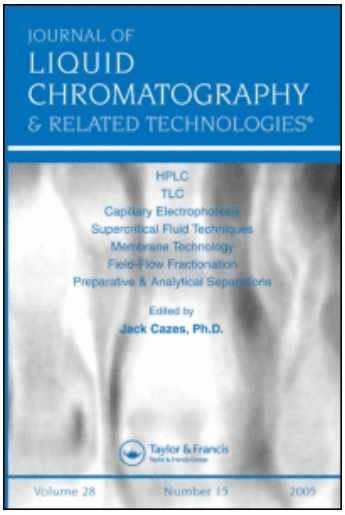

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SEPARATION OF METAL IONS BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING IN-SITU COMPLEXATION. APPLICATION TO DETERMINATION OF Fe(III) AND Fe(II) AS O-PHENANTHROLINE COMPLEXES

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ABSTRACT

The separation of different mixtures of the metal ions Fe(III), Fe(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II), Hg(II), Ca(II), Mg(II), Al(III), Cr(III), Sn(II), and Sn(IV) has been performed by using reversed-phase high performance liquid chromatography and o-phenanthroline in mobile phase. Among others, the separation of Fe(III) and Fe(II) has been achieved and detection limits for these species are given. The detection limit for Fe(II) at 509 nm is 240 times minor than at 340 nm. This last wavelength allows the simultaneous detection of both cations.

INTRODUCTION

The determination of metal ions by reversed-phase high performance liquid chromatography has been extensively studied in the past years. A very common work method is the separation and determination of metal ions as complexes. Several reviews on this sub-

ject have appeared in literature (1-4). The formation of complexes allows the detection of metal ions by UV-VIS spectrophotometry. The complexes can be formed before their introduction in the column or "in situ" within the column, introducing the complexing agent in mobile phase. In-situ complexation allows the direct introduction of metal ions in the chromatographic system in aqueous solution or in mobile phase which eliminates the need to extract them with an organic solvent. However, this technique requires rapid formation of complexes and none or little ligand interference in the detection of metal ions. Generally, the detection is realized at a wavelength at which the background absorbance produced by the presence of ligand in mobile phase is minimum. Despite "in-situ" complexation is faster and easier than pre-column formation, some authors observe a loss of efficiency of the column after a working time with a mobile phase containing ligand (5).

Our goal is the investigation of the capability of complexing agent o-phenanthroline for separating and determining several metal ions on a C-18 reversed-phase column. This ligand has been previously utilized in the separation of Ni(II)-Ru(II)-Fe(II) and Zn(II)-Cd(II) by high performance liquid chromatography using a cation ion-exchange column, and in the separation of Ni(II)-Ru(II)-Fe(II) in a poliestirene-divinylbenzene reversed-phase column (6). Likewise, Fe(II)-Hg(II)-Ni(II)-Cu(II) have been separated by in-situ complexation chromatography with ethyl xanthate and o-phenanthroline as ligands for on-column adduct formation (7).

EXPERIMENTAL

Reagents and Solutions.

All chemical utilized have been of analytical grade purity (Merck and Carlo Erba). 1,10-phenanthroline monohidrate has been a Merck product. The methanol used for preparation of mobile phases has been a Scharlau product quality for HPLC. Ultrapure Milli-Q water (Millipore) has been utilized.

Methanol-water mixtures containing o-phenanthroline, sodium perchlorate and phosphate buffer at pH=6.3 and pH=3.0 have been

employed as mobile phases. The preparation of such solutions has been by weighting methanol and aqueous solution and then, calculating the percentaje in volume. These mobile phases have been filtred in a filtration system (Millipore) with 0.47 μm filters and after, degasified in a ultrason bath.

Metal ion solutions have been obtained by dissolution of the adequate quantity of metal ion salt in the convenient volume of mobile phase to obtain the desired concentration. They were diluted in the mobile phase if neccesary. In the most of cases, nitrates have been employed but sulphates have also been used in some cases.

The samples were eluted in isocratic conditions.

Instrumentation.

The components of the HPLC system (Waters Associates) were the following: a model 510 pump, a UV-VIS Lambda-Max moder 481 variable wavelength detector and a Data Module model 740 Integrator. A 7125 Rheodyne injection valve with a 20 μl loop have been utilized. A 15 cm x 3.9 mm i.d. with end-capping C-18 Novapack column (Waters) has been used (particle diameter 4 μm).

Absorption spectra of the metal o-phenanthroline complexes were obtained with a Lambda 5 UV-VIS spectrophotometer (Perkin-Elmer).

Calibration Graphs.

The calibration graphs for Fe(III) and Fe(II) have been obtained by plotting the peak area against concentration of metal ion injected. For each concentration of metal ion, an average of 8 injections have been realized.

RESULTS AND DISCUSSION

Detection of Metal-o-phenanthroline Complexes.

Absorption spectra of metal-o-phenanthroline complexes obtained in the mixtures employed as mobile phases show that these complexes have a maximum absorption in the UV zone around 300 nm. Fe(II) has, in addition to this absorption, another maximum in the visible zone, around 500 nm. These results allow to select

the wavelength of 305 nm for detection of all metal ions when a mobile phase at pH= 6.3 is used and a wavelength of 509 nm for detection of Fe(II) in the visible zone. When using a mobile phase at pH= 3.0, a wavelength of 340 nm is selected due to the major absorption of o-phenanthroline at 305 nm at this pH value.

Separation of Metal-o-phenanthroline Complexes.

Capacity factors of Fe(III), Fe(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II), Hg(II), Ca(II), Mg(II), Al(III), Cr(III), Sn(II), and Sn(IV) have been determined when a 40:60 (v/v) methanol-water mixture containing o-phenanthroline (10^{-3}M), sodium perchlorate ($5x10^{-2}$M) and phosphate buffer at pH=6.3 (10^{-3}M) is used as mobile phase. The results obtained are grouped in Table 1. Prior to these experiences, it was established that this mobile phase provided adequate times of retention and peak shape, when some metal ions were injected with mobile phases of different content in methanol and perchlorate.

TABLE 1. Capacity factors (k') of metal ions. Mobile phase: 40:60 methanol-water containing $5x10^{-2}$M sodium perchlorate, 10^{-3}M o-phenanthroline, and 10^{-3}M phosphate buffer at pH= 6.3. (Detection at 305 nm).

Metal Ion	k'
Fe(III)	0.3
Fe(II)	2.0
Ni(II)	2.8
Co(II)	3.4
Cu(II)	3.8
Zn(II)	4.2
Cd(II)	15.0
Hg(II)	1.0
Ca(II)	0.0
Mg(II)	0.0
Al(III)	1.1
Cr(III)	1.0
Sn(II)	1.4
Sn(IV)	1.4

The presence of o-phenanthroline in mobile phase was necessary not only to eliminate the overlapping of ligand and complexes when these are injected with mobile phases that do not contain the ligand but also because of the amelliorement of peak shape probably due to the displacement of complexation equilibrium towards the formation of the complex.

From the results grouped in Table 1, it is possible to foresee the separation of different mixtures of metal ions. In Fig. 1, the separation of Fe(III) and Fe(II) (Fig. 1A) as well as other possible combinations (Fig. 1B, 1C, and 1D) are shown. In Fig.1B and 1C, the total separation with good resolution of metal complexes and ligand peaks was obtained. In Fig.1D, metal ions' peaks were separated but one of them, corresponding to Co(II), overlapped with the peak of o-phenanthroline. This is due to the fact that after several months working with the chromatographic column, the retention time of Co(II) increased and its peak experienced a broadening. Similar results of broadening were obtained for Fe(III), Fe(II), and Ni(II) when the same mobile phase was utilized. However, in alike mobile phases except for the pH value (pH= 3.0), the broadening effect greatly decreased at the same time that the retention times of complexes decreased without loss of resolution. Other authors that utilize mobile phases without complexing ligand observe broadening of peaks and a loss in resolution, atributing this fenomena to ligand-exchange reactions with trace amounts of metal oxides present in the chromatographic system (8).

Other mixtures of metal ions having similar times of retention were partially separated though not resolved as it is shown in Fig. 2.

Determination of Fe(III) and Fe(II).

The calibration graphs of complexes of Fe(III) and Fe(II) with o-phenanthroline by using a 40:60 (v/v) methanol-water mixture containing sodium perchlorate ($5x10^{-2}$M), o-phenanthroline (10^{-3}M), and phosphate buffer at pH= 3.0 (10^{-3}M) have been achieved. At this pH value, the separation of Fe(III) and Fe(II) can be performed in the same way that at pH= 6.3 but with the advan-

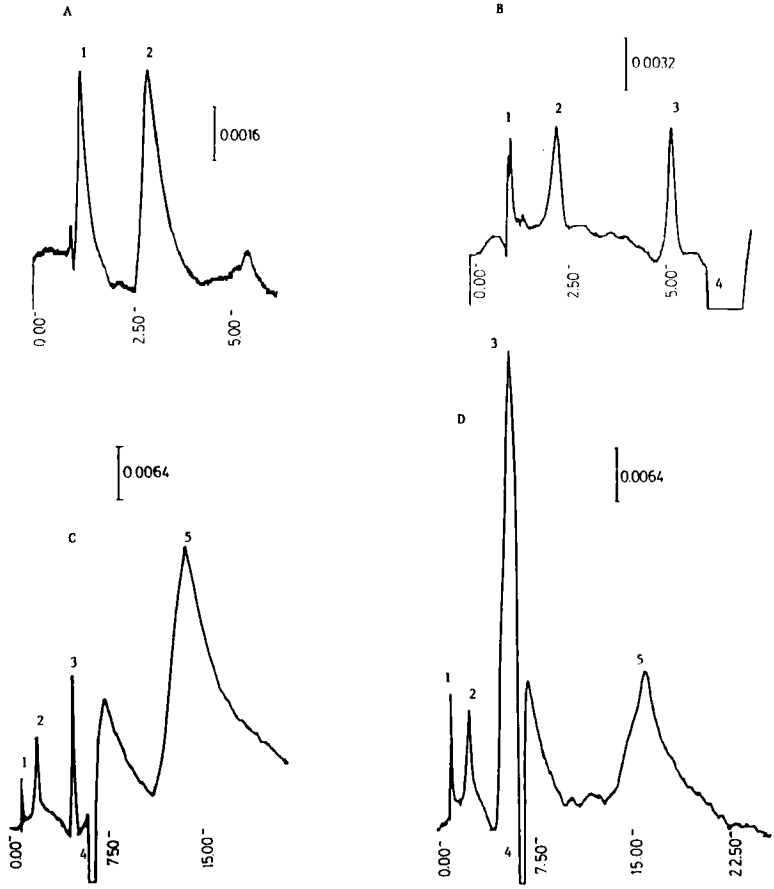

FIGURE 1. Chromatograms showing several separations of metal-o-phenanthroline complexes. A. Separation of 5.0 ppm of Fe(III)(peak 1) and 1.2 ppm of Fe(II)(peak 2). B. Separation of 45.2 ppm of Ca(II)(peak 1), 100.1 ppm of Hg(II)(peak 2), and 11.4 ppm of Zn(II) (peak 3). C. Separation of 28 ppm of Ca(II)(peak 1), 14.7 ppm of Cr(III)(peak 2), 16,8 ppm of Cu(II)(peak 3), and 34.5 ppm of Cd(II)(peak 5). D. Separation of 24.3 ppm of Mg(II)(peak 1), 7.9 ppm of Al(III)(peak 2), 20.2 ppm of Co(II)(peak 3), and 21.9 ppm of Cd(II)(peak 5). Negative peaks (4) correspond to o-phenanthroline. Mobile phase as in Table 1. Detection at 305 nm.

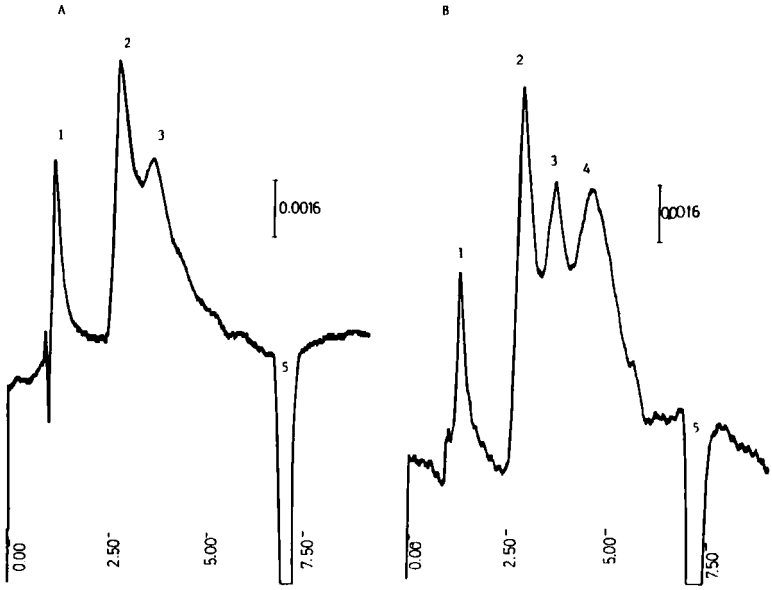

FIGURE 2. A. Separation of 5.0 ppm of Fe(III)(peak 1), 1.2 ppm of Fe(II)(peak 2), and 1.27 ppm of Ni(II)(peak 3). B. Separation of 5.0 ppm of Fe(III)(peak 1), 1.2 ppm of Fe(II)(peak 2), 1.27 ppm of Ni(II)(peak 3), and 2.1 ppm of Cu(II)(peak 4). Negative peaks (5) correspond to o-phenanthroline. Mobile phase as in Table 1. Detection at 305 nm.

tage of eliminating the above-mentioned peaks' broadening which was related with the time of utilization of the column. Also, at pH= 3.0, the analysis time was minor.

Detection limits have been calculated by using a signal-to-noise ratio of 2:1. The results obtained are grouped in Table 2 in which detection limits, expressed as both absolute mass units and concentration units for Fe(III) at 340 nm and for Fe(II) at 340 and 509 nm, are given.

The standard deviation obtained for an average of 8 injections of the same sample ranged from 2 to 4% in most of cases although in some case was 6%.

TABLE 2. Detection limits for Fe(III) and Fe(II). Mobile phase: as in Table 1 but at pH= 3.0 at 340 nm and as in Table 1 at 509 nm.

	340 nm		509 nm	
	mass(ng)	conc.(ppm)	mass(ng)	conc.(ppb)
Fe(III)	1.6	0.08	-	-
Fe(II)	2.0	0.10	0.008	0.42

In view of the important absorption of Fe(II) at 509 nm, the determination of Fe(II) at this wavelength could be very sensible. In fact, the detection limit of this element, that appears also in Table 2, is 240 times minor at 509 nm that at 340 nm.

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