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Separation and quantitation of some metal ions by reversed-phase high-performance liquid chromatography using *in situ* complexation with (±)-*trans*-1,2-diaminecyclohexane-N,N,N′,N′-tetraacetic acid

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ABSTRACT

(±)-*trans*-1,2-Diaminecyclohexane-N,N,N′,N′-tetraacetic acid (DCTA) was used as a mobile phase complexing agent to separate and quantitate the metal ions Fe(II), Fe(III), Cr(VI), Cu(II), Ni(II), Co(II), Pb(II) and Hg(II) in reversed-phase high-performance liquid chromatography. Cations were separated as metal–DCTA complexes formed in the chromatographic system and detected by UV spectrophotometry. Selectivity depended on the methanol content of the mobile phase and the pH. The method was fairly sensitive and detection limits for some metal ions were established at the nanogram level.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) has proved to be an effective technique in the determination of inorganic species. The use of a non-polar chemically bonded phase as the stationary phase in HPLC can be applied to the determination of anions and cations. Ion-pair chromatography (IPC), which uses an ionic hydrophobic agent (counter ion) in the mobile phase, increases the retention of the ionic solutes with an opposite charge. With this method, many different parameters modify the retention of the solute and the selectivity of the separation.

The applications of HPLC using bonded phases to the determination of inorganic species has been reviewed [1]. For inorganic anions, separation is achieved by using tetraalkylammonium salts in the mobile phase and two different methods can be used to separate the metal ions. The first is the formation of an ion pair with the metal ion by placing an anionic counter ion in the mobile phase. This mode requires the detection of the metal ion by refractometry, atomic emission spectrometry or UV spectrophotometry with post-column derivatization. The second method is to form an ion pair consisting of a complex formed between the metal ion and a suitable complexing agent. This method is more often used because it allows detection of the metal ion with either UV–visible spectrophotometry or fluorimetry. The nature of the counter ion used is determined by the charge of the metal ion complex. If the

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complex has a negative charge, the simultaneous separation of metal ions that have been complexed from anions can be carried out with a positively charged counter ion.

Metal ion complexes can be made in one of two ways. (a) The complex is formed outside the chromatographic system (pre-column complexation); in this instance the complexing agent is not introduced into the mobile phase and the complexes are stable enough not to decompose when they are injected into the chromatographic system. Some prior techniques (such as solvent extraction) are often used to obtain the complexes. (b) Metal ion complexes are formed within the chromatographic system itself. In this method the complexing agent is introduced into the mobile phase and then the metal ions injected into the column dissolved in the mobile phase or even in water. This method is called *in situ* complexation and can also consist in injecting the metal ions as complexes if the complexing ligand must be present in the mobile phase to preclude complex decomposition.

Comparison of the two complexation methods shows that *in situ* complexation does not require prior extraction steps to obtain the complexes and thus has the advantage of a faster analysis time. However, this technique places some limitations on the complexing agent: it must be non-corrosive with respect to the chromatographic system and compatible with the detection system used.

Various papers on the separation of metal chelates by HPLC had been published before the review of Marina *et al.* [1]. Veening and Willeford [2–4] described the separation of metal ions as complexes using chemically bonded phases whereas Smith [5], O'Laughlin [6] and Steinbrech [7] reviewed the applications of HPLC in the separation of metal chelates.

All these papers give useful information on the characteristics of the complexing ligand that are required for metal complexation. First, to achieve separation of multielemental mixtures, the reagent must be able to form stable complexes with a great variety of metal ions. Second, the complexes obtained must present a strong UV–visible absorption or emission signal to allow their detection by UV–visible spectrophotometry or fluorimetry. As in the *in situ* complexation mode the metal complexes are formed in the chromatographic column, the formation of the metal complexes must be rapid.

Aminepolycarboxilic acids are known for their ability to form highly stable metal complexes with many different metal ions [8]. However, few reports have been published on the use of these reagents to separate metal ions in RP-HPLC. In fact, the reports that have been published used ethylenediaminetetraacetic acid (EDTA) as the complexing agent [9–11]. The interesting results obtained with this ligand suggest the possibility of using another aminepolycarboxylic acid, (±)-*trans*-1,2-diaminecyclohexanetetraacetic acid (DCTA), which also complexes with many different metal ions.

The purpose of the work reported here was to study the use of DCTA as a complexing agent in the mobile phase for the separation of metal ions by RP-HPLC.

EXPERIMENTAL

Reagents and solutions

All chemicals used were of analytical-reagent grade. Metal ion solutions were prepared from the following salts: $Pb(NO_3)_2$, $Co(NO_3)_2$, $Cu(NO_3)_2$, $FeSO_4$, $K_2Cr_2O_7$, $FeCl_3$, $NiCl_2$ and $HgCl_2$.

DCTA was obtained from Aldrich (Steinheim, Germany). Tetrabutylammonium bromide (TBA), tetrapropylammonium bromide (TPA), NaH_2PO_4 and Na_2HPO_4 were from Merck (Darmstadt, Germany). The methanol used for mobile phase preparation was of HPLC quality (Ferosa, Spain). Ultrapure Milli-Q water (Millipore, Belford, MA, USA) was also used.

Methanol–water mixtures containing DCTA, TBA or TPA and phosphate buffer at pH 4.15, 5.8 or 6.2 were used as mobile phases. These solutions were prepared by weighing the methanol and the aqueous solution and then calculating the percentage by volume. These mobile phases were filtered in a Millipore system with 0.47-μm filters and degassified in an ultrasonic bath.

Metal ion solutions were obtained by dissolving the correct amount of metal ion salt in the appropriate volume of mobile phase to reach the desired concentration.

Samples were eluted under isocratic conditions.

Apparatus

The components of the HPLC system (Perkin-Elmer, Beaconsfield, UK) were the following: a

Model 10 pump, a UV–visible LC-95 variable-wavelength detector and a Model 023 datarecorder. A 7125 Rheodyne injection valve with a 20-μl loop and a 25 cm × 4.5 mm I.D. Spherisorb ODS-2 column (particle diameter 10 μm) were also used.

The detection of the complexes was performed at 254 nm. Absorption spectra of the metal–DCTA complexes were obtained with a Model Lambda 2 UV–visible spectrophotometer (Perkin-Elmer).

Solution pH was measured with a Model M-501 pH meter (Orion Research, Cambridge, MA, USA) with a combined electrode.

Calibration graphs

The calibration graphs for metal ions were obtained by plotting the peak height against the concentration of the injected metal ion. An average of five injections were performed for measurements at each concentration of metal ions used.

RESULTS AND DISCUSSION

Detection of metal complexes

Absorption spectra of the metal complexes indicated an absorption maximum at wavelengths close to 230 nm for Ni(II) and Co(II), 245 nm for Pb(II), 265 nm for Fe(III) and two maxima for Cu(II) and Hg(II) at wavelengths of 230 and 265 nm and 230 and 255 nm, respectively. $Cr_2O_7^{2-}$ has almost the same absorption between 230 and 270 nm.

A wavelength of 254 nm was chosen to detect all the metal complexes investigated as it has a high absorption for most of the complexes, except Ni(II) and Co(II).

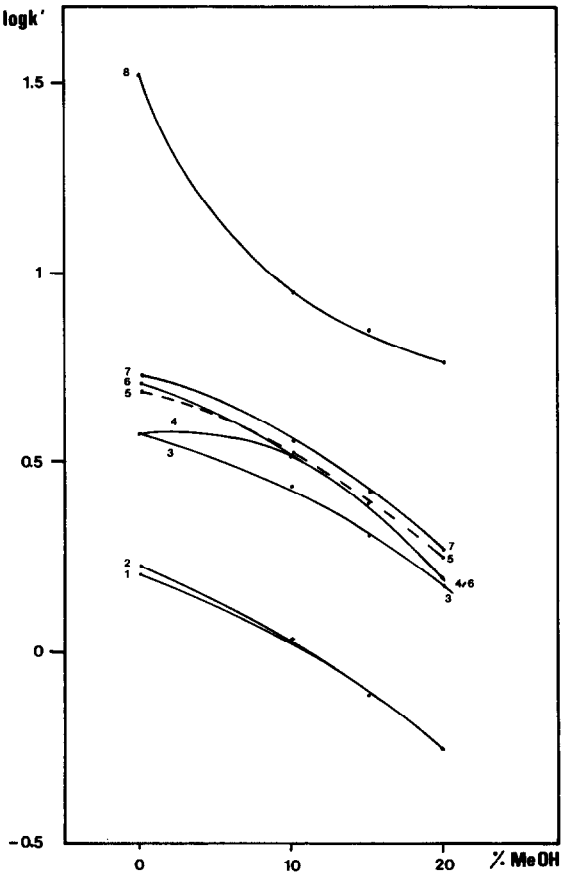

Fig. 1. Variation of the capacity factor logarithm (log k') of species as a function of percentage of methanol (MeOH) in the mobile phase. Mobile phase: methanol–water mixtures, $1 \cdot 10^{-2}$ M DCTA, $1 \cdot 10^{-2}$ M TBA, $1 \cdot 10^{-3}$ M phosphate buffer (pH 5.8). 1 = NO_3^-; 2 = Fe(II); Fe(III); 3 = Cu(II); 4 = Cr(VI); 5 = Ni(II); 6 = Pb(II); 7 = Co(II); and 8 = Hg(II).

Separation of metal complexes

To separate mixtures of the metal ions Fe(II), Fe(III), Cr(VI), Cu(II), Ni(II), Co(II), Pb(II) and Hg(II) as DCTA complexes by RP-HPLC, *in situ* complexation was chosen. This was because when a ligand similar to DCTA, such as EDTA, was used, it was found that the chromatographic peak widened when the concentration of ligand in the mobile phase was low (10^{-4} or 10^{-5} M) [9]. For this reason, the complexing agent DCTA was added to the mobile phase at a concentration of $1 \cdot 10^{-2}$ M. In this way, the formation equilibrium of the complex is favoured, allowing an optimum peak shape and conditions for maximum sensitivity at the wavelength at which the determination was performed.

As metal ions were separated as negatively charged DCTA complexes, a counter ion with a positive charge, TBA, was used to retain the complexes in the stationary phase. The choice of TBA should not only allow the separation of anionic metal–DCTA complexes, but also the separation of other species with a negative charge such as Cr(VI), found in solution as $Cr_2O_7^{2-}$, or of an inorganic anion such as NO_3^-. Preliminary experiments showed a good retention of the metal ions in the system when the concentration of TBA in the mobile phase was $1 \cdot 10^{-2}$ M. The TBA concentration was thus kept constant and equal to this value. The

percentage of organic modifier and the pH of the mobile phase were studied to determine their influence on system selectivity, thus establishing the best conditions for mixture separation.

Influence of percentage of organic modifier

Methanol was chosen as the organic modifier in the mobile phase and the variation of the capacity factor logarithm for either NO_3^-, Fe(II), Fe(III), Cr(VI), Cu(II), Ni(II), Co(II), Pb(II) or Hg(II) with the methanol content of the mobile phase was determined. The results obtained at pH 5.8 (phosphate buffer) are grouped in Fig. 1. Retention of species in the chromatographic system decreased when the methanol content increased, as expected, as when the methanol content increases the amount of counterion adsorbed in the stationary phase decreases, and this decreases the solute retention. Usually the logarithm for the capacity factor of the solutes in IPC decreases linearly with the percentage of methanol [12]. However, Fig. 1 shows that the curve obtained for Cr(VI), Pb(II), and Hg(II) does not concur with the considerations on the retention mechanism.

With respect to selectivity, there is no methanol percentage at which the selectivity is at a maximum as the selectivity depends on the kind of mixture to be separated. In fact, it can be observed (Fig. 1) that

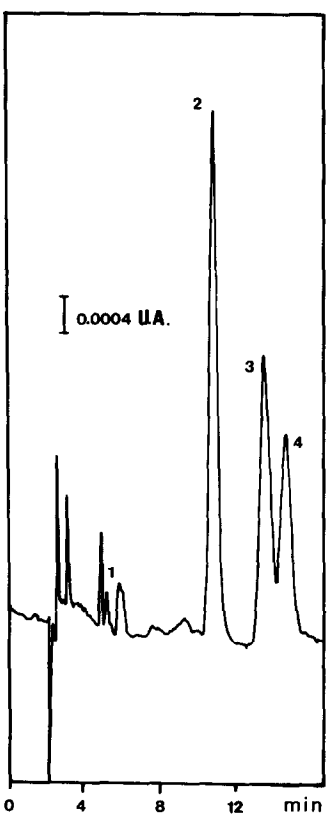

Fig. 2. Chromatogram showing the separation NO_3^-–Cu(II)–Ni(II)–Co(II). Mobile phase: water, $1 \cdot 10^{-2}$ *M* DCTA, $1 \cdot 10^{-2}$ *M* TBA, $1 \cdot 10^{-3}$ *M* phosphate buffer (pH 5.8). 1 = NO_3^-; 2 = Cu(II); 3 = Ni(II); and 4 = Co(II). U.A. = Absorbance units.

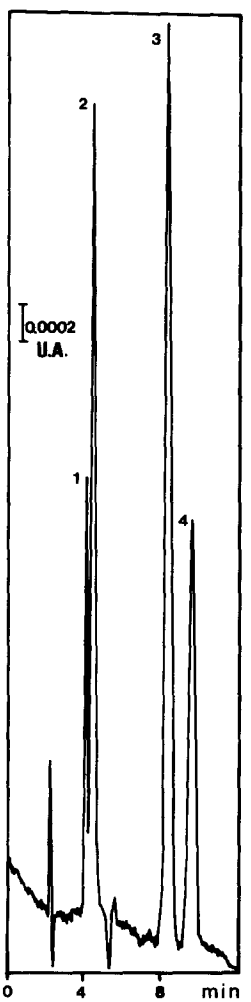

Fig. 3. Chromatogram showing the separation NO_3^-–Fe(II)–Cu(II)–Pb(II). Mobile phase: methanol–water (10:90), $1 \cdot 10^{-2}$ *M* DCTA, $1 \cdot 10^{-2}$ *M* TBA, $1 \cdot 10^{-3}$ *M* phosphate buffer (pH 5.8). 1 = NO_3^-; 2 = Fe(II); 3 = Cu(II); and 4 = Pb(II).

the separation of Ni(II)–Co(II), Cr(VI)–Pb(II) and Cu(II)–Pb(II) are favoured by low methanol contents (0%), the separation of Pb(II)–Ni(II) is favoured in mobile phase with a high methanol content (20%), and the separation of Cu(II)–Cr(VI) is favoured when the methanol content is intermediate (10%). Other separations are possible at any percentage of methanol: Cu(II)–Ni(II) and Cu(II)–Co(II). Finally, there are mixtures that could be separated at low or high methanol contents but not at intermediate ones (10%), such as the mixture Cr(VI)–Ni(II). The behaviour of these ions is roughly similar. However, for Fe(II), Fe(III) and Hg(II), the species Fe(II) as well as Fe(III) can be separated from Hg(II) and all three can be separated from any other ion because of their different retention behaviours. Fig. 1 includes NO_3^-, which can be separated from metal ion mixtures. These variations of selectivity can be illustrated with some simple separations such as those shown in Figs. 2 and 3, in which simply modifying the methanol content at a fixed pH of 5.8 separates the species in the chromatographic column. Fig. 2 shows the separation Cu(II)–Ni(II)–Co(II) at 0% methanol and Fig. 3 shows the separation Fe(II)–Cu(II)–Pb(II) at 10% methanol. It should be noted that although Hg(II) can be separated from all the other species, at this pH there is an important widening of its chromatographic peak.

Influence of mobile phase pH

To study the influence of pH on selectivity, the variation of the retention of the metal ions as a function of the pH of the mobile phase was determined. In these experiments the methanol content of the mobile phase was kept constant and equal to 10%. The results are shown in Fig. 4 in which the variation of the logarithm of the capacity factor for Fe(II), Fe(III), Cu(II), Cr(VI), Pb(II), Ni(II), Co(II) and Hg(II) is plotted for the three pH values studied (4.15, 5.8 and 6.2). It can be observed that the retention of metal ions generally decreases with an increase in the pH of the mobile phase. However, this decrease is more important between pH 5.8 and 6.2 [especially for Cr(VI)] as between pH 4.15 and 5.8, the influence of pH is low.

As the metal ions are retained in the chromatographic system through the formation of complexes with a negative charge, it might be expected that an increase in the pH of the mobile phase would increase the retention of metal ions because of the rise in the conditional complexation constant. However, the results can be explained by another effect. The mobile phase contained the complexing agent DCTA and phosphate buffer and both increase their negative charge with rising pH values. This implies greater retention in the chromatographic system through the formation of an ion pair with TBA and the competing effect provokes a decrease in the retention of the solutes. In fact, the decrease of the capacity factor for Cr(VI) with increasing pH is higher than for all the other metal ions for which the retention takes place by complexation. Regarding selectivity, Fig. 4 shows that it is similar at pH 4.15 and 5.8 whereas at pH 6.2 there is a change caused by the different behaviour of Cr(VI). However, the separations that can be made at pH 6.2 are, in general, also possible when the content of methanol is changed to pH 5.8; for instance, Cr(VI)–Pb(II) or Cr(VI)–Ni(II).

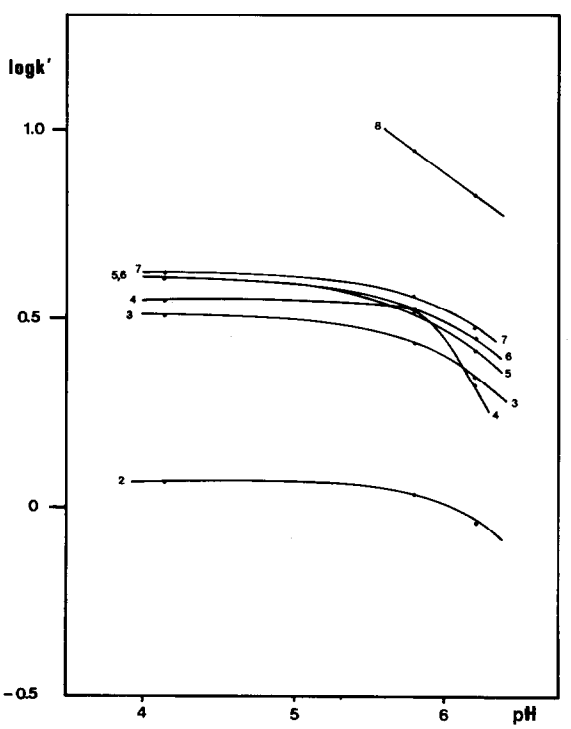

Fig. 4. Variation of the capacity factor logarithm (log k') of species as a function of the mobile phase pH. 2 = Fe(II), Fe(III); 3 = Cu(II); 4 = Cr(VI); 5 = Pb(II); 6 = Ni(II); 7 = Co(II); and 8 = Hg(II).

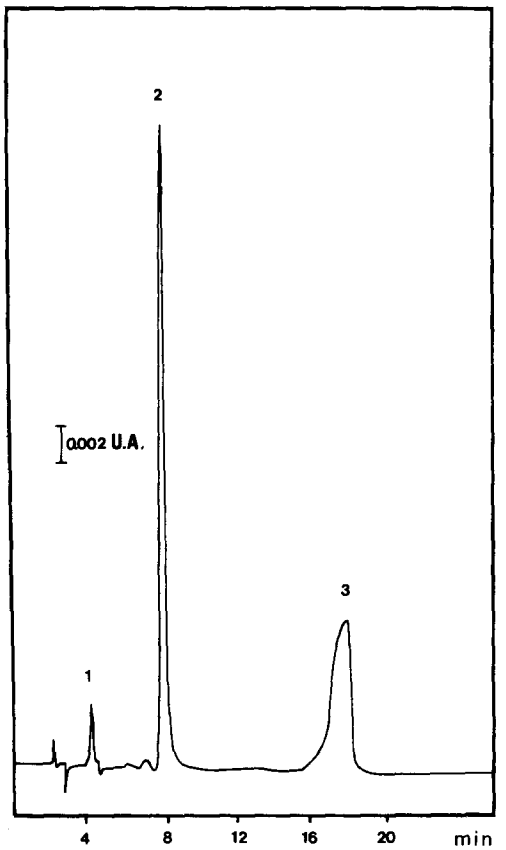

Fig. 5. Chromatogram showing the separation NO_3^-–Pb(II)–Hg(II). Mobile phase: methanol–water (10:90), $1 \cdot 10^{-2}$ *M* DCTA, $1 \cdot 10^{-2}$ *M* TBA, $1 \cdot 10^{-3}$ *M* phosphate buffer (pH 6.2). 1 = NO_3^-; 2 = Pb(II); and 3 = Hg(II).

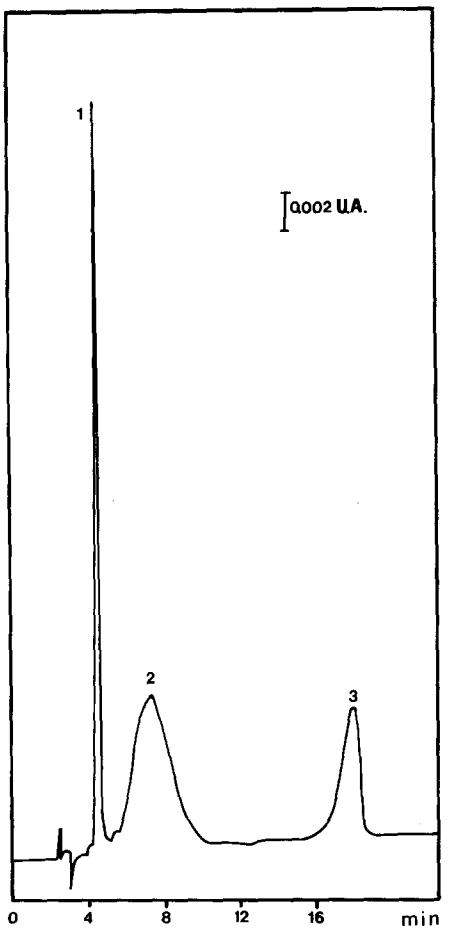

Fig. 6. Chromatogram showing the separation Fe(III)–Cr(VI)–Hg(II). Mobile phase as in Fig. 5. 1 = Fe(III); 2 = Cr(VI); and 3 = Hg(II).

The most interesting effect of a change in pH is the improvement of the peak shape, especially for Hg(II) and Cr(VI). For Hg(II), the peak shape improves considerably between pH 5.8 and 6.2. However, at pH 4.15, a peak for Hg(II) was not obtained. Figs. 5 and 6 show the peak of Hg(II) when it is separated from the others at pH 6.2. Fig. 5 shows the separation Hg(II)–Pb(II) and Fig. 6 shows the separation Fe(III)–Cr(VI)–Hg(II). In this last figure it is possible to observe the significant broadening that is observed for the Cr(VI) peak at pH 6.2. However, at pH 4.15 the peak shape for Cr(VI) improves, allowing separation from other metal ions with a similar retention, as illustrated in Fig. 7. This result for Cr(VI) may be explained by the higher stability of the anion $Cr_2O_7^{2-}$ when the acidity of the medium increases. For Hg(II) the improvement of the peak shape at pH 6.2 could be due to an increase in the conditional complexation constant wiht DCTA that occurs when the pH of the solution is increased.

Quantitation of species

To illustrate the sensitivity of the method, the detection limits (calculated by using a signal-to-noise ratio of 2:1) have been determined for some species. The results are shown in Table I, which groups detection limits in mass as well as in concentration units for Cu(II), Pb(II), Cr(VI) and Hg(II). Table I specifies the mobile phases used to obtain the given

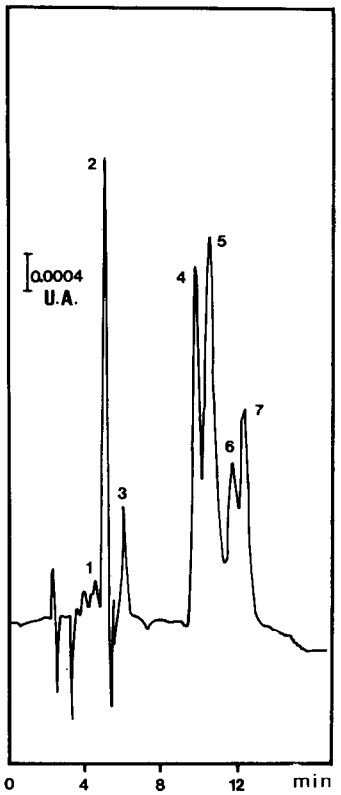

Fig. 7. Chromatogram showing the separation NO_3^-–Fe(III)–Cu(II)–Cr(VI)–Ni(II)–Co(II). Mobile phase: methanol–water (10:90), $1 \cdot 10^{-2}$ *M* DCTA, $1 \cdot 10^{-2}$ *M* TBA, $1 \cdot 10^{-3}$ *M* phosphate buffer (pH 4.15). 1 and 3 = NO_3^-; 2 = Fe(III); 4 = Cu(II); 5 = Cr(VI); 6 = Ni(II); and 7 = Co(II).

detection limits and these phases differ only with respect to the counterion used: TPA was used for Hg(II) to improve the peak shape at pH 5.8. The concentration of TPA was $1 \cdot 10^{-2}$ *M*.

Table I shows that the sensitivity of the method is similar for all the metal ions studied and that detection of these species at the nanogram level is possible. The height of the chromatographic peak varied with the amount of metal ion injected in a linear manner over at least two or three orders of magnitude.

TABLE I

DETECTION LIMITS FOR SOME METAL IONS

Injection volume 20 μl.

Metal ion	Mass (ng)	Concentration (ng/ml)
Cu(II)[a]	4.91	245.6
Pb(II)[a]	1.85	92.57
Cr(VI)[a]	3.23	161.39
Hg(II)[b]	3.87	193.76

[a] Mobile phase: methanol–water (10:90), $1 \cdot 10^{-2}$ *M* DCTA, $1 \cdot 10^{-2}$ *M* TBA, $1 \cdot 10^{-3}$ *M phosphate buffer (pH 5.8)*.

[b] Mobile phase: methanol–water (10:90), $1 \cdot 10^{-2}$ *M* DCTA, $1 \cdot 10^{-2}$ *M* TPA, $1 \cdot 10^{-3}$ *M* phosphate buffer (pH 5.8).

CONCLUSIONS

The following conclusions can be drawn from the results presented. DCTA is a suitable complexing agent for the separation of metal ion mixtures by RP-HPLC. The introduction of DCTA into the mobile phase eliminates the need for preparatory metal complexation steps. The modification of the methanol content and pH of the mobile phase makes it possible to obtain a good peak shape and adequate selectivity for the separation of mixtures. A concentration of $1 \cdot 10^{-2}$ *M* in DCTA, $1 \cdot 10^{-2}$ *M* in TBA, $1 \cdot 10^{-3}$ *M* in phosphate buffer, a methanol content ranging from 0 to 20% and a pH value between 4.15 and 6.2 provide adequate separation conditions. As a positively charged counter ion is used to separate anionic complexes, the separation of inorganic anions from metal ions is also possible. Good sensitivity is obtained and it is possible to detect the metal ions at the nanogram level.

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