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Simultaneous determination of methyl-, ethyl-, phenyl- and inorganic mercury by cold vapour atomic absorption spectrometry with on-line chromatographic separation

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ABSTRACT

A fully automated system for on-line high-performance liquid chromatographic separation, reduction and determination by cold vapour atomic absorption spectrometry (CVAAS) of methyl-, ethyl-, phenyl- and inorganic mercury is described. Reversed-phase chromatography on an ODS column and elution with an acetonitrile–water–ammonium tetramethylenedithiocarbamate buffered mixture was investigated with or without ammonium tetramethylenedithiocarbamate precomplexation. A simple glass flow cell, properly designed as an interface between the chromatographic system and CVAAS, is proposed. The influence of the composition of the eluent, reducing solution and stripping gas flow-rate was investigated and optimized in order to obtain better detection limits. The method, applied to synthetic mixtures and natural samples of tap water furnished satisfactory results. It is shown that, with the on-line preconcentration procedure, sub-ng/ml concentration levels can be determined for all the species considered.

INTRODUCTION

Mercury determinations have been achieved by several methods in recent years, all using basically the same approach [1,2]. A closed reduction purging system converts the mercury compound into a volatile form by reduction to elemental mercury, which is swept from the solution by a gas stream into a detector, sometimes after a preconcentration step (usually a gold trap). The most frequently used detectors are atomic absorption spectrometers, *e.g.*, in cold vapour atomic absorption spectrometric (CVAAS) measurements [3–7].

In view of the high toxicity of organomercury(II) compounds, speciation of the chemical forms is required. Inorganic and organic mercury compounds have been separated by high-performance liquid chromatography (HPLC) as alkyl- or tetramethylenedithiocarbamates [8] and dithizonates [9] after extraction of the complexes, but UV spectrophotometric detection gave too high detection limits. Speciation of inorganic and organomercury compounds was also determined by liquid chromatography, coupling cold vapor generation with inductively coupled plasma emission spectrometry [10] or inductively coupled plasma mass spectrometry [11]. The high instrumental costs in relation to the detection limits obtained (32–62 and 0.6–12 μg/l, respectively) make it difficult to justify. More recently, a speciation of mercury compounds by microcolumn liquid chromatography using a preconcentration column with CVAAS detection furnished 1 μg/l detection limits for all species considered [12].

The purpose of this work was to evaluate the efficiency of ammonium tetramethylenedithiocarbamate [ammonium pyrrolidine dithiocarbamate (APDC)] reagent for the reversed-phase separation of methyl-, ethyl, phenyl- and inorganic mercury, accomplished with on-line reduction and CVAAS

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determination. Experiments were also carried out with a preconcentration procedure in order to achieve lower detection limits.

EXPERIMENTAL

Apparatus and materials

A schematic diagram of the system is shown in Fig. 1. All experiments were carried out on a Varian Model LC 5000 instrument equipped with a Rheodyne injection valve (100-μl sample loop), a Vista 401 Data Station and a Varian UV-100 detector (Varian, Walnut Creek, CA, USA) or a CVAAS detector (Milton Roy, Stone UK). The analytical column was LiChrospher 100 RP-18 (5 μm) (250 × 4 mm I.D.), coupled with a LiChroCART 100 RP-18 (5 μm) guard column (25 × 4 mm I.D.), obtained from Merck (Darmstadt, Germany). The CVAAS signal due to mercury was recorded on a Model 56 strip-chart recorder (Perkin-Elmer, Norwalk, CT, USA). A Model 302 pump and Model 802 manometric module, Gilson, Middleton, WI, USA) ensured, through a T-mixer, the addition of reductive solution to the eluted chromatographic fractions. The mixed solutions containing the dissolved elemental mercury were introduced into a Pyrex flow cell (FC), which also acted as gas–liquid separator. A peristaltic pump (Gilson Minipuls 2) was used for draining the solution from the gas–liquid separator and for ensuring that a constant volume entered the cell. For pH measurements a digital pH meter (Orion, Cambridge, MA, USA) equipped with a combined glass–calomel electrode was used.

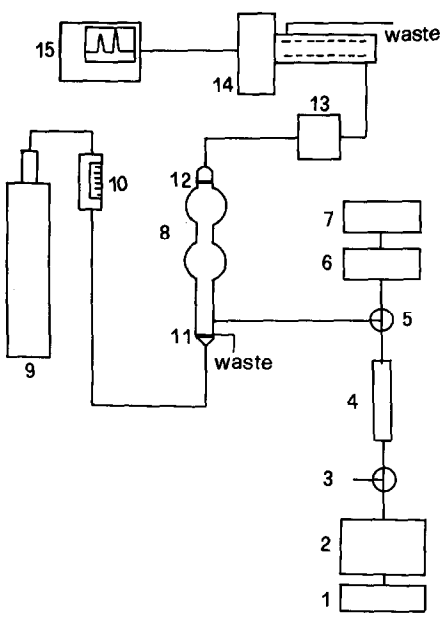

Fig. 1. Schematic representation of the major instrumental components employed: 1 = eluent; 2 = chromatographic system; 3 = injector (100-μl loop or preconcentrator); 4 = chromatographic column; 5 = T-mixer; 6 = manometric pump module; 7 = $NaBH_4$ reservoir; 8 = flow cell; 9 = carrier (N_2); 10 = flow meter; 11, 12 = glass frits; 13 = $Mg(ClO_4)_2$ traps; 14 = CVAAS detector; 15 = recorder.

High-purity water (HPW) obtained from a Milli-Q System (Millipore, Bedford, MA, USA), supplied with deionized water produced with a mixed-bed twin ion-exchange column, was used for preparing all solutions. Acetonitrile used for HPLC, sodium hydroxide, magnesium perchlorate, mercury nitrate and methylmercury chloride were obtained from Merck, ethylmercury chloride from Alfa (Karlsruhe, Germany) and phenylmercury chloride from Aldrich (Steinheim, Germany). Acetic acid (Merck) was purified with a sub-boiling distillation apparatus (Kurner, Rosenheim, Germany). All other reagents employed were of analytical-reagent grade.

APDC (Merck) was used without further purfication. The reducing solution, containing 1.0 g/l of sodium tetrahydroborate (Fluka, Buchs, Switzerland) and adjusted to pH 11.5 with 0.1 *M* NaOH, was prepared and filtered just before use. This solution was purified by bubbling with nitrogen overnight.

Preparation and measurement of solutions

The working methyl-, ethyl-, phenyl- and inorganic mercury solutions were prepared before use by dilution of 100.0, 100.0, 20.0 and 1000 ppm stock standard solutions, respectively. Standards and samples were prepared daily with HPW, stored in the dark and refrigerated.

Chromatographic determinations were performed, unless stated otherwise, with a mobile phase consisting of acetonitrile–water (58:42, v/v), previously buffered to pH 5.5 with ultrapure acetic acid and sodium hydroxide, and added of the appropriate amount of APDC (see below). The eluent composition gave better resolved peaks at a 0.5 m*M* APDC concentration. The flow-rate was 1.5 ml/min.

Sample solutions were introduced into the injection loop (100 μl) or passed through a LiChroCART RP-18 (5 μm) microcolumn (4 × 4 mm I.D.) with the aid of a Model DQP-1 pump (Dionex, Sunnyvalle, CA, USA) for the preconcentration procedure.

Preliminary experiments, using low APDC concentrations, were performed with a UV–VIS detector and the direct injection of aqueous sample solutions was accomplished by incorporating, or not, APDC reagent (1 m*M*) in the samples. For the precomplexation procedure the samples had a composition of acetonitrile-methanol-water (25:25:50, v/v/v) in order to avoid precipitation of complexes. In the optimized procedure metal complexes were formed by on-column derivatization during the separation.

RESULTS AND DISCUSSION

The method is based on the formation, separation and subsequent detection of the metal–APDC complexes (Fig. 2). The on-column in comparison with the precomplexation procedure showed a reduced detection sensitivity only for inorganic mercury species (see below). On the other hand, the on-column complexation procedure avoids tedious manipulations of the sample, due to the low solubility of the APDC complexes, which require a solvent medium for reaction or organic extraction prior to chromatographic separation. Taking into account the high sensitivity of inorganic mercury in comparison with the other species, and that on-line complexation avoids contamination of the sample, the on-line procedure was concluded to be the best approach.

Optimization of procedures

Several factors had to be optimized in order to achieve the maximum selectivity, sensitivity, reproducibility and resolution. These included capacity factor, the width of the peaks at half-height ($W/2$) and ligand concentration (APDC). For detection by CVAAS, the flow-rate of reducing solution and residence times in the reaction coil and in the flow cell must be considered.

It was noted that the peak areas of the separated mercury complexes varied considerably with repeated injections of the sample at low APDC concentration. It is believed that these variations arise from interaction of the charged uncomplexed species, in equilibrium with APDC complexes, with residual silanol groups on the C_{18} column packing. The addition of higher APDC concentrations blocks this effect, in fact the ligand concentration acts on the complexation of mercury species and in addition it avoids adsorption phenomena on the column. In particular, as the concentration of ligand in the mobile phase increased, the retention time of the analytes decreased linearly.

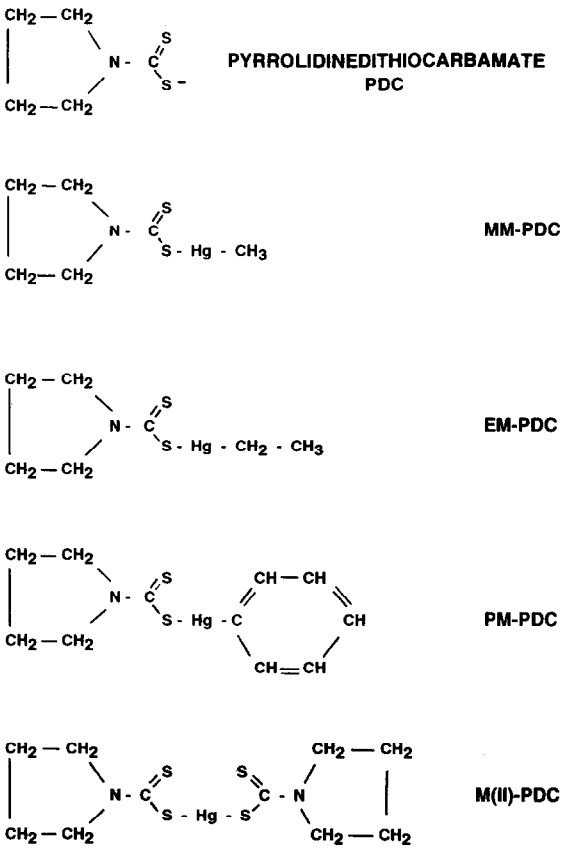

Fig. 2. Structure of APDC complexes: MM = methylmercury; EM = ethylmercury; PM = phenylmercury; M = inorganic mercury.

Fig. 3 shows the behaviour of retention times and $W/2$ as a function of complexing agent (APDC) concentration in the eluent. The UV–VIS detection of mercury complexes was hindered by the increased background absorbance when higher concentrations of reagent were used in the mobile

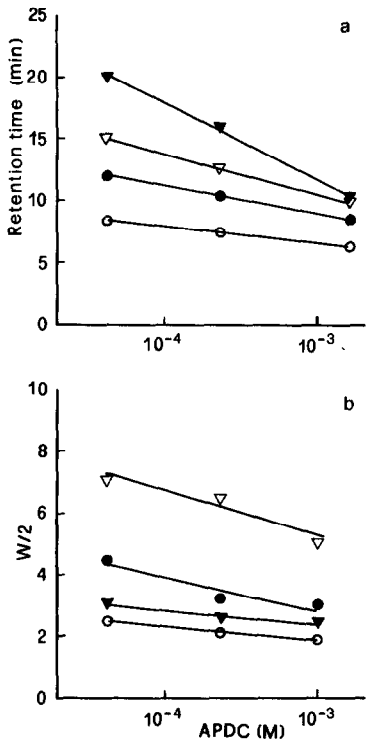

Fig. 3. Effect of APDC concentration on (a) retention times and (b) $W/2$ for a 100-μl sample. Chromatographic conditions: mobile phase, acetonitrile–water (58:42, v/v) containing 20.0 mM acetic acid, APDC as shown and sodium hydroxide up to pH 5.5; flow-rate, 1.5 ml/min. ○ = CH_3Hg^+ (100 ng as Hg); ● = $C_2H_5Hg^+$ (100 ng as Hg); ▽ = $C_6H_5Hg^+$ (100 ng as Hg); ▼ = Hg^{2+} (20 ng).

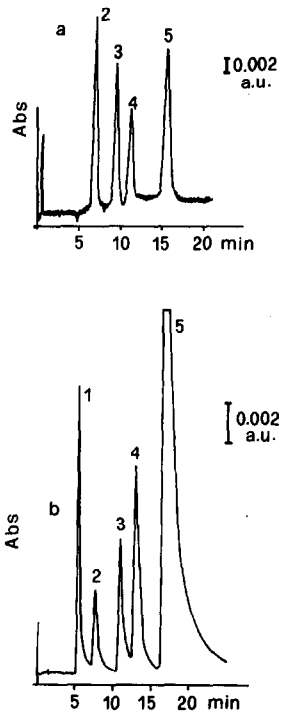

Fig. 4. Comparison between UV absorption and CVAAS detection of mercury–APDC complexes separated by HPLC. (a) UV detection, 254 nm; (b) CVAAS detection, 254 nm. Chromatographic conditions: mobile phase, acetonitrile–water (60:40, v/v) containing 20.0 mM acetic acid, 0.1 mM acetic acid, 0.1 mM APDC and sodium hydroxide up to pH 5.5; flow-rate, 1.5 ml/min; 100-μl sample. Peaks: 1 = ghost peak (CH_3OH); 2 = CH_3Hg^+ (200 ng as Hg); 3 = $C_2H_5Hg^+$ (200 ng as Hg); 4 = $C_6H_5Hg^+$ (200 ng as Hg); 5 = Hg^{2+} (200 ng).

phase. CVAAS was employed for APDC concentrations >0.1 mM to optimize the method. The removal of adsorption phenomena during the chromatographic separation, due to increased APDC concentration, gave better reproducibility and enhanced the detection signal, and on the basis of the results obtained, a 0.5 mM APDC concentration was selected. In fact, shorter retention times are obtained with a decrease in broadening of the ethylmercury peak and a good separation of components in a shorter analysis time and with total recovery of the investigated species.

Comparison of the spectrophotometric detection of metal complexes based on their UV–VIS adsorption around the maximum allowed APDC concentration in the eluent (0.1 mM) and the CVAAS determination is shown in Fig. 4. The precomplexation procedure was adopted. It must be noted that if the samples are not freshly prepared, an anomalous peak, due to inorganic mercury, appears during the analysis of methyl-, ethyl- or phenylmercury samples as a result of decomposition of the latter. In addition, a ghost peak appears when standard solutions of organic species involve the use of methanol.

In order to perform CVAAS determinations, the detector was interfaced with the chromatographic system through a flow cell (FC) which permitted the recovery of Hg(0) after the reduction of the eluted species. The chromatographic eluate, connected by a T-joint with reducing reagent, was passed to the FC through a reaction coil. The lenght of the tube (300 × 0.3 mm I.D.) allows the reduction of mercury species before the sample is introduced into the FC and the design of the cell was such as to ensure a

residence time of chromatographic fractions sufficient to permit complete removal of mercury without overlap of the previously separated species.

Fig. 5 shows the FC optimized to reduce the residence time of the eluate, to minimize the vapour dispersion and to permit the maximum flow-rate of carrier according to the maximum detection sensitivity. A countercurrent nitrogen flow into the FC swept out the reduced mercury from an outlet located on top of the separator and then introduced it into the CVAAS system, after removal of water through a trap filled with $Mg(ClO_4)_2$, which was replaced daily.

The effect of reducing solution flow-rate on detection was also investigated. Fig. 6 shows that the maximum peak height is obtained with a flow-rate of 0.1–0.2 ml/min and an increase in this parameter affects the peak width and symmetry, probably owing to dilution effects, without improving the sensitivity. Different flow-rates of the reductant also affect the pH value, which ranged from 6 to 11, but this did not affect the detection sensitivity. Therefore, a flow-rate of 0.1 ml/min was selected for the reductant.

In order to optimize the sensitivity, the effect of nitrogen flow-rate was investigated. Fig. 7 shows

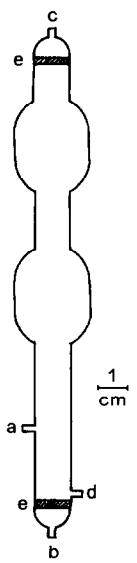

Fig. 5. Flow cell: a = inlet of effluent from HPLC column and reductant mixture; b = inlet of nitrogen flow; c = outlet of mercury vapour; d = outlet of waste; e = glass frits.

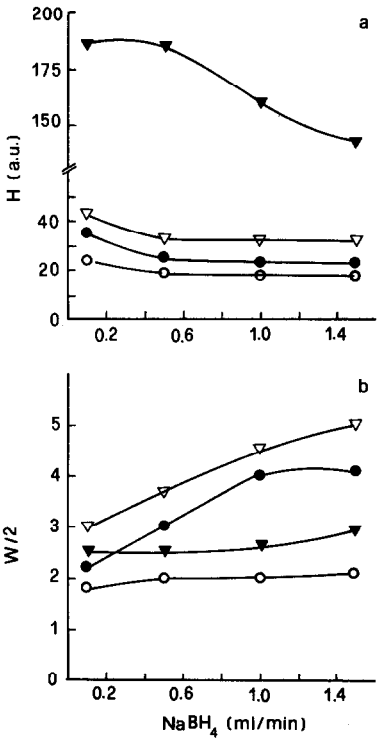

Fig. 6. Dependence of (a) peak height (H) and (b) $W/2$ on flow-rate of $NaBH_4$. Chromatographic conditions: mobile phase, acetonitrile–water (58:42, v/v) containing 20.0 mM acetic acid, 0.5 mM APDC and sodium hydroxide up to pH 5.5; flow-rate, 1.5 ml/min; 100-μl sample. ○ = CH_3Hg^+ (100 ng as Hg); ● = $C_2H_5Hg^+$ (100 ng as Hg); ▽ = $C_6H_5Hg^+$ (100 ng as Hg); ▼ = Hg^{2+} (20 ng).

the behaviour of peak height, $W/2$ and the time delay as function of flow-rate. The time delay is defined as

$$\text{time delay} = \frac{t_{rCV} - t_{rUV}}{t_{rUV}} \cdot 100\%$$

where t_{rCV} = retention time for the CVAAS detector and t_{rUV} = retention time for the UV–VIS detector.

As expected, at too low a flow-rate, the sensitivity is reduced and the peaks become broadened. On the other hand, the flow-rate corresponding to the maximum peak height cannot be selected because in this range the peak broadening is still too large with poor resolution; moreover, a slight variation in the nitrogen flow-rate can dramatically affect the time delay and sensitivity, reducing the reproducibility

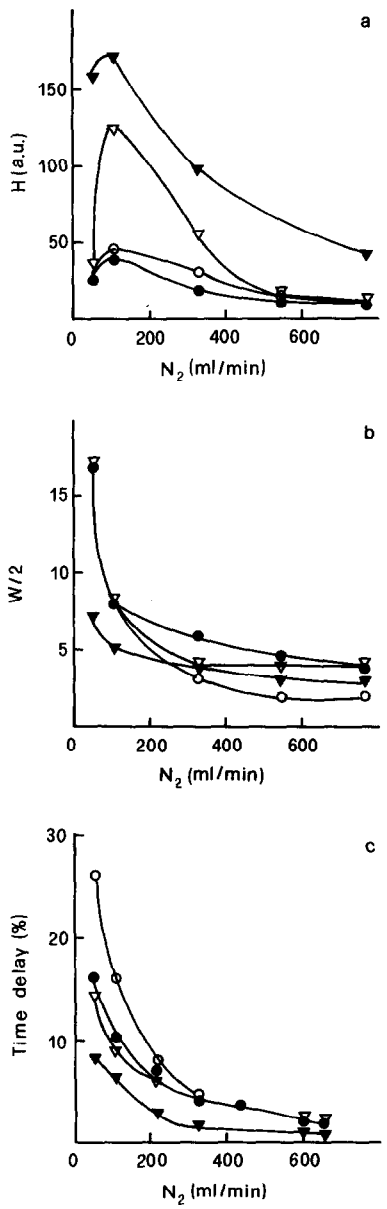

Fig. 7. Effect of flow rate of carrier (N_2) on (a) peak height (H), (b) $W/2$ and (c) time delay. Chromatographic conditions as in Fig. 6.

of the measurements. In order to avoid these phenomena, a flow-rate of 550 ml/min was selected, at which a relatively reduced sensitivity is shown but good symmetry and reproducibility are achieved.

Preconcentration procedure

Preliminary studies were made of the ability of the microcolumn to preconcentrate samples with the on-line complexation procedure. The 100-μl loop on the Reodyne valve was replaced with a LiChroCART RP-18 (5 μm) microcolumn (4 × 4 mm I.D.). Samples of 100 ml were passed at 4.0 ml/min through the microcolumn, in the same direction as the elution step, followed with washing with 10.0 ml of HPW to remove the matrix. Usually a counter-flow is preferred for the preconcentration procedure in order to avoid interferences in the chromatographic behaviour; in this case the limited length of the preconcentrator did not affect the separation. Each sample solution was analysed in triplicate and the amounts ranged from 50 to 500 ng (as Hg) for inorganic and organic species, respectively. The analyte recoveries were evaluated by comparing the height of the peaks obtained for samples analysed by this procedure with those obtained for 100-μl samples, containing the same absolute amounts of mercury species, analysed without preconcentration. Recoveries of 40% were obtained except for methylmercury, which showed an anomalous recovery of only 16%. The expected preconcentration factor of 1000 was so reduced to 400 for ethyl-, phenyl and inorganic mercury and 160 for methylmercury, but good reproducibility was obtained over the range of concentrations studied. The calibration graph (Fig. 8) shows the linear response for the species considered and illustrates the order of sensitivity of CVAAS, which increases in the order phenyl-, methyl- and ethylmercury.

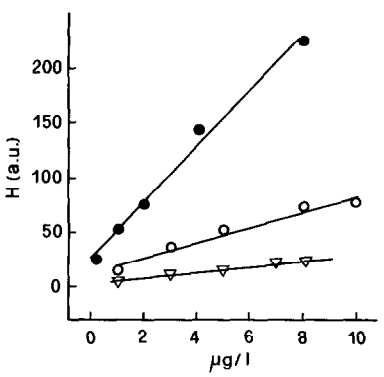

Fig. 8. Calibration graphs for on-line preconcentration procedure. Chromatographic conditions as in Fig. 6; 100-ml sample volume. ○ = CH_3Hg^+; ● = $C_2H_5Hg^+$; ▽ = $C_6H_5Hg^+$.

Detection limits

As shown above (optimization of the parameters), there is a large difference between the sensitivity of inorganic mercury and organic species. The detection limits (DL) were determined for each analyte in ultrapure water with a 100-μl loop and for 100 ml in the on-line preconcentrated sample procedure. Table I compares the DLs (three times the blank value) calculated for both procedures and indicates that the DLs for inorganic mercury with or without precomplexation and DLs of organic species were the same for both procedures.

Real samples

The performance of the proposed system was also evaluated on a real sample of tap water. The sample was filtered (0.45 μm) and analysed as such as or spiked with 0.1–0.3 ng/ml concentrations for inorganic mercury. Fig. 9 compares the chromatogram of tap water with that for samples spiked with organic species at ng/ml and sub-ng/ml concentration levels. The chromatograms show that the method is unaffected by the matrix composition, and moreover a good reproducibility is obtained for this kind of sample. The sample concentration, evaluated by the standard addition procedure, was of 170 ng/l of inorganic mercury with a linear regression coefficient $R = 0.995$. In the light of these results, it is concluded that the developed method is well suited to speciation studies on natural and polluted waters.

Fig. 9. Analysis of tap water by on-line preconcentration procedure: (a) as such; (b) spiked sample. Peaks: = 1 CH_3Hg^+; 2 = $C_2H_5Hg^+$; 3 = $C_6H_5Hg^+$; 4 = Hg^{2+}. Chromatographic conditions as in Fig. 6.

TABLE I

DETECTION LIMITS FOR DIRECT INJECTION AND ON-LINE PRECONCENTRATION PROCEDURE

Signal-to-noise ratio = 3. Chromatographic conditions: mobile phase, acetonitrile–water (58:42, v/v) containing 20.0 m*M* acetic acid, 0.5 m*M* APDC and sodium hydroxide up to pH 5.5; flow-rate 1.5 ml/min; sample loading flow-rate 4.0 ml/min; CVAAS detection, 253.7 nm.

Species	Detection limit (ng/ml)	
	100 μl[a]	100 ml[b]
CH_3Hg^+	100	0.50
$C_2H_5Hg^+$	50	0.09
$C_6H_5Hg^+$	300	0.50
Hg^{2+}	8; 1[c]	0.015

[a] Direct injection.
[b] On-line preconcentration procedure.
[c] Without and with precomplexation, respectively.

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