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Short Communication

Interfacing high-performance liquid chromatography and cold-vapour atomic absorption spectrometry with on-line UV irradiation for the determination of organic mercury compounds

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Abstract

A liquid chromatographic method with on-line UV irradiation was developed for the determination of organic mercury compounds by cold-vapour atomic absorption spectrometry (AAS). Methyl-, ethyl-, phenyl- and inorganic mercury were separated on RP C_{18} columns. An UV-irradiation lamp was used for the on-line destruction of the organomercury compounds. Sample and $NaBH_4$ solution were continuously fed to the reaction vessel where mercury was reduced. The volatilized mercury was swept into the absorption cell of a cold-vapour AAS system by nitrogen. The detection limit for methylmercury is 80 pg absolute ($S/N = 3$).

1. Introduction

In recent years various high-performance liquid chromatography (HPLC) methods for the determination of inorganic and organomercury have been developed. The most simple method is the interfacing of HPLC with UV detection. The use of RP C_{18} columns with chelating agents such as substituted dithiocarbamates or thiols enabled simultaneous separation and detection by UV absorption [1–5]. Another, but more selective method was the coupling of HPLC with atomic absorption spectrometry (AAS) or atomic fluorescence spectrometry (AFS), to suppress interferences which occur with UV detection [6,7]. The most difficult procedure was the transformation of the usually stable organomercury complexes into elementary mercury for detection. Several powerful oxidizing solutions like potassium dichromate or peroxodisulphate with following reduction have been used on-line to increase the detection limits. Former papers reported about oxidizing photodigestion by UV irradiation with high-pressure UV lamps (150 W) for batch samples [8–10].

A new method was developed to avoid the on-line wet oxidation, by using a 8-W low-pressure UV lamp with a surrounding PTFE tubenet, to reach detection limits for the investigated organo mercury compounds in the range of 80 pg absolute. Three chelating agents have been tested.

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2. Experimental

2.1. Apparatus

A schematic diagram of the detection system is shown in Fig. 1. The HPLC system consists of a LDC Analytical pump module with a Rheodyne injector (20-μl sample loop), Model ConstaMetric 3200 and a LDC analytical membrane degasser. The detection was carried out on a cold-vapour AAS mercury monitor 3200 (the absolute detection limit of the detector for elementary mercury is 15 pg) by LDC Analytical with integration software LDCtalk. The 8-W UV lamp and the irradiation PTFE coils were obtained from ICT. The peristaltic pump was made by Ismatec and the gas–liquid separator was part of the cold-vapour mercury generator module by LDC Analytical. The drying tube (7 cm × 1 cm I.D.) was filled with calcium chloride. For lengthening the reducing time of the sodium borohydride a PTFE reaction tube (1.5 m × 0.8 mm I.D.) was used. Three RP C_{18} columns were used for the experiments: (1) Chromosphere ODS (5 μm) 30 × 4 mm, (2) Chromosphere ODS (5 μm) 15 × 4 mm and (3) Chromosphere ODS (3 μm) 8 × 4 mm.

2.2. Reagents

Mercury chloride and methylmercury chloride were purchased from Merck, ethylmercury chloride from Alfa and phenylmercury chloride from Aldrich. All compounds for the eluents like methanol, acetonitrile, HPLC-grade water, acetic acid, ammonium acetate and the reducing substances tin(II) chloride and sodium borohydride were of analytical-reagent grade (Baker). 2-Mercaptoethanol, sodium pyrrolidinedithiocarbamate and cysteine from Merck were used without further purification. Before application the sodium borohydride solution was purified from mercury by bubbling with nitrogen for 1 h.

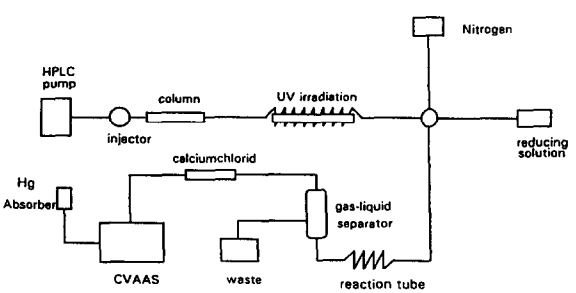

Fig. 1. The HPLC–cold vapor (CV) AAS system coupled with on-line UV irradiation and mercury vapour generator for the separation and detection of mercury compounds.

2.3. Preparation of solutions

The stock solutions of methyl-, ethyl-, phenyl- and inorganic mercury were prepared in water, stored in the dark and refrigerated. The standards were prepared weekly. The eluent was prepared by using acetonitrile–water (65:35, v/v) buffered with ammonium acetate–acetic acid at pH 5.5 containing 0.5 m*M* pyrrolidinedithiocarbamate. A solution of 1% sodium borohydride adjusted to pH 13 with 1 *M* NaOH was used for reduction.

2.4. Irradiation PTFE coil

Several lengths of irradiation coils were tested. The irradiation coil consisted of a PTFE tube of 0.3 mm I.D. A hand-knitted net of 5, 10, 15 and 20 m length is commercially available. For the shorter lengths we knitted the net ourselves. The UV lamp has a length of 30 cm and a diameter of 15 mm and was put in a box for eye protection. The irradiation coils were pulled over the lamp and to better exploit the irradiation they were enveloped with aluminum foil. The PTFE coil shows no change after 1000 operation hours.

3. Results and discussion

3.1. Selection of the complexing agent

2-Mercaptoethanol, cysteine and sodium pyrrolidinedithiocarbamate (SPDC) form stable complexes with organic and inorganic mercury compounds [11–15], which can be separated by HPLC and reduced by tin(II) chloride or sodium

borohydride after on-line oxidation by UV irradiation [3]. After gas–liquid separation, the mercury vapour was detected with AAS. Fig. 2 shows a typical chromatogram for inorganic mercury, methylmercury, ethylmercury and phenylmercury with and without on-line UV irradiation of the mercury pyrrolidindithiocarbamate complexes. The comparison of the two chromatograms shows that with UV irradiation the sensitivity is considerably increased. The inorganic mercury peak has the same area in both chromatograms, so that under these conditions a supplementary UV irradiation does not improve the peak height.

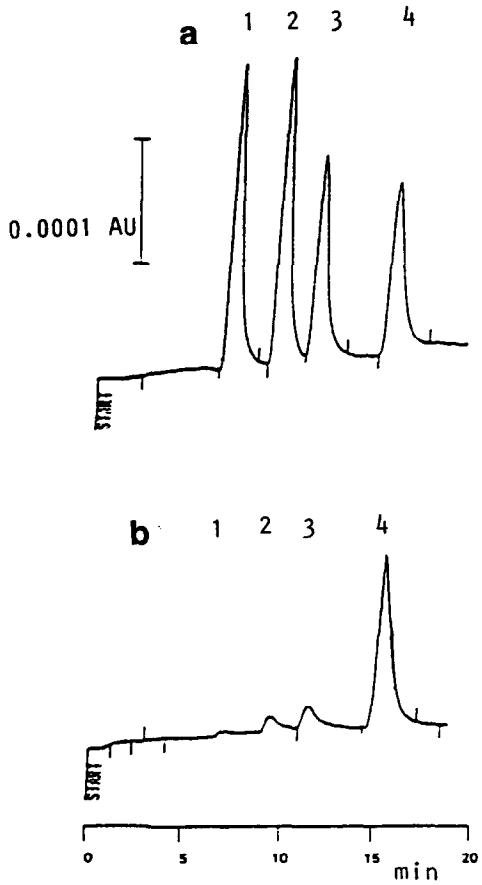

Fig. 2. Comparison between a separation with and without UV irradiation of mercury–SPDC complexes by HPLC–cold-vapour AAS. (a) UV irradiation (90% at 254 nm; reaction coil length 10 m); (b) without UV irradiation. Chromatographic conditions: mobile phase, acetonitrile–water (65:35, v/v) containing 25 mmol ammonium acetate, 0.5 mmol SPDC and acetic acid up to pH 5.0. Column, Chromosphere ODS (5 μm) 30 × 4 mm; flow-rate, 0.9 ml/min; 20-μl sample loop. Peaks: 1 = CH_3Hg^+ (5 ng as Hg); 2 = $CH_3CH_2Hg^+$ (4.5 ng as Hg); 3 = $C_6H_5Hg^+$ (5.5 ng as Hg); 4 = Hg^{2+} (4 ng).

2-Mercaptoethanol, cysteine and SPDC as complexing agents are tested by different eluent conditions, reduction with tin(II) chloride and sodium borohydride as reducing compounds (see Fig. 3). We found that the best results are obtained by using the SPDC as complexing compound with sodium borohydride reduction. An advantage is the order of separation of the different mercury compounds with SPDC, because Hg^{2+} is eluted at the end. With 2-mercaptoethanol, Hg^{2+} is eluted first and might cover in higher concentrations the methylmercury peak during elution [12].

3.2. Selection of the UV-irradiated reaction coil length

The irradiation source is a glass tube filled with argon and mercury under low pressure (about 10^{-3} Torr; 1 Torr = 133.322 Pa). The low-pressure mercury lamp transmits 90% of the light at 254 nm. The reaction coil is a handmade knitted PTFE coil (0.3 mm I.D.) which covers

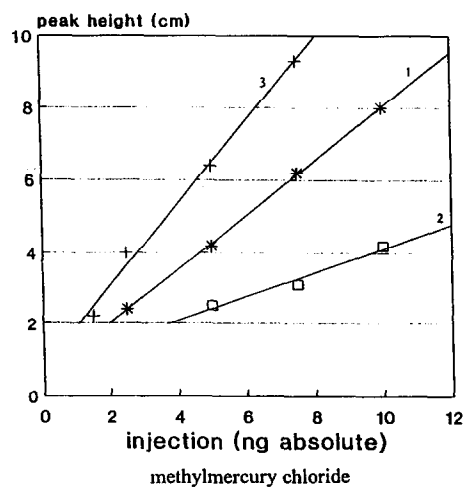

Fig. 3. The increase of the peak height for 2-mercaptoethanol (1), cysteine (2) and sodium pyrrolidinedithiocarbamate (3) as complexing compound. Sodium borohydride for reduction.

the lamp. The peak width, the peak height, the irradiation coil length, the reducing tube and the flow-rate of the eluent had to be optimized in order to achieve maximum sensitivity. There was good linearity for the SPDC–methylmercury complex (regression coefficient $r = 0.994$) between 200 pg and 20 ng Hg absolute (20-μl injection). One must also take into consideration that the pressure of the eluent pump increases with the length of the irradiation coil, so that we have an upper limit at 20 m coil length. Consequently the procedure for SPDC is optimized with an eluent flow of 0.9 ml/min (tested range between 0.5 and 1.5 ml/min), nitrogen flow 120 ml/min (tested range between 50 and 200 ml/min), a 10-m irradiation coil (tested range between 0 and 200 cm) and a reducing tube (tested range between 20 and 200 cm).

3.3. Detection limits for direct injection

The limit of detection for the four tested compounds (Hg^{2+} and the three organomercury compounds) are the same [80 pg Hg absolute ($S/N = 3$)]. This means: Hg^{2+} forms a stable compound with SPDC which is completely destroyed by sodium borohydride and serves as reference basis; the other organomercury compounds need an additional oxidizing step for a complete destruction (*e.g.* UV irradiation). From that we infer the conversion is almost 100%. Comparing to Sarzanini *et al.* [16] the enhanced sensitivity is due to the quantitative destruction of the organomercury compounds.

The relative standard deviations for the methylmercury, ethylmercury and inorganic mercury measurement at 5 ng level are 5%, for phenylmercury 6%.

4. Conclusions

The HPLC–AAS coupling with on-line UV irradiation is a new powerful method for the determination of methylmercury and other organomercury compounds. All components are commercially available. In comparison to earlier methods ours is a simpler one and overcomes the wet oxidation problem by using the on-line UV irradiation. Therefore this procedure does not have the problem of an increasing volume as wet oxidizing has.

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