least, if the extracts were stored in a brown glass bottle in a refrigerator. The pH value influenced the stability of the mercury species-2-mercaptophenol complexes. If the pH value was in intervals between 3 and 5, the complexes were stable more than 10 h.

3.2. Isolation of mercury species from sediment and zoobenthos samples

Isolation of mercury species from samples is the most complicated and the most important part of the analytical procedure. Recovery, repeatability and preservation of the initial distribution of mercury species were the most important criteria for selection of an extraction method. The isolation of mercury species was performed in the microwave-assisted extractor (MAE). The certified reference material CRM 580, spiked sediment (Loučka) and spiked zoobenthos sample (Loučka) were used for optimization of extraction procedure. The extraction recoveries were calculated as percentage difference between total mercury content in solid sediment sample and in its extract solution. The sample mass (0.5–4 g) was optimized to fulfill the need of extraction procedure and subsequent HPLC/CV-AFS. No observable differences in results were found in the given range.

Four experimental parameters (solvent type and its volume, extraction temperature and time) were selected for extraction procedure applied to sediment and zoobenthos samples. Several extraction agents (see Fig. 1a–c) were tested for extraction of mercury species from sediment and zoobenthos samples. The highest extraction efficiencies (higher than 90%) were obtained only while using mixed extraction agents on the basis of 3 M hydrochloric acid and also while using 6 M hydrochloric acid and 6 M nitric acid. Hydrochloric acid mixtures were further tested as extraction agents since low acid concentration was preferred. The mixed extraction agents based on 3 M HCl in combination with 1 mol L⁻¹ CuCl₂, 1 mol L⁻¹ KBr, 0.1 mol L⁻¹ NaCl and 0.2 mol L⁻¹ citric acid provided extraction efficiency higher than 95%.

A mixture containing 3 mol L⁻¹ HCl and 0.2 mol L⁻¹ citric acid and 50% methanol was selected as the most suitable extraction agent due to the next processing of the extract (pH adjusting and addition of 2-mercaptophenol). Citric acid in the extraction agent eliminates interferences of co-extracted Fe³⁺, which would be separated out from extract solution during pH adjusting. The addition of methanol into extraction agent improved extraction efficiency (approximately 5%). It also increased solubility of 2-mercaptophenol that is added to the extract solution before analysis. The stability of mercury species in this extraction agent was validated by analyses of the reference material CRM 580, spiked sediment (Loučka) and spiked zoobenthos sample (Loučka). The results were in good agreement with the certified values. No transformations of mercury species were observed during the extraction procedure.

An extraction temperature and duration of the MAE were changed from 25 to 65 °C for 2–15 min to gain the highest extraction recovery and reproducibility. The extraction temperature (Fig. 2) as well as duration of the extraction (Fig. 3) influenced the extraction recoveries of mercury species. Influence of temperature was not so important. Vázques et al. [10] noted the same effect. The lowest possible temperature (45 °C), whereas the extraction recoveries >95% were obtained, was selected as optimal. Any transformations of mercury species were not observed, if the extraction of the samples was performed with the selected temperature. The most suitable extraction time was 7 or 10 min for sediment and zoobenthos samples, respectively. At least 10 mL of extraction agent
was used considering the construction of MAE. Use of a higher volume of extraction agent did not influence extraction recoveries. Quantitative extraction (R = 95–101%) was reached if the selected extraction conditions were applied. Precision (R.S.D.s) was better than 6%. The optimum operating conditions for microwave extraction are given in Table 1. The short extraction time, high throughput, high precision and accuracy were the most remarkable advantages of MAE for extraction of mercury species from sediment and zoobenthos samples.

### 3.3. Selection of HPLC/CV-AFS conditions

An HPLC/CV-AFS method was optimized with respect to individual parameters of both steps—separation in HPLC part and CV-AFS detection. The type of sorbent, the flow rate of the mobile phase and its composition were the main parameters responsible for obtaining the best chromatographic separation.

The best chromatographic separation of all mercury species (MeHg+, EtHg+, Hg2+, PhHg+) as their 2-mercaptophenol complexes was obtained on a Zorbax SB-C18 chromatographic column with reverse phase. The presence of the complexing agent forming kinetically robust complexes with mercury species in the sample was necessary for effective separation of mercury species on C18 column. 2-mercaptophenol enabled to overcome the significant differences in chemical and physical characteristic of mercury species and thus permitted determination of widely differing compounds (inorganic mercury cations, alkylmercury compounds (MeHg+, EtHg+) and low-polar PhHg+) in a single separation step.

The pH value influenced the stability of mercury species-2-mercaptophenol complexes. The complexes were stable more than 10 h in the pH interval 3–5. In contradiction to other complexing agents e.g. APDC, the 2-mercaptophenol do not form insoluble compounds with Fe3+ ions that are present in sediment samples in a large quantity. The application of 2-mercaptophenol is preferable since presence Fe3+ ions disable the other agents for the speciation analysis of mercury in sediment samples.

The elution profile was optimized to obtain the highest resolution of the mercury-2-mercaptophenol complexes (R > 1.5) and the shortest time of chromatographic analyses (less than 30 min). The best resolution of all peaks was obtained by using the isocratic profile of the mobile phase containing methanol (65%) and water (35%). Composition of the mobile phase influenced markedly sensitivity of the cold vapour fluorescence detection. The mobile phase consisting of water and acetonitrile was also suitable for the chromatographic separation, but not suitable for the cold vapour fluorescence detection due to serious decrease of sensitivity.

Increasing the concentration of 2-mercaptophenol in injected sample declined the resolution of mercury species; nevertheless, the concentration of 2-mercaptophenol must be sufficient for full complexation of mercury species. Because of the higher concentration of ferric ions (Fe3+ in g kg⁻¹) that interfere with 2-mercaptophenol, 30 mmol L⁻¹ concentration of 2-mercaptophenol was selected for the speciation analysis of mercury in sediment samples. The 5 mmol L⁻¹ concentration of 2-mercaptophenol was sufficient for the speciation analysis of mercury in enriched waters due to a lower amount of ferric ions. The optimum operating conditions for HPLC/CV-AFS are given in Table 1.

### 3.4. Analytical performance characteristics and method validation

The analytical performance of the extraction method was evaluated by the determination of the recovery, repeatability and preservation of the initial distribution of mercury species during extraction procedure. HPLC/CV-AFS method was evaluated by the determination of linearity, sensitivity and repeatability and preconcentration procedure was evaluated by the determination of recovery, repeatability and total retention capacity of the SPE column.

Quantitative extraction (R = 95–101%) mercury species from sediment and zoobenthos samples was reached if the selected extraction conditions (extraction agent: 10 mL of 3 mol L⁻¹ HCl and 0.2 mol L⁻¹ citric acid and 50% methanol, 45 °C, 7–10 min) were applied. Precision (R.S.D.s) of microwave extraction was better than 6%. The stability of mercury species during extraction procedure was validated by analyses of the reference material CRM 580, spiked sediment (Loučka) and spiked zoobenthos sample (Loučka). The results were in good agreement with the certified values. No transformations of mercury species were observed during the extraction procedure.

The standard addition calibration method was used for calibration of HPLC/CV-AFS device. Calibration curves showed

#### Table 1 – Optimum operating conditions for HPLC, CV-AFS, microwave extraction and preconcentration procedure

<table>
<thead>
<tr>
<th>Procedure</th>
<th>HPLC</th>
<th>CV-AFS</th>
<th>Microwave Extraction</th>
<th>Preconcentration</th>
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good linearity with regression coefficients higher than 0.98 in tested concentration range (10–1000 µg L⁻¹). The HPLC/CV-AFS chromatogram of the mercury species standards (20 µg L⁻¹ of each) is demonstrated in Fig. 4. The accuracy of the results was controlled by analyses of the CRM 580 reference material, spiked sediment (Loučka) and spiked zoobenthos sample (Loučka). The LODs (3 S/N criterion) estimated from the measurement of standard solution at the concentration of 20 µg L⁻¹ of each mercury species and from the measurement of river water samples enriched with mercury species were 4.3 µg L⁻¹ (2.0%) for MeHg⁺, 1.4 µg L⁻¹ (3.5%) for EtHg⁺, 0.8 µg L⁻¹ (3.3%) for Hg²⁺ and 0.8 µg L⁻¹ (4.5%) for PhHg⁺ with corresponding R.S.D.s at 20 µg L⁻¹ (n = 6) given in parentheses. Absolute LODs were determined from 100 µL injections and were 430 pg for MeHg⁺, 140 pg for EtHg⁺, 80 pg for Hg²⁺ and 80 pg for PhHg⁺. The LODs (3 S/N criterion) estimated from the measurement of enriched extract of sediment and zoobenthos samples were 7.6 µg L⁻¹ for MeHg⁺, 3.0 µg L⁻¹ for EtHg⁺, 1.9 µg L⁻¹ for Hg²⁺ and 1.9 µg L⁻¹ for PhHg⁺. Higher peak expansion is the main reason for the deterioration of LODs values. The results of proposed method were in good agreement with those obtained by the method described in Ref. [17]. The results deviation of both methods was less than 7% (Table 2).

Generally, 90–95% of mercury was trapped from the water samples in the column during preconcentration procedure. Elution recoveries of mercury species were higher than 90%. The total capacity of the SPE columns was dependent on the amount of the Spe-ed C18 sorbent and for 300 mg of the C18 sorbent reaches up to 155 µg of mercury. The reproducibility of the preconcentration procedure was better than 10% (n = 6).

3.5. Preconcentration

Because the mercury species concentration in water samples was lower than the detection limits of the proposed method, a preconcentration procedure utilizing homemade C18 SPE microcolumns was developed to enhance sensitivity of the mercury species determination. The preconcentration procedure uses the same mercury species-2-mercaptophenol complexes formation as HPLC/CV-AFS method thus the preconcentrated sample can be directly injected into the chromatographic column. We assume that the trapping mechanism is based on the reaction of thiol groups of 2-mercaptophenol bound to the sorbent surface and the mercury species from the sample.

The 20 mL volume of 14 mmol L⁻¹ 2-mercaptophenol was sufficient for the speciation analysis of mercury in enriched river water samples (0.5 µg L⁻¹). Typically, 100–500 mL of the water sample was preconcentrated on the 100–300 mg C18 microcolumn. The column with 300 mg of the sorbent had sufficient total retention capacity (up to 155 µg of mercury) for preconcentration of 500 mL river water sample. Generally, 90–95% of mercury was trapped from the water samples in the column. The flow rates of the sample and the 2-mercaptophenol (up to 5 mL min⁻¹) did not influence the efficiency of the mercury trapping in the column. The total capacity of the columns can be simply increased by increasing the amount of the Spe-ed C18 sorbent.

In the elution step, methanol of various concentrations was pumped through the column in counter-current flow at 0.1 mL min⁻¹. The obtained recoveries of mercury species were higher than 90% for the methanol concentration higher

<table>
<thead>
<tr>
<th>Sample site, year 2006</th>
<th>Complexes with 2-mercaptophenol</th>
<th>Complexes with 2-mercaptophenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeHg⁺ (mg kg⁻¹)</td>
<td>MeHg⁺ (%)</td>
</tr>
<tr>
<td>Jihlava-Hrubčice – sediment</td>
<td>0.046</td>
<td>32.0</td>
</tr>
<tr>
<td>Loučka-Strážek – sediment</td>
<td>0.022</td>
<td>18.5</td>
</tr>
<tr>
<td>Bečva-Choryné – sediment</td>
<td>0.031</td>
<td>22.3</td>
</tr>
<tr>
<td>Jihlava-Hrubčice – zoobenthos</td>
<td>0.168</td>
<td>66.0</td>
</tr>
<tr>
<td>Loučka-Strážek – zoobenthos</td>
<td>0.158</td>
<td>43.6</td>
</tr>
<tr>
<td>Bečva-Choryné – zoobenthos</td>
<td>0.082</td>
<td>33.7</td>
</tr>
</tbody>
</table>

Comparison results obtained proposed method and method described in Ref. [17].
than 70%. The preconcentration factor as much as 1000 was obtained since 0.5 mL of the methanol completely eluted the mercury species trapped from 500 mL of the original sample.

Iron ions (Fe³⁺) can interfere with the mercury species in the preconcentration procedure, because they react also with 2-mercaptophenol. No measurable interferences were observed for concentrations of ferric ions in tested river water samples in the range 7–15 µg L⁻¹ (preconcentration from 500 mL). The reproducibility of the preconcentration procedure was better than 10% (n=6). The optimum operating conditions for preconcentration method are given in Table 1.

3.6. The speciation analysis of mercury in environmental samples

The proposed method was applied for the determination of the mercury species in sediment, zoobentos and water samples. The samples were collected from three Moravian rivers (Jihlava, Loučka, Bečva). More detailed description of the study area and sample preparation procedures are given in Sections 2.3 and 2.4.1, respectively.

The sediments contained 119–144 µg kg⁻¹ of the total mercury in dry matter. The MeHg⁺ contents in the river sediments ranged from 18.5% to 32.0% (in percents of the total mercury content). Zoobentos samples contained 236–358 µg kg⁻¹ of the total mercury in dry matter. The relative contents of MeHg⁺ were from 33.7% to 66.0% in these samples. The final contents of the mercury species in the sediment and the zoobentos samples are given in Table 2.

Enriched river water samples (0.5 µg L⁻¹) and the real (not enriched) river water samples were analyzed after their preconcentration. If 500 mL of enriched river water samples (0.5 µg L⁻¹) were preconcentrated, results corresponding to the added quantity of mercury species were obtained (recoveries 85–90%). The contents of the mercury species in the real un-enriched river water samples were below the limits of detection of the proposed method even after preconcentration. Any significant industrial mercury pollution of the river water samples was not proven. The permitted limit [52] of Hg contamination (0.1 µg L⁻¹) was not exceeded in any sample.

4. Conclusions

The sensitive and robust HPLC/CV-AFS method was proposed for the speciation analysis of four mercury species (MeHg⁺, EtHg⁺, PhHg⁺, Hg²⁺) in sediment, zoobentos and water samples. The mercury species were separated as the complexes with 2-mercaptophenol. The preconcentration procedure utilizing the homemade C18 SPE microcolumns was developed to enhance sensitivity of mercury species determination. The same complexation reagent (2-mercaptophenol) was used for preconcentration of the mercury species as well as their chromatographic separation.

The simple, universal and very fast extraction method using MAE with newly proposed extraction agent (3 mol L⁻¹ HCl + 0.2 mol L⁻¹ citric acid + 50% methanol) was developed for the simultaneous extraction of the mercury species from the sediments as well as zoobentos samples. The proposed extraction method has wide applicability and can be combined also with other than the proposed separation method. The short extraction time, high throughput, high precision and high accuracy were the most remarkable advantages of the MAE.

The proposed method enabled the speciation analysis of mercury in environmental samples.

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REFERENCES


