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Short communication

Interfacing supercritical fluid chromatography with atomic fluorescence spectrometry for the determination of organomercury compounds[1]

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Abstract

The development of an interface for a system consisting of supercritical fluid chromatography (SFC) and atomic fluorescence spectrometry for the determination of organomercurials is presented. The compounds escaping from the restrictor of the SFC column are transported with an argon stream into a tube furnace to decompose the organomercurials. The formed elemental mercury is detected with an atomic fluorescence spectrometer. Diorganomercury compounds can be analysed with the developed system without any problems. Because of their lower solubility in supercritical carbon dioxide monoorganomercury (II), compounds must be derivatised to compounds of lower polarity. This can be done by complexing the monoorganomercurials with diethyldithiocarbamate. Even thermolabile compounds, like alkoxyethylmercurials, can be quantified with the new system. © 1997 Elsevier Science B.V.

Keywords: Interfaces; Atomic fluorescence spectrometry; Detection, SFC; Organomercury compounds

1. Introduction

Because of their high toxicity and the methylation of inorganic mercury in water by microorganisms, the determination of organomercury compounds is very important.

Many different analytical methods are used for their determination. After their separation by gas chromatography (GC) or high-performance liquid chromatography (HPLC), the organomercurials may be determined using many different detectors. The most common system for the analysis of organomercurials is GC followed by electron-capture detection (ECD) [1]. However, element-specific detectors, such as atomic absorption spectrometry (AAS) [2], atomic emission spectrometry (AES) [3], atomic fluorescence spectrometry (AFS) [4], mass spectrometry (MS) [5] and inductively coupled plasma mass spectrometry (ICP-MS) [6] are also used in combination with GC. GC methods are restricted to the relatively thermostabile compounds like the alkylmercurials. Other species, such as the alkoxyethylmercury compounds, which are used as seed dressing and fungicides, decompose under GC conditions.

These problems can be avoided by using HPLC as the separation method. The species are determined using various detection methods, especially element-

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specific ones like AAS [7], AES [8] and AFS [9]. The disadvantages of HPLC techniques in comparison with GC methods are higher detection limits and more complicated interfacing with element-specific detectors. In this context, the application of supercritical fluid chromatography (SFC) represents an interesting alternative for the determination of organomercurials. There are two main advantages: The interfacing with element-specific detectors is similar to GC and thermolabile compounds can be determined.

Because AFS is the most sensitive detection method for mercury [10], a SFC–AFS system seems to be particularly interesting for the determination of organomercury compounds.

2. Experimental

2.1. Apparatus

All experiments were carried out using an SFC Series 600 from Dionex. The samples were injected by time split injection (0.2 s) using a 0.5-μl sample loop. The resulting injection volume depends on the restrictor and the pressure of the supercritical fluid. At 100 bar (which was the start pressure that was usually used in our investigations), approximately 0.2 μl are injected. A 10 m×50 μm I.D. SB-Methyl 100 column, fitted with a frit-restrictor (Dionex), was used. For the determination of volatile compounds such as dimethylmercury, a retention-gap (5 m deactivated fused-silica tubing) was installed.

An AFS Model 2500 from Tekran was used as the detector. The interface was constructed in the laboratory. The parts that are in contact with the fluid consist of PTFE or quartz.

For data acquisition, evaluation and control operations, AI-450 Release 3.32 software (Dionex) was used.

2.2. Gases

Carbon dioxide is used as the supercritical fluid. It was purchased from Air Products and was of SFE/SFC-grade. The argon was 'for spectrometry' grade (Linde).

2.3. Reagents

Sodium diethyldithiocarbamate (Merck) and *n*-hexane (Merck) were of analytical-reagent grade. Methanol (Promochem) was of HPLC-grade.

Stock solutions of mercury dichloride (Merck), methylmercury chloride (Merck), methoxyethylmercury chloride (O. Tropitsch, Marktredwitz, Germany) and ethoxyethylmercury chloride (synthesized as described by Schoeller et al. [11]) were prepared in water; ethylmercury chloride (Merck), phenylmercury chloride (Merck) and tolylmercury chloride (O. Tropitsch) were dissolved in methanol. Stock solutions of dimethylmercury (Merck) and diphenylmercury (Fluka) were prepared in hexane.

2.4. Complexation of monoorganomercurials with sodium diethyldithiocarbamate

A 100-μl volume of 4% sodium diethyldithiocarbamate in methanol and about 0.5 g of sodium chloride were added to 10 ml of an aqueous solution containing monoorganomercurials. After shaking for about 1 min, the solution was extracted twice with 1 ml of hexane. The hexane containing the complexes was injected into the SFC–AFS system.

3. Results and discussion

At the new interface between SFC and AFS, the compounds escaping from the restrictor must be transported into the detector. Because of the low flow-rates in capillary SFC (about 0.1 ml/min), a make-up gas is necessary to attain flow-rates that are sufficiently high. All molecules cause quenching by partly absorbing the fluorescence radiation in the detection cell of the AFS so that the use of argon is favourable. Furthermore, the organomercurials must be decomposed in the interface to form elemental mercury, because only elemental mercury can be detected by AFS. For this purpose, the pyrolysis of compounds is promising.

3.1. Pyrolysis in the supercritical fluid

Because of the longer time of direct contact in the tube furnace and expected better peak shapes in the

interface, the pyrolysis was first carried out under supercritical conditions.

The organomercurials coming from the SFC column are decomposed in a tube furnace. After the pyrolysis unit, the supercritical fluid is expanded to normal pressure in a frit restrictor. The compounds escaping from the restrictor are transported into the AFS detection cell by an argon stream. Although the temperature in the tube furnace is about 700°C, restrictor heating is necessary. Otherwise, the restrictor would freeze up because of the Joule–Thomson effect.

Organomercurials can be analysed with the described system. A disadvantage of the system presented is that the restrictor become plugged, over time, with pyrolysis products. This results in a shifting of the retention times and it is necessary to change the restrictor after about 100 injections.

3.2. Pyrolysis after restriction

The problems presented should not occur when pyrolysis takes place after the restrictor.

The argon is pre-heated in the SFC oven and transports the compounds escaping from the restrictor into the tube furnace, where the organomercurials are thermally decomposed in a quartz tube. The formed mercury (0) is detected in the atomic fluorescence spectrometer.

As expected, there were no problems caused by the restrictor being plugged when the system described was used. The retention times are reproducible over a long period. The standard deviation for four injections performed one after another is typically 0.1%.

3.3. Quenching caused by the carbon dioxide used as the supercritical fluid

In the detection cell, all molecules cause quenching by partly absorbing the fluorescence radiation. By using carbon dioxide as the mobile phase in SFC, the carbon dioxide is transported into the AFS system and causes quenching. To determine the loss in sensitivity, the dependence of the quenching on the carbon dioxide pressure in the SFC column was investigated.

Behind the restrictor (at normal pressure), mercury-saturated air was injected, through a septum with a microliter syringe, into the argon–carbon dioxide mixture that reaches the detection cell. For the mercury gas standards, a set-up analogous to that of Dumarey et al. [12] was used and 10 μl of mercury-saturated air were injected at 10°C (corresponding to 55.7 pg mercury). The results are shown in Fig. 1.

As expected, the peak area decreased with increasing carbon dioxide pressure, because, with rising pressure, more carbon dioxide escapes from the restrictor. At 400 bar, the signal is a factor of six smaller than that obtained without carbon dioxide being present in the detection cell. Therefore, it is favourable to elute the organomercurials at low pressures. However, this gives rise to longer retention times.

The problem can be avoided by using xenon as the supercritical fluid, because no quenching occurs. Xenon has advantageous critical parameters (T_c, 16.6°C; p_c, 58.4 bar) and similar chromatographic behaviour to carbon dioxide. Unfortunately, xenon is very expensive, which has restricted its use to date.

3.4. Optimisation of the SFC–AFS system

There are two variables that must be optimised for the SFC–AFS interface: The temperature in the tube furnace and the flow-rate of argon. For these investigations, diphenylmercury standard solutions were injected by the time split injector. Diphenylmercury was chosen because of its good chromatographic behaviour and its UV activity. It is

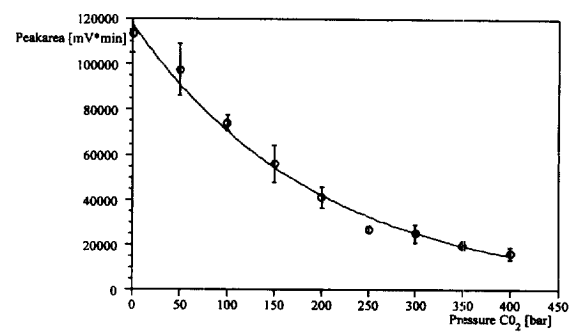


Fig. 1. Effect of carbon dioxide pressure on quenching in AFS. Argon flow-rate, 35 ml/min. (The error bars shown represent the confidence interval with $P=95\%$ and $F=4$).

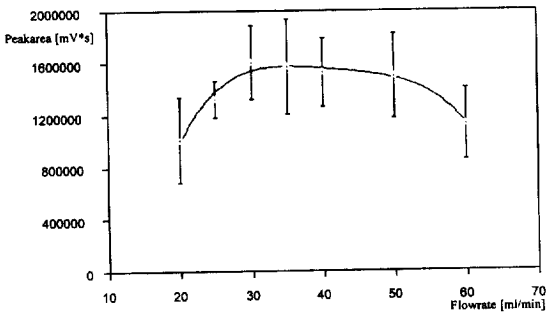


Fig. 2. Effect of the flow-rate of argon on the peak area of diphenylmercury (100 μg/ml). A temperature of 700°C was used for the tube furnace. (The error bars shown represent the confidence interval with $P=95\%$ and $F=4$).

advantageous to check the injected amount using an UV detector.

The argon flow-rate was varied between 20 and 60 ml/min and the temperature of pyrolysis was varied between 500 and 800°C. The results are shown in Figs. 2 and 3.

As can be seen in Fig. 2, the argon flow-rate shows a broad optimum in the range of 30–50 ml/min. The decrease in peak areas with higher flow-rates can be explained by the shorter time of direct contact in the tube furnace, so not all of the diphenylmercury is converted into elemental mercury. With lower flow-rates, not only the time of direct contact increases, but also the share of carbon dioxide in the argon–carbon dioxide mixture in the detection cell. This causes more quenching, such that, with lower flow-rates of argon, smaller peaks are detected.

Optimisation of the temperature in the tube furnace shows that a temperature of about 650°C is sufficient for the maximum formation of elemental mercury. Higher temperatures show no further effect.

For further investigations, an argon flow-rate of 35 ml/min and a temperature in the tube furnace of 700°C were used.

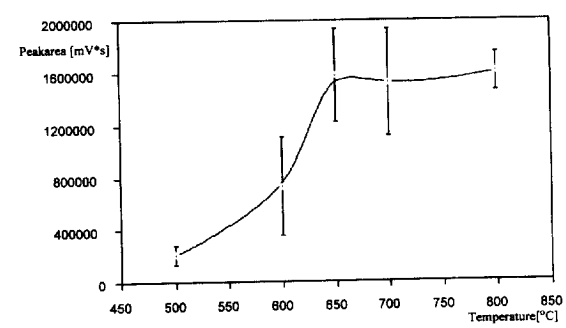


Fig. 3. Effect of the temperature in the tube furnace on the peak area of diphenylmercury (100 μl/ml). The flow-rate of argon was set at 35 ml/min. (The error bars shown represent the confidence interval with $P=95\%$ and $F=4$).

3.5. Investigation of diorganomercurials with SFC–AFS

Because of their low polarity and the resulting good solubility in supercritical carbon dioxide, diorganomercurials can be analysed well using SFC–AFS. For the determination of volatile compounds like dimethylmercury (boiling point, 97°C), a retention gap (5 m deactivated fused-silica tubing) and injection in the subcritical region (50 bar) is necessary to get sufficient separation of the analyte from the solvent.

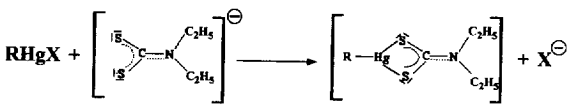
The instrumental detection limits for diorganomercurials, calculated using a signal/noise ratio of three, are about 0.1 mg/l (20 pg Hg absolute) (see Table 1). It is expected that this value could be lowered by raising the injected sample volume and decreasing adsorption in the SFC–AFS system. If one assumes that the injected sample passes through the column without any losses and that the organomercurials are completely converted to elemental mercury at the interface, the instrumental detection limits will be about 1 μg/l.

Table 1

Compound	Detection limit	
	mg of Hg/l	pg of Hg absolute
Dimethylmercury	0.08	15.87
Diphenylmercury	0.14	28.30
Methylmercury chloride	0.23	46.15
Ethylmercury chloride	0.25	50.00
Methoxyethylmercury chloride	3.00	600.0
Ethoxyethylmercury chloride	1.00	200.0
Phenylmercury chloride	0.68	136.36
Tolylmercury chloride	1.00	200.0

3.6. Investigation of monoorganomercurials using SFC–AFS

Because of their high polarity, monoorganomercury compounds cannot be analysed satisfactorily by SFC with carbon dioxide as the mobile phase. To improve the chromatographic behaviour, one possibility is the use of modifiers like methanol to increase the solubility of the organomercurials in the supercritical fluid. Another way is to convert them into compounds of lower polarity. For example, monoorganomercurials can be complexed with sodium diethyldithiocarbamate and then analysed by SFC–AFS.



An exemplary chromatogram is presented in Fig. 4.

Even the thermolabile alkoxyethylmercurials and inorganic mercury ions can be analysed with the SFC–AFS system. The detection limits for the investigated monoorganomercury compounds range between 0.2 and 3 mg/l (50–600 pg Hg absolute) (see Table 1). As explained for the diorganomercurials, it is expected that these detection limits could be lowered by further improvements of the SFC–AFS system.

In comparison with our new method, the instrumental detection limits for methylmercury using GC–AFS are 0.6 pg Hg absolute [4], and using HPLC–AAS, they are 80 pg Hg absolute [7].

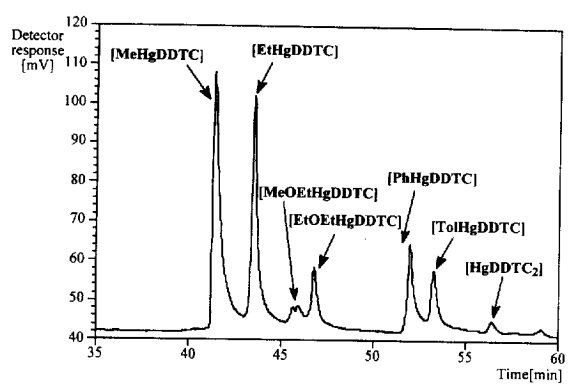


Fig. 4. Chromatogram of a standard mixture after complexation with sodium diethyldithiocarbamate. Composition of the standard: Mercury dichloride, methylmercury chloride, ethylmercury chloride, methoxyethylmercury chloride, ethoxyethylmercury chloride, phenylmercury chloride and tolylmercury chloride (100 mg/l each). Chromatographic conditions: 80°C, 10 min at 100 bar, then a pressure gradient of 2 bar/min.

4. Conclusions

The developed SFC–AFS system is suitable for the analysis of organomercury compounds. While diorganomercurials can be analysed without prior treatment, monoorganomercurials and inorganic mercury compounds must be converted into compounds of lower polarity by complexation. Even thermolabile organomercurials, such as the alkoxyethylmercurials, which decompose under GC conditions, can be analysed with the new system.

In comparison with HPLC, the easy and stable working interface is advantageous. In addition the operating costs are much lower than those for HPLC.

The main disadvantage of the new SFC–AFS system is the high detection limits in comparison to comparable element-specific systems. In further investigations, we will attempt to lower the detection limits.

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