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TRACE ENRICHMENT AND SEPARATION OF As^{III}, Sb^{III} AND Bi^{III} AS DIETHYLDITHIOPHOSPHATE COMPLEXES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

As^{III}, Sb^{III} and Bi^{III} were separated and detected as their tris(diethyldithiophosphate) (DTP) complexes by reversed-phase high-performance liquid chromatography (HPLC). The advantage of using DTP as a complexing reagent in comparison with, *e.g.*, dithiocarbamates is that metals present in the metallic parts of the HPLC system, such as Ni^{II} and Co^{II}, do not interfere because they form only weak complexes with DTP and that DTP complexes are stable under acidic conditions, therefore permitting extraction and complexation at low pH. The formation of the metal–DTP complexes takes place on a PRP-1 precolumn which is loaded with a cetrimide dithiophosphate ion pair. The injected metal ions react instantaneously with DTP to form apolar complexes, which are efficiently retained by the PRP-1 material. Subsequently, the metal complexes are eluted on-line to a C_{18} column, separated with acetonitrile–water (85:15) containing 10 m*M* of the dithiophosphate ligand and detected at 280 nm. Interfering apolar compounds can be removed with a second PRP-1 clean-up pre-column, which renders this method suitable for the analysis of complex samples. Detection limits of about 2 ng were obtained for As^{III}, Sb^{III} and Bi^{III} and the detector response was linear over a concentration range of at least three orders of magnitude.

INTRODUCTION

In recent years, the determination of metal ions by high-performance liquid chromatography (HPLC) has been studied intensively as a possible alternative to methods such as atomic absorption spectrometry (AAS), inductively coupled plasma–atomic emission spectrometry (ICP–AES) and differential-pulse polarography[1]. The metals were separated as ions[2] or complexes[3–8] using electrochemical, conductivity or UV detection. Coupling of HPLC with ICP–AES or AAS has also been reported, which allows the detection of different metal species[9,10].

The separation of metal ions as their dithiocarbamate (DTC) complexes by reversed-phase liquid chromatography (RPLC) has been thoroughly studied[5–10]. Hg^{II}, Cd^{II}, Pb^{II}, Cu^{II}, Ni^{II}, Co^{II} and Bi^{III} can be separated on C_{18}-bonded silica columns

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and detected by UV or electrochemical detection with detection limits in the nanogram range. Elements such as arsenic, however, cannot be determined with these methods because they form only weak complexes with the various dithiocarbamates, which probably dissociate or undergo ligand-exchange reactions[3]. The dissociation of arsenic tris(diethyldithiocarbamate), $As(DTC)_3$, may be caused or accelerated by the formation of strong hydroxo complexes of As^{III} at higher pH[11]; on the other hand, it is not possible to work under acidic conditions (pH 1–4) owing to the instability of the DTC ligand at low pH[12]. An alternative may be the use of a ligand that forms strong, stable and neutral As^{III} complexes at the low pH values suitable for RPLC. Such a chelating agent with a dithio functional group is diethyldithiophosphoric acid (DTP). The extraction properties of apolar DTP complexes have been investigated in detail by Busev and Ivaniutiu[13], Bode and Arnswald[14,15] and Handley and Dean[16–19]. DTP forms weaker complexes than DTC with most metal ions, which can be explained by its higher dissociation constant[14]. The DTP complexes, however, are generally very stable under strongly acidic conditions. Bode and Arnswald[14] reported a half-life of sodium diethyldithiophosphate, Na(DTP), in 1 *M* hydrochloric acid of about 250 h, whereas the value for Na(DTC) under similiar conditions is only a few seconds. Cardwell *et al.*[20] chromatographed $Co(DTP)_3$ and $Cr(DTP)_3$ on a silica column with hexane–ethanol as the mobile phase. However, no application of DTP in RPLC has been reported previously. Because As^{III} forms a strong and neutral DTP complex, in this work the RPLC of $As(DTP)_3$ and also of $Sb(DTP)_3$ and $Bi(DTP)_3$ was investigated. The separation and detection of As^{III}, Sb^{III} and Bi^{III} as DTP complexes was combined with the on-precolumn derivatization–pre-concentration system described earlier[8].

EXPERIMENTAL

Chemicals

All reagents were of analytical-reagent grade, except for diethyldithiophosphate, which was of 97% purity (E. Merck, Darmstadt, F.R.G.). A 1 m*M* stock solution of As^{III} was prepared by dissolving arsenic trioxide (Baker, Deventer, The Netherlands) in 0.1 *M* sodium hydroxide, neutralizing the solution with sulphuric acid and diluting it to 100 ml with doubly distilled water. A 1 m*M* stock solution of Sb^{III} was prepared by dissolving antimony(III) chloride (Baker) in 1 *M* hydrochloric acid and diluting the solution to 100 ml with doubly distilled water. A 1 m*M* stock solution of Bi^{III} was prepared by dissolving bismuth(III) nitrate (Baker) in 0.1 *M* nitric acid. DTP used for the preparation of metal complexes and the HPLC mobile phase was purified by flushing a 0.1 *M* stock solution through a 100 × 4.6 mm I.D. stainless-steel column packed with 5 μm Hypersil ODS.

Instrumentation

All experiments were carried out in an HPLC system consisting of a Waters (Milford, MA, U.S.A.) Model 510 HPLC pump, a Valco six-port injection valve, a 200 × 4.6 mm I.D. stainless-steel separation column packed with 5-μm Hypersil ODS (Shandon Southern, Cheshire, U.K.) and a Pye Unicam (Cambridge, U.K.) PU 4025 UV detector. The pre-columns (10 × 4.6 mm I.D.) used for the clean-up and derivatization procedure were purchased from Chrompack (Middelburg, The Netherlands) and hand-packed with 10-μm PRP-1 styrene–divinylbenzene copolymer (Hamilton, Bonaduz, Switzerland). The pre-concentration pump was a Kontron (Zürich, Switzerland) 414 LC pump used at a flow-rate of 0.5 ml/min. The HPLC mobile phase was acetonitrile–water (85:15) containing 10 m*M* DTP and the flow-rate was 1.5 ml/min.

Procedure for the simultaneous derivatization and pre-concentration of As^{III}, Sb^{III} and Bi^{III}

The cetrimide–diethyldithiophosphate ion pair (CTAB–DTP) was formed off-line by mixing equal volumes of equimolar (1 m*M*) solutions of cetyltrimethylammonium bromide and diethyldithiophosphate. A 10-ml volume of this solution was pumped to the derivatization pre-column (No. 3, Fig. 1a), thus loading it with 10 μmole of CTAB–DTP. Subsequently, 1000 μl of the sample (adjusted to pH 1.0 with 0.1 *M* perchloric acid) were injected on to the derivatization pre-column. For the pre-concentration of larger volumes the sample was pumped directly to this pre-column. For urine samples a second PRP-1 clean-up pre-column (No. 6, Fig. 1b) was inserted in front of the derivatization pre-column and switched on after the loading of the derivatization pre-column with CTAB–DTP. The metal dithiophosphates that were formed and retained on the pre-column were eluted isocratically to the analytical column (Fig. 1c), separated and detected with UV detection at 280 nm.

RESULTS AND DISCUSSION

Although much is known about the extraction behaviour of metal–DTP complexes[13–19], only a few stability constants are available[21,23]. In general, the order of stability corresponds to that of dialkyldithiocarbamate complexes, with the important exception that Ni^{II} and Co^{II} form only weak, water-soluble DTP complexes[19,22]. $Ni(DTP)_2$ has even been used for the extraction of other metal ions via ligand exchange[22]. The relative displacement order is reported[19,22] to be $Pd^{II} > Au^{III} > Cu^{I} > Hg^{II} > Ag^{I} > Cu^{II} > Sb^{III} > Bi^{III} > Pb^{II} > Cd^{II} > Ni^{II} > Zn^{II}$. Recently, Hayashi *et al.*[23] published data on the thermodynamic properties of $As(DTP)_3$ and $Sb(DTP)_3$. They reported conditional stability constants ($\log \beta'_3$) of 11.2 for $As(DTP)_3$ in 2 *M* sulphuric acid and 10.4 for $Sb(DTP)_3$ at pH 1.6. The distribution constants (K_d) for the hexane–water system are $10^{3.0}$ and $10^{2.4}$, respectively, under these conditions. It was shown that pH and ligand concentration are the critical parameters for the extraction of the metal complexes into an organic phase. For the quantitative extraction of $As(DTP)_3$ a sulphuric acid concentration of at least 1 *M* was necessary; the pH for complete extraction of $Sb(DTP)_3$ was about 2.5, and at pH > 4.2 neither metal complex could be extracted into the organic phase. Moreover, a DTP concentration of over 1 m*M* was required for complete extraction.

Chromatography of $As(DTP)_3$, $Sb(DTP)_3$ and $Bi(DTP)_3$

The first attempts to chromatograph $As(DTP)_3$, $Sb(DTP)_3$ and $Bi(DTP)_3$ by RPLC under similar conditions to those for the chromatography of metal dithiocarbamates [*e.g.*, with acetonitrile–acetate buffer (70:30), pH 5.8[6]] failed. No signal was obtained at all after injection of the complexes which were formed off-line. Further experiments with the yellow $Bi(DTP)_3$ showed that the complex is strongly adsorbed

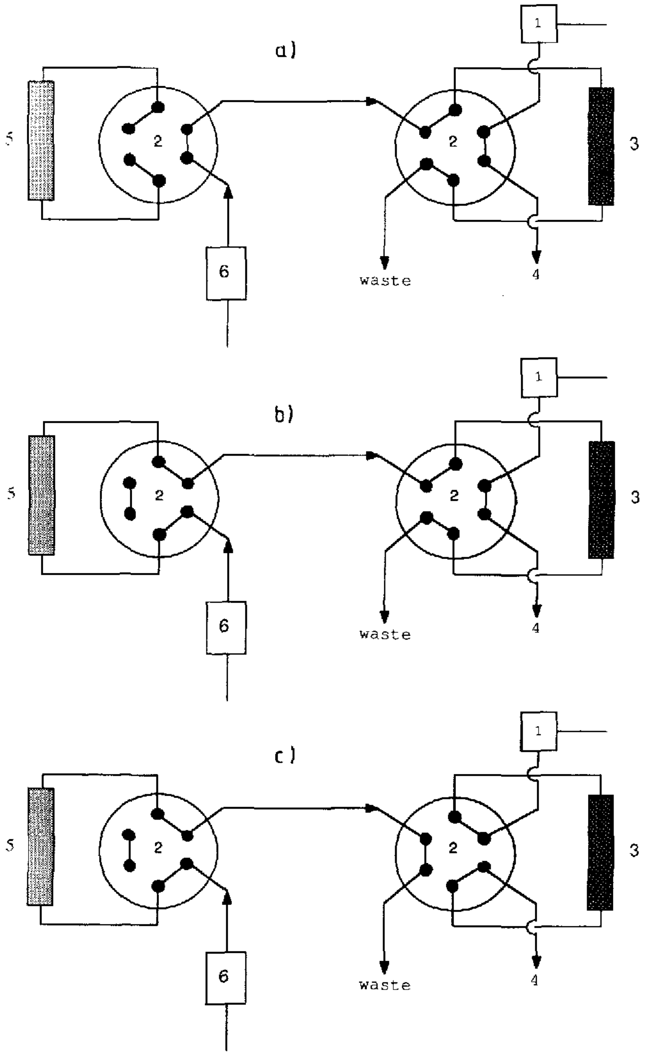

Fig. 1. Scheme of the loading and elution procedure. 1, HPLC pump; 2, six-port injection valve; 3, derivatization pre-column; 4, to analytical column and UV detector; 5, clean-up pre-column; 6, pre-concentration pump. (a) Loading of the derivatization pre-column with CTAB–DTP; (b) pre-concentration of the metal ions as metal–DTP complexes; (c) elution of the metal DTP complexes to the analytical column.

on top of the analytical C_{18}-bonded silica column and cannot be eluted even with pure acetonitrile. The same phenomenon occurred when the polymeric PRP-1 phase was used; hence, the reason for the strong adsorption probably is not an interaction with free silanol groups, as suggested for $Cd(DTC)_2$[10]. With $Bi(DTP)_3$, the problem of strong adsorption was overcome by choosing a lower pH. At pH 3.0 (adjusted with acetic acid) $Bi(DTP)_3$ was chromatographed and a good peak shape was obtained. Lowering the pH did not have any effect on the chromatographic behaviour of $As(DTP)_3$ and $Sb(DTP)_3$, however, even if a pH of 1.5 was chosen.

Because the results of Hayashi *et al.*[23] suggested that both a low pH *and* a 1 m*M* concentration of DTP were required for quantitative extraction, DTP was added to the mobile phase. Using acetonitrile–water (85:15) with 1 m*M* DTP (pH 3.0) as the mobile phase it was possible to chromatograph and separate $Bi(DTP)_3$ and $Sb(DTP)_3$, whereas $As(DTP)_3$ still did not elute from the column. At a DTP concentration of 5 m*M*, a broad $As(DTP)_3$ peak was obtained, and a further increase in the DTP concentration to 10 m*M* (pH 2.0) resulted in improved peak symmetry and a good peak width. At higher DTP concentrations no further improvement in peak shape was observed. Fig. 2 shows the final separation of $As(DTP)_3$, $Sb(DTP)_3$ and $Bi(DTP)_3$ with acetonitrile–water (85:15) containing 10 m*M* DTP as eluent.

The addition of a ligand to the mobile phase in order to reduce peak tailing has been used before, *e.g.*, for metal–DTC complexes[4,6]. The major drawback of this method, *viz.*, the high UV background signal caused by the excess of DTC (absorption maximum at 254 and 282 nm), does not occur with DTP, which has low absorbance in the whole 250–300 nm region. Using an eluent containing 10 m*M* of purified DTP, detection between 260 and 280 nm was satisfactory. Using UV detection at 280 nm, detection limits of about 2 ng (signal-to-noise ratio = 3) were obtained for As^{III}, Sb^{III} and Bi^{III}. The detector response was linear over a concentration range of at least three orders of magnitude, and the relative standard deviation for 10^{-4} *M* injections ($n = 5$) was 1.4%.

On-column trace enrichment of As^{III}, Sb^{III} and Bi^{III}

Recently we reported a new derivatization principle based on the formation of metal–DTC complexes on a pre-column packed with C_{18}-bonded silica, which had previously been loaded with a cetrimide–DTC ion pair[8]. The metal ions could then simply be injected on to this pre-column, where they react immediately with DTC to form apolar metal–DTC complexes, which are efficiently pre-concentrated on the C_{18}-bonded silica. Because of its structural similarity to DTC, the use of DTP in the described system was investigated.

Because of the low pH that is required for the complexation of As^{III} and Sb^{III}, PRP-1, which is stable from pH 1 to 13[24], was chosen as the packing material for the derivatization pre-column. PRP-1 also shows a higher hydrophobicity than

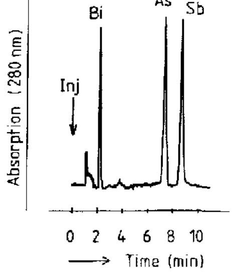

Fig. 2. Chromatography of $As(DTP)_3$, $Sb(DTP)_3$ and $Bi(DTP)_3$. HPLC conditions: 200 × 4.6 mm I.D. analytical column packed with 5-μm Hypersil ODS; eluent, acetonitrile–water (85:15) containing 10 m*M* DTP (pH 2.0); flow-rate, 1.5 ml/min; injection volume, 10 μl; detection wavelength, 280 nm; attenuation, 0.02 a.u.f.s.

C_{18}-bonded silica[24], and therefore the retention of CTAB–DTP and metal–DTP was increased. Because DTP is a strong acid with a pK_a of about 0[4], it is completely ionized at pH > 1 and, therefore, strongly adsorbed (even at low pH) as an apolar ion pair. It is important to mention that the cetrimide concentration in the solution used to load the derivatization pre-column should not be higher than 1 m*M* in order to prevent micelle formation (the critical micellar concentration of cetrimide is 0.99 m*M*[25], which, as we observed, leads to a lower retention of DTP on the pre-column. The optimal concentration range for CTAB was 0.7–1.0 m*M*.

The stability of the CTAB–DTP-loaded pre-column was mainly determined by the presence of anions in the sample that will elute the DTP ions. For example, samples that have been dissolved in nitric acid possess a high ionic strength, which negatively influences the breakthrough volume of CTAB–DTP and, therefore, the maximum pre-concentration volume. However, using 0.1 *M* nitric acid, pre-concentration of sample volumes up to 20 ml still provides a quantitative recovery at a column loading of 10 μmole of CTAB–DTP and a metal concentration of 1 μM. The reaction time for the complexation of As^{III}, Sb^{III} and Bi^{III} with DTP was not critical if the pH of the sample was 1.5 and a flow-rate of 0.5 ml/min was used.

The DTP complexes of As^{III}, Sb^{III} and Bi^{III}, which are formed during the on-column reaction, are strongly apolar (*cf.*, the use of 85% acetonitrile in the HPLC mobile phase) and they are therefore efficiently pre-concentrated on the PRP-1 material from a purely aqueous sample solution. A second PRP-1 clean-up pre-column, which is inserted in front of the derivatization system (see Fig. 1) in order to remove interfering non-polar sample constituents, does not affect the on-column formation of the metal–DTP complexes. In Fig. 3 a comparison of three different pre-concentration modes is demonstrated. In all three experiments the same peak height is obtained for $As(DTP)_3$, demonstrating that recoveries of >90% are obtained in the on-pre-column formation mode compared with direct pre-concentration, and also that the clean-up pre-column does not cause losses. In the on-pre-column formation mode a relative standard deviation of 3.4% for 1-ml injections of 10^{-6} *M* As^{III}

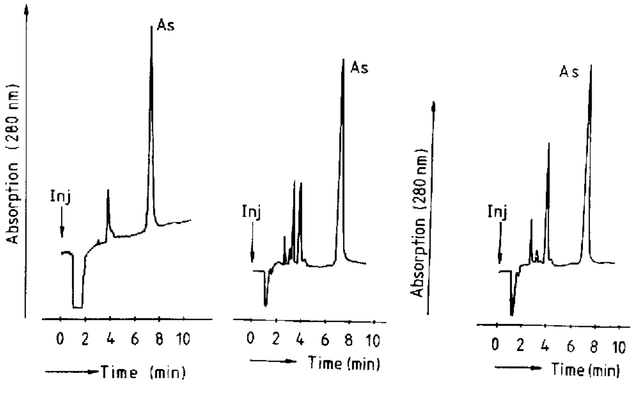

Fig. 3. Comparison of different pre-concentration modes. HPLC conditions as in Fig. 2; clean-up and derivatization pre-column, 10 × 4.6 mm I.D. packed with 10-μm PRP-1; attenuation, 0.08 a.u.f.s. (a) Direct pre-concentration of 75 ppb of $As(DTP)_3$ formed off-line; (b) on-pre-column formation of 75 ppb of $As(DTP)_3$, without an extra clean-up column; (c) on-pre-column formation of 75 ppb of $As(DTP)_3$, with an extra clean-up column.

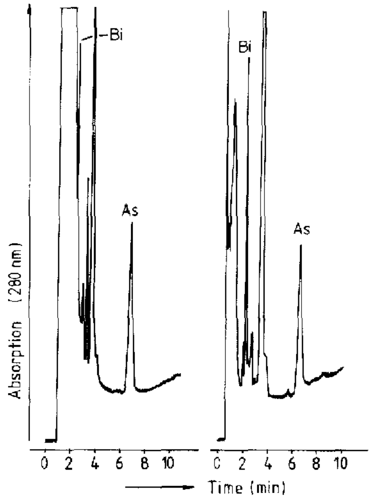

Fig. 4. Chromatogram of a urine sample spiked with 19 ppb of As^{III} and 57 ppb of Bi^{III} (1 ml pre-concentration). HPLC conditions as in Fig. 3. On-pre-column formation of the metal–DTP complexes occurred (a) without or (b) with a PRP-1 clean-up pre-column.

($n = 5$) and a detection limit of about 20 ppb (10^9) were obtained. Even at low As^{III} concentrations, no interferences were observed due to ligand-exchange reactions with, *e.g.*, Ni^{II} or Co^{II}. This is in agreement with the stability constant data and the relative displacement order[21,23] mentioned above.

Determination of As^{III} and Bi^{III} in urine

The trace-level determination of As^{III} and Bi^{III} in urine was investigated in order to demonstrate the clean-up potential of the described method for complex samples. Fig. 4 shows two chromatograms corresponding to the determination of 19 ppb of As^{III} and 57 ppb of Bi^{III} in urine (pre-concentration of 1 ml) without and with a PRP-1 clean-up pre-column. The use of an extra clean-up pre-column almost eliminates the broad unretained peak, allowing the determination of Bi^{III}. It should be mentioned that the length of the clean-up pre-column does not affect the peak width of the metal DTP peaks, as peak compression occurs on the derivatization pre-column owing to the rapid formation of apolar complexes. Therefore, the length of the clean-up pre-column (typically 1.0 cm) can be adapted to the complexity of the sample. When pre-concentrating 10 ml of urine, a detection limit of 1 ppb was obtained for As^{III} and Bi^{III}.

CONCLUSIONS

The use of diethyldithiophosphate as a complexing agent offers the possibility of determining As^{III}, Sb^{III} and Bi^{III} by RPLC with UV detection at 280 nm. Separation of the three metal complexes requires the addition of the ligand to the mobile phase. Nevertheless, low detection limits of about 2 ng for all metals ions still can be achieved. The metal–DTP complexes can be formed on-line on a pre-column that has been loaded with cetrimide–DTP ion pair. With this system even complex samples such as urine can be analysed with high selectivity also owing to the removal of interfering apolar compounds by an extra pre-column. No interferences due to ligand-exchange reactions, *e.g.*, with Ni^{II} or Co^{II} present in the metal parts of the HPLC systems were observed. Using the on-pre-column formation/pre-concentration system, detection limits in the sub-ppb range can be obtained for As^{III}, Sb^{III} and Bi^{III}.

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