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Short Communication

Determination of organic ionic lead and mercury species with high-performance liquid chromatography using sulphur reagents

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ABSTRACT

We have verified the suitability of different sulphur-containing complexing reagents for the HPLC separation of ionic lead and mercury compounds with subsequent photometric detection. For the optimization of the separations, different parameters, *e.g.* the pH value of the mobile phase, were varied and their influence on the retention of the species determined. For the first time on-column derivatization with mercaptoethanol was used for the separation of lead compounds. Based on the optimized chromatographic conditions, an on-line enrichment system was developed with recoveries between 75 and 88%. The use of methyl thioglycolate as the second complexing reagent made it possible to determine all analytes, organolead and organomercury compounds, simultaneously. Finally, both methods are compared briefly.

INTRODUCTION

In recent years the importance of speciation analysis in the field of trace element analysis has grown enormously. Because of several disasters, the organometal compounds of the elements lead and mercury have become of particular interest [1–4]. First, the ionic di- and trialkyllead and monoalkylmercury compounds [dimethyllead (DiML), diethyllead (DiEL), trimethyllead (TriML), triethyllead (TriEL), methylmercury (MMM) and ethylmercury (MEM)] are the subjects of analytical research because of their high toxicity and bioavailability compared with the inorganic ions [2,5]. In addition to GC, liquid chromatography has often been employed for the determination and separation of the analytes [6–8]. A disadvantage of separating these compounds by GC is the need to perform a derivatization step, such as propylation or butylation, to form volatile compounds. It is not necessary, though it may be easily accomplished, to perform a derivatization step for the HPLC separation. Table I summarizes the methods using different sulphur compounds which are often used for the derivatization and separation.

The possibility of separating mercury compounds using on-column derivatization with mercaptoethanol led us to apply a similar system for the determination of organolead compounds. Separation was carried out on an RP-18 column

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TABLE I

METHODS USING SULPHUR COMPOUNDS FOR THE SEPARATION OF LEAD AND MERCURY COMPOUNDS WITH HPLC

Analytes	Sulphur compound	Detector[a]	References
Pb^{2+}, DiML, DiEL, TriML, TriEL	Dithizone	QTAAS	7, 9
Hg^{2+}, MMM, MEM	Dithizone	UV–VIS	10, 11
Pb^{2+}, DiML, DiEL, TriML, TriEL	Alkyldithiocarbamate	QTAAS	7, 9
Hg^{2+}, MMM, MEM	Alkyldithiocarbamate	UV–VIS	12
Hg^{2+}, MMM, MEM	Mercaptoethanol	ED	13
Hg^{2+}, MMM, MEM	Mercaptoethanol	GFAAS	14
Hg^{2+}, MMM, MEM	Mercaptoethanol	UV–VIS	15, 16
Hg^{2+}, MMM, MEM	Mercaptoethanol	MIP–AES	16
Hg^{2+}, MMM, MEM	Mercaptoethanol	ICP–MS	17
Hg^{2+}, MMM, MEM	Cysteine	CVAAS	18

[a] QTAAS = quartz tube atomic absorption spectrometry; UV–VIS = ultraviolet/visible; ED = electrochemical detector; GFAAS = graphite furnace atomic absorption spectrometry; MIP–AES = microwave-induced plasma atomic emission spectrometry; ICP–MS = inductively coupled plasma mass spectrometry; CVAAS = cold vapour atomic absorption spectrometry.

and with a methanolic citric acid buffer as mobile phase; for detection at UV–VIS detector at 235 nm was used. Chromatographic separation of dialkyllead compounds and trimethyllead was achieved by systematically varying mobile phase parameters; for the elution of triethyllead a gradient had to be established. Furthermore, the separation was completed by developing an on-line enrichment method involving the preconcentration of mercaptoethanol complexes of the lead compounds on an RP-18 precolumn.

Methyl thioglycolate was employed as a second sulphur compound for the complexation of the ionic lead and mercury species and allowed us to determine simultaneously all the organometal compounds mentioned above in a 40-min isocratic run.

EXPERIMENTAL

Reagents

For the preparation of the mobile phases methanol AR, trisodium citrate dihydrate AR (both obtained from Merck, Darmstadt, Germany) and twice-distilled water were used. The pH was adjusted with hydrochloric acid AR (Baker, Deventer, Netherlands) and sodium hydroxide AR (Merck). For the derivatization mercaptoethanol AR (Merck) and methyl thioglycolate 98% (Riedel de Haën, Seelze, Germany) were used. Trialkyllead and organomercury compounds were obtained from Alfa Products (Karlsruhe, Germany) as their chlorides. Dialkyllead species were self-prepared [19]. All analytes were stored at 4°C and dried in the desiccator ($CaCl_2$) prior to use.

HPLC equipment

The HPLC system consisted of the following set-up: a consta Metric 4100 gradient pumping system (LDC Analytical, Gelnhausen, Germany), an HPLC pump 64 and a UV–VIS detector variable-wavelength monitor (both obtained from Knauer, Bad Homburg, Germany), a six-way injection valve with 20-μl sample loop and the same injection valve (Knauer) with a precolumn (Nucleosil C_{18} 120-5, 30 mm × 4 mm I.D.). The analytical columns were RP-18 columns (Hypersil ODS, 100-5, 250 mm × 4 mm I.D., and Nucleosil C_{18} 120-5, 250 mm × 4 mm I.D.), and for their protection precolumns (5 mm) with the same filling were used. The chromatograms were evaluated by a C-R6A integrator (Shimadzu, Duisburg, Germany). The mobile phases and the sample solutions were

degassed in a Sonorex TK 52 ultrasonic bath (Bandelin, Berlin, Germany).

Sample preparation

The concentrations of the prepared stock solutions (analytes in methanol/twice-distilled water; 50/50, v/v) were about 1 mg/ml. This solutions were diluted daily with twice-distilled water to concentrations from 0.5–100 μg/ml. For the injections methanolic solutions of the derivatization reagent (0.08%, v/v) were added because of the need for pre-column complexation. The concentrations were about 0.02% (v/v).

RESULTS AND DISCUSSION

Mercaptoethanol as complexing reagent

Mercaptoethanol is widely used in the complexation and chromatographic separation of mercury compounds, as shown in Table I. Lead and its organo compounds also show a high affinity for sulphur compounds, and mercaptoethanol was tested for its usefulness in the separation of ionic lead compounds. The primary reactions during the pre- and on-column derivatization are summarized in eqn. 1.

$$R_{4-n}PbCl_n + nHSCH_2CH_2OH \rightleftharpoons R_{4-n}Pb(SCH_2CH_2OH)_n + nH^+ + nCl^- \quad n = 1, 2 \qquad (1)$$

The UV spectra of the resulting complexes showed characteristic peaks of absorption between 225 and 240 nm [20]. Further measurements were carried out at a wavelength of 235 nm.

The first parameter varied for the optimization of the chromatographic separation was the concentration of mercaptoethanol. High concentrations of mercaptoethanol displace the formation equilibrium (eqn. 1) to the right with the effect of increasing the capacity factors. For all subsequent investigations the concentration of mercaptoethanol was held constant at 0.02% (v/v).

The influence of the concentration of citric acid in the range 0.05–0.15 mol/l is negligible, but at 0.2 mol/l citric acid a decrease in the capacity factors could be observed. Owing to the limited solubility of citric acid in the methanol–water mixture used, it was not possible to investigate higher concentrations. A concentration of 0.2 mol/l was chosen for the separations. The next parameter to be varied in order to optimize the separation conditions was pH value (Fig. 1). Particularly in the pH range 6.7–7.0, the capacity factors increase enormously with pH. This phenomenon is based on two effects: (1) with increasing pH the formation equilibrium (eqn. 1) is displaced to the right and (2) the deprotonation of mercaptoethanol is favoured by decreasing concentration of protons.

For separation a pH value of 6.7 was suitable. If the composition of the mobile phase was held constant, it was possible to separate the three organolead compounds (DiML, DiEL and TriML) on the reversed-phase column. For the elution of triethyllead it was necessary to increase the concentration of methanol up to 55% (v/v). The gradient conditions are given in Table II. A chromatogram recorded at the conditions described is shown in Fig. 2.

Calibrations, detection limits

During the calibration experiments we found that the standard solutions were light sensitive. Further examinations were carried out to explain this phenomenon by storing the solutions in daylight for a defined period. The influence on the stability of the complexes is shown in Fig. 3.

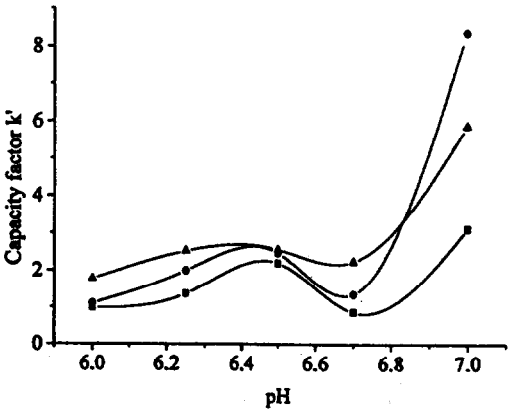

Fig. 1. Influence of the pH value on the capacity factors. ■ = DiML; ● = TriML; ▲ = DiEL.

TABLE II

GRADIENT FOR THE ELUTION OF THE LEAD COMPOUNDS

Stationary phase, Nucleosil C_{18} 120-5, 250 mm × 4 mm; Mobile phase A, methanol–0.2 mol/l citric acid (20/80, v/v), adjusted to pH 6.7, 0.02% (v/v) mercaptoethanol; mobile phase B, methanol–0.2 mol/l citric acid (55/45, v/v); adjusted to pH 6.7, 0.02% (v/v) mercaptoethanol; flow, 1.0 ml/min.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
10	100	0
12	0	100
22	0	100
24	100	0

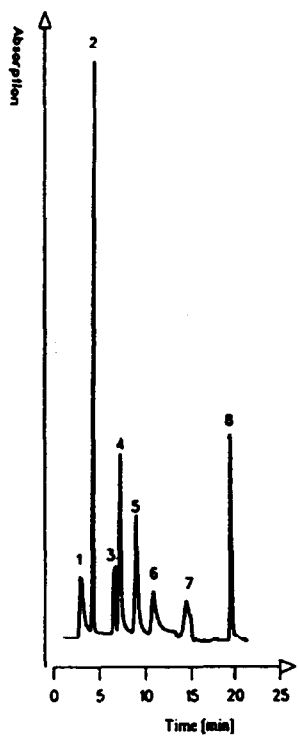

Fig. 2. Chromatogram of the lead compounds. For chromatographic conditions see Table II. UV detection: 235 nm; 0.04 a.u.f.s. Peak 1 = 620 ng of Pb^{2+}; peak 2 = mercaptoethanol; peak 3 = impurity from mercaptoethanol; peak 4 = 321 ng of DiML; peak 5 = 493 ng of TriML; peak 6 = 982 ng of DiEL; peak 7 = system peak; peak 8 = 756 ng of TriEL.

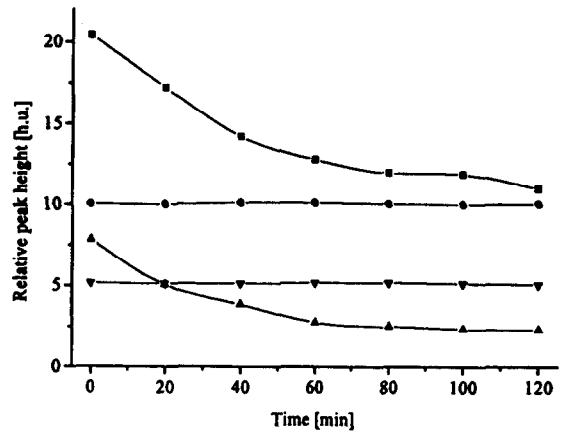

Fig. 3. Influence of daylight on the stability of mercaptoethanol complexes. ■ = DiML; ● = TriML; ▲ = DiEL; ▼ = TriEL.

Only the complexes of the dialkyllead compounds and not those of the trialkyllead species decomposed in daylight. Additional investigations proved that it was the mercaptoethanol complexes and not the metal ions themselves that were sensitive to light. Consequently, the samples were stored in the dark before injection.

The chromatographic conditions used for the calibrations are summarized in Table II.

Linearity with correlation coefficients between 0.997 and 0.999 was obtained for each analyte in the range between approximately 50 and 1500 ng (the specifications refer to the organometallic compounds and not to the elements). The detection limits ($S/N = 3:1$; $n = 10$) were from 18 ng for DiML to 61 ng for TriEL (see also Table III).

For the determination of trace amounts an enrichment system had to be tested, and is described below.

Enrichment from water samples

For enrichment the HPLC system used was enlarged by installing a second injection valve with an RP-18 precolumn (30 mm). An HPLC pump delivered the solutions onto the precolumn and then the analytes were loaded into the chromatographic system by switching the injection valve. The chromatographic conditions for the enrichment from water samples are summarized as follows: flow, 5.0 ml/min; pH, 7.0; citric

acid concentration, 0.1 mol/l; mercaptoethanol, 0.02% (v/v).

The recoveries were 75.2 ± 7.9% for DiML, 87.4 ± 4.2% for TriML, 77.9 ± 9.9% for DiEL and 88.2 ± 5.1% for TriEL. The results obtained were not satisfactory, so further investigations should improve the recoveries and their reproducibility.

Methyl thioglycolate as complexing reagent

Mercaptoethanol was used to obtain a simultaneous determination of the organolead and organomercury species. Because of peak overlaps and co-elutions methyl thioglycolate, another sulphur compound, was preferred to solve this analytical problem. Its reactions with the analytes were similar to those shown in eqn. 1. The complexes could be determined photometrically at 235 nm [21]. For the separation a methanolic citric acid buffer was again used. The concentration of methanol was kept at 40% (v/v) and the concentration of citric acid buffer at 0.1 mol/l. The effects of pH and concentration of methyl thioglycolate were similar to the effects on mercaptoethanol. With increasing proton concentration and decreasing concentration of the complexing reagent in the mobile phase, the capacity factors decreased for the lead compounds. On the other hand, mercury compounds were not affected. For the separation, a pH of 5.8 and 0.02% (v/v) methyl thioglycolate proved to be suitable. A chromatogram recorded under these conditions is shown in Fig. 4.

To prevent the decomposition of the complexed analytes the solutions were stored in the dark before the injections. Calibration graphs were linear in the range between approximately 50 and 600 ng for each analyte with correlation coefficients between 0.993 and 0.998. A comparison of the detection limits with both complexing reagents is demonstrated in Table III. However, the complexation with methyl thioglycolate resulted in lower detection limits for the trialkyllead compounds.

Following the enrichment procedure described above, the lead and mercury compounds could be preconcentrated in the same way. The recoveries for all the analytes were between 70 and 80%, thus there was no improvement compared with the recoveries obtained with mercaptoethanol.

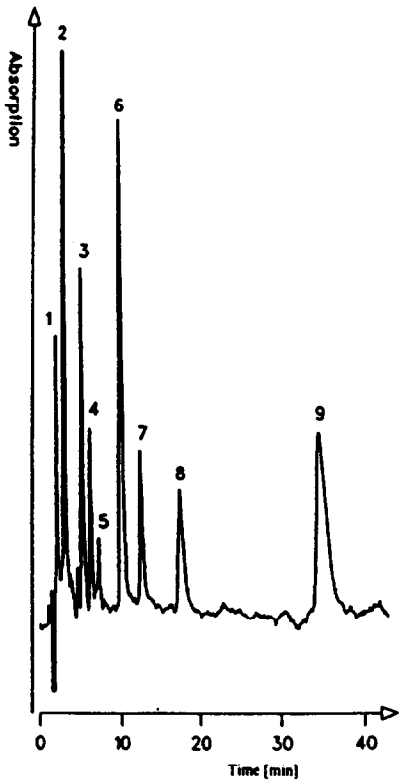

Fig. 4. Chromatogram of the lead and mercury compounds. Stationary phase: Hypersil ODS 100-5, 250 mm × 4 mm. Mobile phase; methanol–0.1 mol/l citric acid (40/60, v/v), adjusted to pH 5.8, 0.02% (v/v) methyl thioglycolate. UV detection: 235 nm; 0.04 a.u.f.s. Peak 1 = methanol; peak 2 = methyl thioglycolate; peak 3 = 181 ng of TriML; peak 4 = 226 ng of MMM; peak 5 = impurity from methyl thioglycolate; peak 6 = 129 ng of DiML; peak 7 = 208 ng of MEM; peak 8 = 238 ng of DiEL; peak 9 = 246 ng of TriEL.

CONCLUSIONS

Two methods for the enrichment and determination of lead compounds are presented. New methods of complexation of the analytes using sulphur compounds were considered, and proved to be alternative methods of separation and determination. The use of methyl thioglycolate made it possible to determine simultaneously organolead and organomercury compounds in a 40-min isocratic run. For the enrichment an on-line method was tested but the recoveries ob-

TABLE III

DETECTION LIMITS OF THE ANALYTES, COMPLEXED WITH METHYL THIOGLYCOLATE AND MERCAPTOETHANOL

$S/N = 3/1$; $n = 10$.

Analytes	Detection limits in ng absolute	
	Methyl thioglycolate	Mercaptoethanol
DiML	12	18
TriML	17	35
DiEL	31	30
TriEL	32	61
MMM	25	–
MEM	19	–

tained for both complexing reagents were unsatisfactory.

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