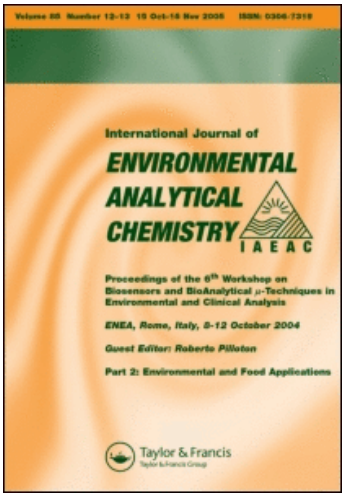


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# A Sensitive Method for Simultaneous Determination of Airborne Organolead Compounds

## Part I: Chromatographic Separation and Chemical Reaction Detection

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Reversed phase high performance liquid chromatography coupled to a sensitive chemical reaction detector has been developed for the separation and detection of inorganic lead and organolead compounds. The separation of $Pb^{2+}$, $Me_3Pb^+$, $Et_3Pb^+$, $Me_4Pb$, and $Et_4Pb$ can be achieved within 50 minutes. The eluate from the column flows continuously through a system of mixing coils where the lead species are transformed into compounds which undergo complexation with 4-(2-pyridylazo)resorcinol. The red-coloured complex is detected spectrophotometrically at 546 nm. In the lower measuring range of 0.3 to 1.5 nmol (abs.) for the five compounds linear calibration curves are obtained. The requirements and characteristics of the separation and post-column reactions are illustrated and the linearity, reproducibility, and sensitivity of the system are discussed.

KEY WORDS: Organolead compounds, HPLC, chemical reaction detector.

## INTRODUCTION

Many heavy metals and organometallic compounds belong to the group of very toxic substances. Their noxious effects result not only from acute intoxications but also from accumulation of these hazardous compounds over long periods up to high concentration levels. The toxicokinetics that are incorporation and resorption, distribution and deposition, metabolism and excretion as well as the toxicodynamics, that is the molecular response mechanism in the different organs, are strongly depending on the oxidation state and the binding of a metal to certain organic or inorganic groups. In general organometallic compounds (e.g. $Me_4Pb$, MeHgCl) are

more toxic than inorganic metal species, and higher oxidation states are more toxic than lower ones (e.g. in the system Cr(III)/Cr(VI)). Gosio[1], Kehoe[2], and Takeuchi[3] have reported on intoxications by organometallics as trimethylarsine, tetraalkyllead, and methylmercuric chloride already decades ago. But although the toxicity of these substances has been recognized very early their pathways in ecosystems as well as their toxicological behaviour remained unknown to a high degree. In order to enable investigations on the behaviour of certain species which gain increasing importance also from the point of view of occupational health and industrial toxicology, analytical methods with high precision are needed for qualitative and quantitative speciation analyses in the range of MAK values and below. In recent years the development of analytical techniques, as capillary gas chromatography, high pressure liquid chromatography, and flameless atomic absorption spectrometry[4] has improved the preconditions for the analyses of metal species. Several authors are involved in the problems of determining organolead compounds. De Jonghe[5] and Chau[6] separated tetraalkyllead species by gas chromatography (GC) followed by an atomic absorption spectrophotometric (AAS) detection. However, gas chromatography without pre-column derivatization can be easily applied only to uncharged organolead compounds. Another approach to the quantitative determination of organometal species is to use high performance liquid chromatography (HPLC) in combination with a selective detection system. Graphite furnace atomic absorption spectroscopy (GFAAS) with an automatic, periodic sampling and atomization of the chromatographic eluent has been used frequently as a detection system.[7] The disadvantage of this detection mode is the low atomization efficiency of organometals because of their volatile nature. MacCrehan[8] described the combination of LC and electrochemical detection (EC) but only for cationic organometals for which good detection limits can be obtained. However, electrochemical detection in the reductive mode requires ultrapure reagents, electrolytically purified solvents, and the exclusion even of traces of oxygen by purging the whole system with high-purity nitrogen.

This paper describes the separation of organolead species by RP-HPLC with a detection approach based on the spectrophotometric absorption measurement of a coloured lead complex formed in a continuous flow reaction system.[9]

## EXPERIMENTAL

### Procedure

Reversed phase high performance liquid chromatography (RP-HPLC) was

chosen for the separation of divalent inorganic lead ($Pb^{2+}$) and the organolead compounds $Me_3PbAc$, $Et_3PbCl$, $Me_4Pb$, and $Et_4Pb$. In comparison to GC this technique offers some advantages. HPLC packing materials show very high column efficiency. As several organolead compounds are relatively unstable in substance as well as in solution, liquid chromatography provides mild eluting conditions during the separation process. Another advantage is that both cationic and uncharged lead species can be investigated chromatographically without any expensive derivatization reactions, leading to thermally stable, strong complexes of the cationic organometals.

The outlet of the HPLC column is connected to the chemical reaction detector consisting of a continuous flow system and a UV-VIS absorbance detector. The eluted lead compounds which do not undergo complexation with 4-(2-pyridylazo)resorcinol (PAR) are decomposed in exactly controlled chemical reactions. After this the eluted lead species or their decomposition products form red-coloured PAR complexes which are detected spectrophotometrically.[10] These post-column reactions must proceed under exact and reproducible conditions which are determined mainly by the concentration of the reagents, the pH-values, and the temperature. The potential of this technique as a sensitive, selective, and at the same time universal detector is apparent.



## Apparatus

| | |
|---|---|
| High pressure pump: | Waters M6000A, Waters GmbH, D-6240 Königstein; |
| Injection valve: | Rheodyne, Latek 7010, LATEK, D-6900 Heidelberg; |
| Peristaltic pump: | Gilson, Minipuls II, Abimed, D-4000 Düsseldorf; |
| Pump tubing and accessories: | Biotechnic GmbH, D-2000 Hamburg; |
| Spectrophotometric detector: | Waters M440 absorbance detector, Hg-lamp, 546 nm-filter, Waters GmbH, D-6240 Königstein; |
| Recorder: | Corning recorder 840, IMA Analysentechnik, D-6300 Gießen; |
| Thermostat: | Immersion thermostat T51, Heidolph-Elektro KG D-8420 Kelheim. |

## Reagents and solutions

*Iodine solution*: 0.005 M $I_2$ in $H_2O$ by diluting a 0.05 M stock solution (20 g KI + 12.7 g $I_2$/L) with bidistilled water.

*Sodium thiosulfate*: 0.01 M $Na_2S_2O_3$ in $H_2O$ by diluting a 0.1 M stock solution (24.8 g $Na_2S_2O_3 \times 5H_2O$/L) with bidistilled water.

*Buffer*: $NaOH/Na_2B_4O_7$ of different composition, dependent on the used mobile phase, to adjust the solution to pH 10.

*Dye stock solution*: 255 mg 4-(2-pyridylazo)resorcinol-Na $\times H_2O$ (PAR) in 100 mL bidistilled water; $10^{-2}$ M PAR (stored in the cold and in the dark).

*Dye solution*: 10 mL of the dye stock solution diluted to 1000 mL with a 0.10 M acetic acid/0.14 M triethylamine buffer (pH 10); $10^{-4}$ M PAR; 0.4 mL Triton X 100/1000 mL (C. Roth KG Chem, Fabrik, D-7500 Karlsruhe) is added to the 0.01 M $Na_2S_2O_3$ and the dye solution to improve the regularity of the bubble pattern in the chemical reaction system.

*Organolead compounds*: Tetramethyllead (with 25 Vol% toluene), tetraethyllead (AK Chemie, D-6081 Biebesheim); trimethyllead acetate and triethyllead chloride (Ventron, D-7500 Karlsruhe). The organolead compounds should be stored in the cold and the dark to minimize decomposition. Unless otherwise noted, all chemicals (E. Merck, D-6100 Darmstadt) are of analytical reagent grade quality, and are used as received.



## RESULTS

### Chemical reaction detector

Liquid chromatography was favoured for the separation of both, cationic and uncharged organolead compounds, because of the reasons described above. The methods of detection mostly used for LC are AAS, EC, and UV-VIS absorption spectrometry. As an atomic absorption spectrometer was not available and the expense of the apparatus and of high-purity chemicals for EC is very high, a simple detection system was developed, using VIS absorption spectrometry (Figure 1). The investigated organolead compounds and the lead ion in aqueous solution are colourless substances. In order to detect them by VIS absorption spectrometry, they have to be transformed into a coloured form. A great number of complexing reagents for lead ions are known, the complexes of which are soluble in water or in organic solvents. For the detection system under discussion here, 4-(2-pyridylazo)resorcinol (PAR), a well investigated complexing reagent for many metal ions, was chosen. In aqueous alkaline buffered solutions it reacts with lead(II) as well as with dialkyllead ions to intensely red-coloured complexes. Their water solubility facilitates the

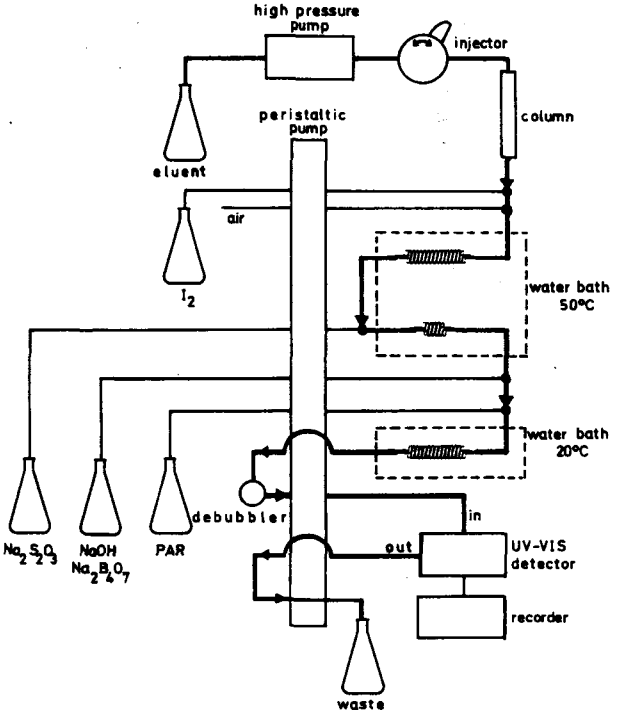


FIGURE 1 Schematic diagram of chromatograph and detection system.

application of this reagent in continuous flow chemical reaction systems. The maximum absorbance of lead(II)-PAR and dialkyllead(II)-PAR is observed at 520 nm (pH 10) and 516 nm (pH 9) with molar absorptivities of about 50,000 and 40,000 liters/mole·cm resp.[11]. The two latter values reveal the good sensitivity of absorption spectrometry with PAR lead complexes. The maximum colour intensity of the complex was found to develop immediately and is stable for several hours. The spectrophotometric determination of the ligand-lead complex is carried out with a fixed-wavelength detector at 546 nm (Hg-line). The absorption at this wavelength is 30–35% lower compared to the maximum absorption at 520 nm. In the series of tetraalkyllead ($R_4Pb$), trialkyllead ($R_3PbX$), dialkyllead ($R_2PbX_2$), and lead(II) only the latter two species are complexed with PAR. Monoakyllead ($RPbX_3$) is too unstable to be detected with this system. After separation on the chromatographic column $R_4Pb$ and $R_3PbX$ have to be decomposed in a post-column

reaction in a way as to form the detectable PAR lead complexes. Strong oxidizing agents e.g. potassium permanganate, sulfuryl chloride, and calcium hypochlorite effect complete decomposition of alkyllead compounds already at low temperatures. These reagents are used for decontamination of tetraalkyllead spills and for final cleaning of equipment contaminated with these substances or their partial decomposition products. The reaction of organolead compounds with halogens involves cleavage only of the lead-carbon bond. The initial products are dependent on the reaction temperature and the amount of halogen introduced into the system.[12] Because of the aggressive character of most of the named agents against material and equipment iodine is used for the decomposition of organolead species. All investigated tetra- and trialkyllead compounds are decomposed by a 0.005 M iodine solution at 50°C at a sufficient rate to achieve low detection limits. Only little attack on the material coming into contact with the iodine is observed. In methanolic solutions which are used for the chromatographic separation of organolead compounds iodine does not lose its activity. Decomposition and complexing reaction take place in the chemical reaction system, shown schematically in Figure 2. The peristaltic pump conveys the



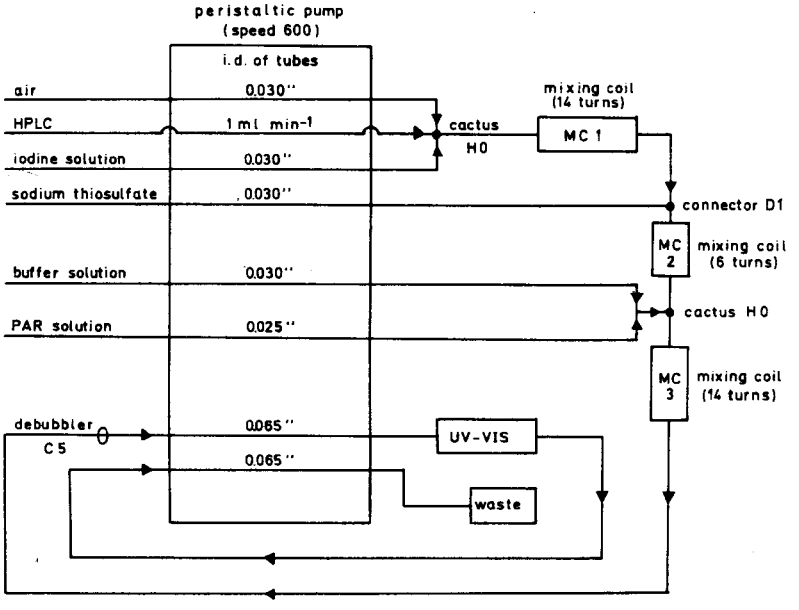


FIGURE 2 Schematic diagram of the chemical reaction system including pump tubings and accessories.

solutions determining the chemical reactions. The column effluent is mixed with the iodine solution and then segmented by air bubbles. The stream passes through a mixing coil (MC1) for decomposition reaction and the excessive iodine is then reduced by sodium thiosulfate in a second mixing coil (MC2). After this a buffer for adjustment of pH to 10 and the PAR reagent solution are added successively. The pH-adjustment and the formation of the coloured lead or dialkyllead complex take place in a third mixing coil (MC3). The air bubbles escape through a debubbler. The stream has to be pumped to the spectrophotometric detector because the flow-through cuvette with its narrow 1.26 mm bore builds up a considerable pressure against the flowing stream (flow rate 3.2 mL/min at the end of the chemical reaction system). At the given concentration levels, the chemicals which are used in this system diminish the formation of the PAR lead complex only insignificantly, so that the sensitivity of the method is negligibly reduced. The decomposition reaction should take place at higher temperatures whereas the complexation shows a higher rate of formation at room temperature. A precondition for the well functioning of the chemical reaction system is an exact and continuous flow rate of the high pressure pump and the peristaltic pump, a symmetrical bubble pattern, a correct pH-value, and a constant temperature of the stream when it passes through the detector cell. Disturbances in the system may cause background signals and/or a noisy baseline which are registered by the detector. Table I shows the calibration data of the various lead species obtained by measuring peak heights after separation on a Nucleosil 5-$C_{18}$ column. For the five compounds linear calibration curves can be drawn. The mean detection limits are estimated to be in the range of 100 ng (abs.).



## HPLC separation

Considering the chemical forms of the lead species that are the charge and the binding of the lead atom to hydrophobic groups, two chromatographic modes promise good separations: ion exchange and reversed phase chromatography. As $Pb^{2+}$ has one of the strongest sorptions of all metal cations to strong acid cation exchangers, it can only be eluted with highly concentrated strong acids often in combination with organic solvents. The application of such aggressive solutions, however, should be avoided in HPLC as they lead to fast corrosion of the high pressure pump, the injector, the stainless steel columns etc. Therefore, the separation of lead species is investigated using reversed phase HPLC. For the chromatographic studies the following five lead compounds lead(II)nitrate, trimethyllead acetate, triethyllead chloride, tetramethyllead,

system was its reappearance in the river downstream. Peaks 33 and 84 are examples.

Compound 82 would have been overlooked if detection only by flame ionization and mass spectrometry had been used. Fortunately, it was easily detected with the ECD in the discharge water (Figure 3) as well as in the Esches river, 1 km downstream from the plant (Figure 4). A closer examination of the mass spectra at retention indices approximately equivalent to the ECD chromatogram eventually revealed peak 82 to be 2,3-dichloronitrobenzene.

While the mass spectra of peaks 220 and 230 indicated they were identical to other peaks identified as acrylates of long chain alcohols (no.'s 233–240), the ECD response suggested possible halogenation. This not only increased our interest in the compounds due to their increased toxicity potential but it also improved their detectability in the river. In the Esches river extract chromatogram (Figure 4) peak 220 is indeed quite evident. However, peak 230 has disappeared. Evidently, peaks 220 and 230 are not as closely related as their mass spectra and response factors indicate.

The nonyl phenols (148–158) and alkyl acrylates (233–240) were found in the Esches river at concentrations consistent with a simple river water dilution of the discharge. The dichlorobenzenes and 2,3-dichloronitrobenzene are found at relatively lower concentrations suggesting that removal mechanisms have already affected these pollutants 1 km from the plant. This chromatogram provides valuable information as to which compounds have the greatest potential of being found further downstream at Méry/Oise.



## Verification of a source identification

Factory C which produces dyes has the highest discharge rate on the river and a high concentration of toxic compounds, as the chromatogram of one of the factory's three discharges illustrates (Figure 5). All three discharges were quantitated in terms of their mass flux, in kg/day, which reflects the discharge flow rates as well as their concentrations, as shown in Table III. Not surprisingly, at Méry/Oise, 29 km downstream, the pollutants from factory C of greatest environmental concern (nitrobenzene, nitrotoluenes, and di- and tri-chlorobenzenes) were present in the river (Figure 6) at mass flux values (considering the flow rate of the river) within a factor of 10 of the summation of all three discharges (Table III). In contrast, peaks 26 and 31 have been reduced substantially.

Equally important in confirming this source was the non-detection (Table III) of these rather common pollutants either in the river upstream

chromatographic separation study and showed well shaped peaks even at long retention times. Using this column, all five lead compounds are separated within 50 minutes, when changing the mobile phase after 25 minutes from acetate buffer/MeOH (80+20) to acetate buffer/MeOH (10 +90). The first solvent separates the ionic compound $Pb^{2+}$, which is not retarded, from $Me_3Pb^+$ and $Et_3Pb^+$. The second less polar mobile phase elutes $Me_4Pb$ and $Et_4Pb$ (see Figure 3).

As the polarity of the five species ranges from very polar ($Pb^{2+}$) to non-polar ($Et_4Pb$) a separation of all compounds within a reasonable time could not be achieved under isocratic conditions. The application of a linear gradient elution would lead to great difficulties, concerning the chemical reaction detector. The increasing amount of methanol during gradient elution causes a constant decrease of viscosity of the liquid in the reaction system. This change of the eluate permanently effects disturbances in the bubble pattern and in the mixing ratios of the added chemicals. The

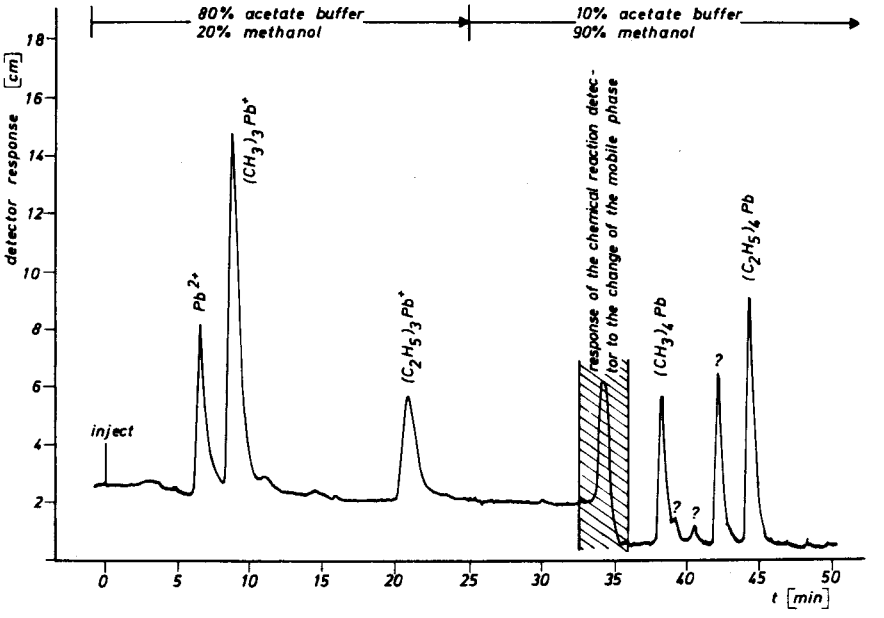


FIGURE 3 Separation of organolead compounds (column: Nucleosil 5-$C_{18}$, 200 × 6 × 4 mm; acetate buffer: 0.1 M/0.1 M, pH 4.6; column temperature: 21°C; mixing coil temperature: MC 1: 60°C, MC 2 and 3: 0°C; detector sensitivity: 0.1 A.U., 546 nm; amount per 20 μL injection volume: 1 μg $Pb^{2+}$, 1 μg $Me_3PbAc$, 3 μg $Et_3PbCl$, 5 μg $Me_4Pb$, 4 μg $Et_4Pb$; pH adjustment: 3.43 g $Na_2B_4O_7$/4.68 g NaOH per 1000 mL; delay time of the chromatogram due to the chemical reactor: 5.8 min).

result would be an increase of the baseline noise which would make correct measurements impossible.

The high pressure pump used is equipped with a solvent select valve which enables a selection between three solvents by turning a control knob so that there is no delay in the chromatogram. As can be seen from Figure 3, the sudden change of the mobile phase also effects disturbances in the chemical reaction detector. The resulting detector response, however, only lasts for about four minutes (dashed area in Figure 3), a fact by which the chromatogram is not seriously influenced.

## CONCLUSIONS

The described method, which bases on separation by high performance liquid chromatography and a chemical reaction detector, shows the possibilities for simultaneous determination of organolead species at low concentration levels. It should be pointed out that besides the conditions described here, up to now, no further investigations on the influence of the chromatographic parameters have been performed. Further investigations, facing the optimization of the chromatographic system and especially the chemical reaction system, could possibly improve the chromatographic separation and the sensitivity of the detection. The applicability of this method to real air samples is strongly depending on the specificity and selectivity of the used sampling technique. Studies on this are proposed and on their way. The applicability of the analytical method described here to other organometallics like compounds of tin and mercury, will also be examined.



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