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FURTHER STUDIES OF DIETHYLDITHIOCARBAMATE COMPLEXES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

S. DILLI*, P. R. HADDAD and A. K. HTOON
Department of Analytical Chemistry, School of Chemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. (Australia)
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SUMMARY

Normal- and reversed-phase high-performance liquid chromatography have been used to study the behaviour of the diethyldithiocarbamate complexes of Cu(II), Co(III), Cr(III), Ni(II), Hg(II), Pb(II), Se(IV), Zn(II), Fe(III), As(III) and Cd(II). A variety of commercially available columns was employed in an effort to identify significant differences in column performance. An analytical procedure suitable for the determination, in biological materials, of the more stable chelates (Cu, Co, Cr, Ni and Hg) by reversed-phase high-performance liquid chromatography is presented, following clarification of the optimal reaction conditions for formation of the Cr(III) complex.

INTRODUCTION

Numerous reports[1–23] dealing with the separation of metal diethyldithiocarbamate complexes by normal (NP) or reversed-phase (RP) high-performance liquid chromatography (HPLC) now exist. In fact, RP-HPLC appears to have overtaken the earlier work[1–10], published mainly before 1980 and based on normal-phase separation of important ions such as Cu(II), Co(III), Cr(III), Ni(II), Hg(II), Pb(II), Se(IV), Zn(II), Fe(III), Te(IV), Bi(III), Pd(II) and Pt(II). In the main, the requirements for these separations are straightforward although there is still some uncertainty regarding the ideal column and the stability of certain of the chelates. Thus, in RP-HPLC, stainless steel, glass, microbore and inert polymers have been used with evident success as column materials, although stainless-steel frits have been implicated in the decomposition of less stable metal chelates.

In an earlier paper[21], we reported the results of a study of the separation of eight stable metal diethyldithiocarbamates using RP-HPLC and non-metallic columns. We present here a further investigation of the chromatographic behaviour of a range of important metal ions as their diethyldithiocarbamate complexes, using a variety of columns and mobile phases in both NP- and RP-HPLC. In addition, studies of the optimal reaction and extraction conditions for the slow-reacting chromium(III) ion have been examined and extended to establish the recovery efficiency and precision of a procedure suitable for routine multi-element, trace level analysis of samples of biological origin.

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EXPERIMENTAL

Preparation of dithiocarbamate chelates

In most cases, metal chelates were prepared directly from sodium diethyldithiocarbamate and the stoichiometric amount of common metal salts[24]. The products were filtered off, washed with water and dried. Generally, no more than two recrystallizations were needed to give micro-analytically pure compounds. The only exceptions to this procedure, were the chromium(III)[25] and iron(III)[24] chelates which were conveniently extracted into chloroform and precipitated from the solution by the addition of ethanol. Reprecipitation of these compounds yielded pure products.

Instrumentation

The liquid chromatograph used for this work was a Millipore Waters system consisting of an M6000A pump, a U6K injector, a Model 441 UV detector with fixed wavelength of 254 nm, and an Omniscribe B-5000 strip-chart recorder (Houston Instruments) adjusted for 10-mV input. A 10-μl loop was filled with a 25-μl Hamilton syringe.

Unless stated otherwise, instrument parameters (at 20–25°C) were: mobile phase flow-rate 2.0 ml, recorder chart speed 0.5 cm/min and detector sensitivity 0.02 a.u.f.s.

Columns

With one exception, all columns were made of stainless steel and representative of commercially available columns suitable for both normal-phase [column 1 (Waters μPorasil column, 10-μm particle size, 30 cm × 3.9 mm I.D.; 8600 plates/m) and column 2 (Alltech LiChrosorb SI-60 column, 10 μm, 25 cm × 4.6 mm I.D.; 10 000 plates/m] and reversed-phase chromatography [column 3 (Kortec C_{18}-column, 5 μm, 25 cm × 4.6 mm I.D.; 10 500 plates/m), column 4 (Chromatographic Systems C_{18} column, 5 μm, 25 cm × 4.6 mm I.D.; 5800 plates/m), column 5 (standard Waters μBondapak C_{18}-column, 10 μm, 25 cm × 4.6 mm I.D.; 9500 plates/m), column 6 (Waters "Resolve" column, 5 μm, 15 cm × 4.6 mm I.D.; 14 500 plates/m) and column 7 (Waters Radial-Pak column, 10 μm, 10 cm × 8 mm I.D. PTFE cartridge; 45 000 plates/m)].

Reagents and solvents

Reagents were of analytical grade and solvents were either of HPLC grade (acetonitrile, tetrahydrofuran) or analytical quality and redistilled through an all-glass system. Water was prepared using a Millipore Milli-Q purification unit. All solvents were degassed and filtered through suitable membrane filters (Millipore types FH or HA, 0.45 μm) before use.

Acetate and ammonia–ammonium chloride buffer solutions were treated with a small quantity of ligand (5 ml, 0.05 *M*) followed by extraction with chloroform, then stored in plastic containers prior to use.

Chelate solutions

In preliminary work, stock solutions were prepared by dissolving the metal chelates, individually or in mixtures, in methanol for reversed-phase studies or in chloroform for normal-phase investigations.

Optimization of the formation of chromium(III) chelate

The optimal conditions for the formation of the chromium(III) chelate involved examining the reaction products following the use of five different reaction conditions. This was achieved by placing five aliquots of a chromium(III) acetate solution (2 ml, 10 ppm) in separate beakers and adding an excess of the dithiocarbamate reagent solution (2 ml, 0.025 *M*) to each beaker. One mixture was then allowed to react (a) at room temperature, (b) at 80°C for 20 min, (c) at 80°C for 20 min in the presence of sodium sulphite (1 ml, 2%, w/v solution), (d) at 80°C for 20 min in the presence of ascorbic acid (1 ml, 2%, w/v solution), and (e) at 80°C for 20 min in the presence of hydroxylamine hydrochloride (1 ml, 2%, w/v solution). Each reaction mixture was quantitatively transferred to a clean separating funnel (125-ml capacity, fitted with a polyethylene stopcock), acetate buffer (pH 5.8, 10 ml) added and the resulting mixture extracted with chloroform (4 × 5 ml). After washing out excess ligand from the combined extract with an equal volume of water[21], the solvent was removed by evaporation at room temperature and the residue dissolved in methanol (10 ml). Aliquots (10 μl) of this solution were injected into the chromatographic column.

Calibration curves and recovery studies

Including "blanks', aliquots (1–5 ml) of an aqueous mixture of Ni(II), Co(II), Cr(III), Cu(II) and Hg(II) were taken and extracted as outlined in the previous section. Analyses were made using column 5 and methanol–water (66:34, v/v) as mobile phase. Sensitivity of the detector was varied, depending on the absorptivity of each chelate. Calibration curves were prepared by plotting the amount of each metal injected (in ng) against the corresponding peak height (in mm).

Recovery efficiencies for known amounts of the five metals were determined using the same experimental conditions. All statistical calculations are based on data for triplicate injections of each extract.

Detection limits

Detection limits were determined using the same procedure given above for extraction of a mixture of the five metal ions into chloroform. For this purpose, the criterion chosen was an absorbance signal equal to twice the background noise, using the highest detector sensitivity available (0.005 a.u.f.s.).

Sample treatment and analysis

The method developed in the previous work was applied to the determination of trace levels of the metals in two NBS reference materials. These were the brewer's yeast (SRM 1569) and orchard leaves (SRM 1571). Portions of the yeast (0.500 g) and leaves (0.150 g) were accurately weighed and separately transferred to small Kjeldahl flasks (100 ml), then refluxed for 4 h with a mixture of nitric and perchloric acids (30 ml, 25:5, v/v) on a boiling waterbath. Each digestion residue was reduced to a small volume, additional nitric acid (15 *M*, 5 ml) added and the mixture again reheated briefly. When cold, water (25 ml) was added to the residue and ammonium citrate (10%, w/v, 10 ml) added to mask any iron and manganese, then the solution was neutralized and adjusted to pH 9.5 (ammonia–ammonium chloride buffer, 20 ml). Chelation was achieved by adding sodium diethyldithiocarbamate reagent (0.025 *M*,

3 ml) and heating the mixture for 15 min at 60°C. When cool, extraction was carried out as above using chloroform. The final residue was dissolved in methanol and made to volume (2.0 ml). As before, 10-μl volumes were examined on the μBondapak C_{18} column. Blanks and duplicate determinations were made on each sample.

RESULTS AND DISCUSSION

Chromatographic behaviour of diethyldithiocarbamate complexes

Normal-phase chromatography. The work of Liska *et al.*[6] indicated that the diethyldithiocarbamate of Zn(II), Cu(II), Mn(II), Ni(II), Pb(II), Cr(III), Co(III), Cd(II) and Fe(II) were satisfactorily separated on a LiChrosorb SI-60 (10 μm, stainless steel) column using cyclohexane–chloroform (90:10, v/v) as the mobile phase. For the experimental conditions described, a typical separation is shown in Fig. 1 for nanogram amounts of six of the most stable diethyldithiocarbamates. The separation, however, is not entirely satisfactory because of the failure to separate the Co(III) and Cr(III) chelates, and the inability to separate the oxidized ligand (the disulphide "disulfiram") from the Cu(II) complex. Attempts to separate the less stable metal

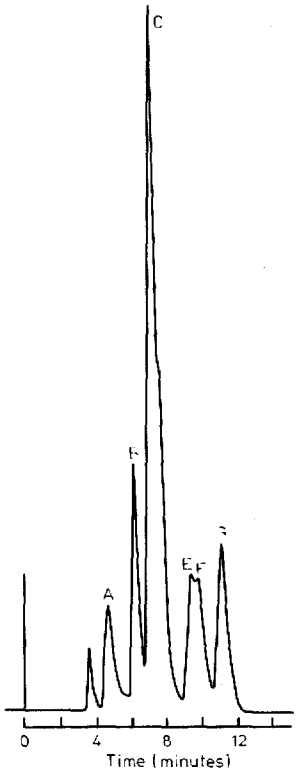

Fig. 1. Chromatogram showing the separation of a mixture of six metal diethyldithiocarbamates on column 1. Mobile phase: cyclohexane–chloroform (90:10, v/v) at 1 ml/min. Identity is: A, Se(IV) 50 ng; B, Hg(II) 20 ng; C, ligand disulphide; D, Cu(II) 6 ng; E, Co(III) 2 ng; F, Cr(III) 2 ng; G, Ni(II) 4 ng. Conditions (for other figures also) are as given in the Experimental section.

chelates [such as those of Zn(II), As(III), Pb(II) and Cd(II)] were totally unsuccessful on both columns 1 and 2 and no chromatographic peaks were obtained other than those corresponding with the retention times of the Cu(II) and Ni(II) diethyldithiocarbamates, presumably as a result of their decomposition to yield the stable copper and nickel chelates. Retention times for the stable complexes are given in Table I. In fact, better peak shapes were achieved for the Se(IV), Ni(II) and Cu(II) chelates with column 2 but the Co(III) and Cr(III) chelates were not well-resolved until the percentage of chloroform was reduced to 4%. At this concentration, although not a critical need in either case, separation of the disulphide and the Cu(II) chelate was successful but, then, the Se(IV) and Hg(II) chelates co-eluted. Another possibly more serious disadvantage for normal-phase liquid chromatography is the appearance of the disulphide peak in the midst of metal chelate peaks.

Other binary solvents, such as cyclohexane–tetrahydrofuran and chloroform–tetrahydrofuran, were also examined, but without providing any improved separation of the complexes.

Reversed-phase chromatography. Some initial work was carried out using column 3 after it had been washed with a solution of the ligand (0.001 *M*; in methanol–water, 70:30, v/v) to remove any accessible, on-column traces of metal impurities. On this column, the separation of metal chelates was best achieved with a ternary solvent system containing acetonitrile–methanol–water (35:30:35, v/v/v) and indicated that the dithiocarbamates of Ni(II), Co(III), Cr(III), Se(IV), Cu(II) and Hg(II) are stable enough to be used at the nanogram level (10–20 ng) in quantitative work for multi-element trace metal analysis.

Other elements produced multiple peaks similar to those previously reported[15] representing decomposition products and disulphide. The group of less stable metal complexes, referred to above, produced similar chromatograms (on the five reversed-phase columns) consisting mainly of three peaks. The first, a large peak, was observed at the column void volume and showed some decomposition products were unretained by the column (see peaks I in Fig. 2). The peak was also observed when the ligand was chromatographed on the column and suggests that this behaviour is due to

TABLE I

RETENTION TIMES OF METAL DIETHYLDITHIOCARBAMATES IN NORMAL-PHASE LIQUID CHROMATOGRAPHY

Diethyldithiocarbamate complex	*Retention time (min)*	
	System 1[a]	*System 2*[b]
Se(IV)	4.3	4.8
Hg(II)	6.3	6.2
Ni(II)	7.2	11.1
Cu(II)	8.5	7.6
Cr(III)	9.5	9.8
Co(III)	10.0	9.4
Ligand disulphide	8.2	7.3

[a] Using column 1 with cyclohexane–chloroform (90:10, v/v) at 1 ml/min.
[b] Using column 2 with cyclohexane–chloroform (90:10, v/v) at 1 ml/min.

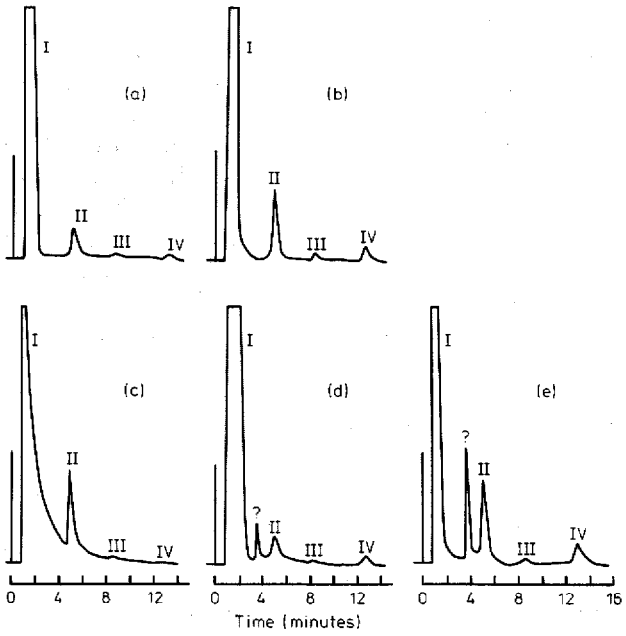

Fig. 2. Reversed-phase chromatograms of metal diethyldithiocarbamates on column 3. Identity is: (a) Cd(II), 200 ng; (b) As(III), 200 ng; (c) Fe(III), 50 ng; (d) Pb(II) 300 ng; (e) Zn(II), 300 ng. See text for identification of species I–IV.

the elution of the ligand anion or a solute–mobile phase interaction product. The latter is not unknown and is illustrated by the reported precipitation[26] of the Pb(II) chelate in the column when the mobile phase was deficient in chloroform and by the decomposition[27], in 4-methyl-2-pentanone, of the Pb(II) chelate of the more stable ligand, ammonium pyrrolidinedithiocarbamate. The second peak (see peaks II in Fig. 2) is one of medium height whose retention time corresponds to that of the disulphide and appears to be directly related to the stability of a metal chelate. Finally, two small peaks corresponding to the retention time of the Ni(II) and Cu(II) chelates (see peaks III and IV in Fig. 2) are presumably due to interaction with stainless-steel components of the column or the injector (or both). Retention times for the various diethyldithiocarbamate on column 3 are given in Table II. Separation of the chelates on this column was also examined with a small amount of disodium ethylenediaminetetraacetate (Na_2EDTA, 0.005 *M*) in the mobile phase in an effort to bind more effectively any free metal ions in the system contributing to the on-column decomposition of the group of less-stable diethyldithiocarbamates. However, as expected, the stable chelates merely showed longer retention and on-column decomposition of the less-stable chelates was unaffected. These results, together with those obtained with a lower concentration of sodium diethyldithiocarbamate (*ca.* 0.0001 *M*) in the mobile phase are included in Table II and Fig. 3.

Since the dissociation of the diethyldithiocarbamate complexes[5] (and other dialkyldithiocarbamate complexes) at very low concentrations limits their utility for trace analysis by HPLC unless free ligand is present in the mobile phase [22,23,28–33], some additional work was carried out with the ligand incorporated at the level of 20

TABLE II

RETENTION TIMES OF INDIVIDUAL METAL DIETHYLDITHIOCARBAMATES IN REVERSED-PHASE CHROMATOGRAPHY

Using column 3. System 1, acetonitrile–methanol–water, 35:30:35 (v/v/v); system 2, as for system 1, but also containing 0.005 *M* Na_2EDTA; system 3, as for system 1, but containing 0.0001 *M* sodium diethyldithiocarbamate.

Diethyldithiocarbamate complex	*Retention time (min)*		
	System 1	*System 2*	*System 3*
Cd(II)	–	–	3.1
Pb(II)	–	–	6.6
Ni(II)	8.0	9.4	8.4
Co(III)	9.9	12.1	10.3
Cr(III)	10.9	13.4	11.5
Se(IV)	11.4	13.3	11.2
Cu(II)	12.3	14.7	12.8
Hg(II)	14.2	17.3	14.9

mg/l (or *ca.* 0.0001 *M*). This low concentration is dictated by the strong UV absorption of the ligand and the need to detect the chelates at 254 nm. It may be noted that addition of free ligand to the mobile phase was accompanied by a steady drifting of the baseline at the sensitivity setting of 0.02 a.u.f.s. and this was only slowly overcome or, alternatively, circumvented by using a much more insensitive detector setting, such as 0.5 a.u.f.s. In this way, it was possible to deal more satisfactorily with the Pb(II) and Cd(II) chelates among the less stable metal diethyldithiocarbamates. A further account of the effect of the ligand in the mobile phase is the subject of a later communication[34].

Other aspects of the preliminary studies of the chromatography of these chelates included comparison of their behaviour on column 7, the wall of which is made of an inert polymer and end-fittings are of thin, stainless-steel metal spreader-plates. Despite the minimal contact with metal components of the analytical system[21,35], the less stable metal complexes were not chromatographed successfully or in any way better than on the stainless-steel columns. A typical chromatogram obtained with the stable metal chelates on this column is shown in Fig. 4.

Simultaneous determination of metal ions

From the above considerations, column 6 and a ternary mobile phase consisting of methanol–acetonitrile–water (45:20:35, v/v/v) were chosen for the simultaneous, quantitative determination of the elements Ni(II), Co(II), Cr(III), Cu(II) and Hg(II). However, it was necessary to abandon this approach because of the unsatisfactory features of the chromatographic separation of mixtures containing the Hg(II) complex. Although interference caused by the Hg(II) chelate is not expecially evident in typical chromatograms at higher concentrations, at lower levels a pronounced and elevated baseline becomes obvious, as shown in Fig. 5 for three different amounts of the complex, observed under the same experimental conditions. This unsatisfactory behaviour with the Hg(II) complex was observed also on column 5, again using the same experimental conditions. In addition, there was a marked deterioration in the

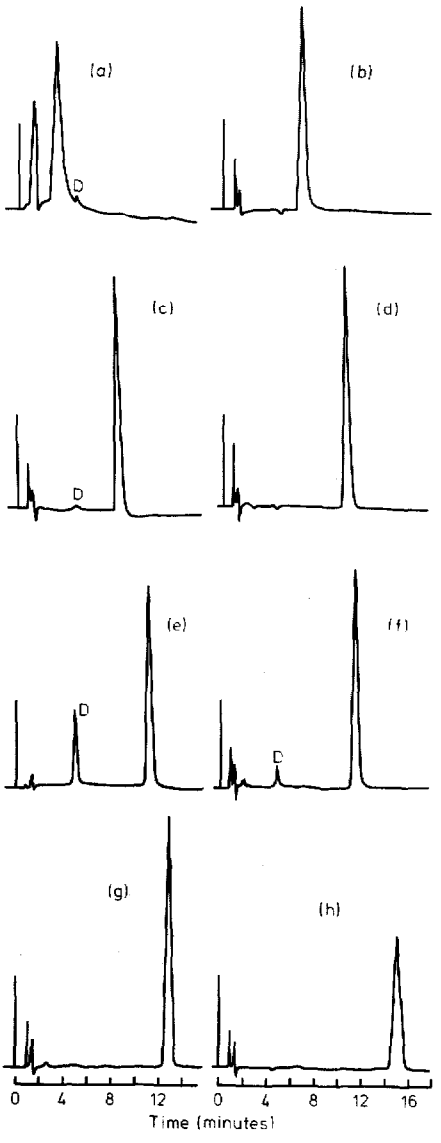

Fig. 3. Chromatographic behaviour of metal diethyldithiocarbamates on column 3. Identity is: (a) Cd(II), 60 ng; (b) Pb(II), 600 ng; (c) Ni(II), 30 ng; (d) Co(III), 30 ng; (e) Se(IV), 400 ng; (f) Cr(III), 50 ng; (g) Cu(II), 50 ng; (h) Hg(II), 100 ng; D = ligand disulphide.

detector response for the complex when a solution in acetonitrile was examined and compared with a methanolic solution.

The most plausible explanation of this phenomenon appears to implicate acetonitrile in complex formation with mercury, and possibly one of the other metals present in the mixtures when the behaviour was observed [in this case, the Ni(II) and Cu(II) chelates]. The idea is not new, and several instances of ternary complexes involving mercury[33,36] and various donors[37–40] have been reported. Whatever the

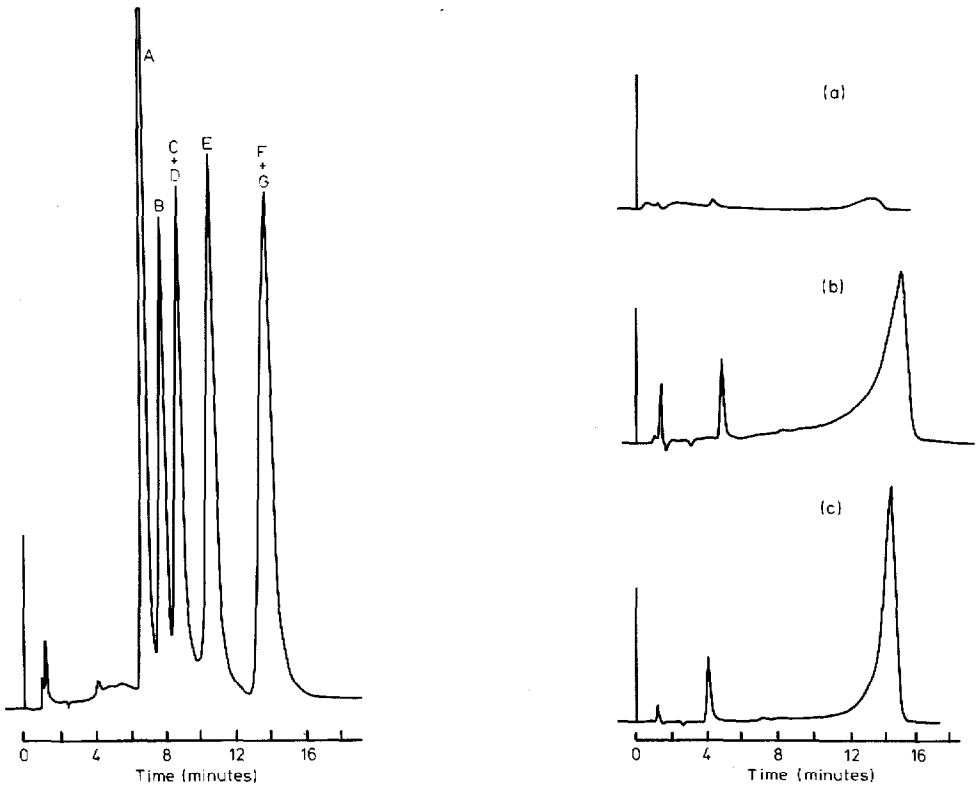

Fig. 4. Chromatogram showing the separation of six metal diethyldithiocarbamates on a non-metal, reversed-phase column (column 7) with a mobile phase of methanol–chloroform–water (50:30:20, v/v/v) at 1.5 ml/min. Peaks: A = disulphide; B = Ni(II) 30 ng; C = Se(IV) 75 ng; D = Cu(II) 40 ng; E = Hg(II) 100 ng; F = Co(III) 15 ng; G = Cr(III) 20 ng.

Fig. 5. Chromatograms showing the retention and elevated baseline of the Hg(II) diethyldithiocarbamate on a stainless-steel column (column 6) with the mobile phase methanol–acetonitrile–water (45:20:35, v/v/v) at 1 ml/min. Quantities are (a) 20 ng Hg(II); (b) 80 ng Hg(II); (c) 120 ng Hg(II).

complete explanation, Fig. 6 shows chromatograms for the separation of a mixture of five diethyldithiocarbamates prepared in methanol and examined on column 5. Fig. 6a shows that the presence of as little as 5% acetonitrile in the mobile phase produces the characteristic elevated baseline preceeding the peak of the Hg(II) complex. In marked contrast, with methanol–water as mobile phase the separation of the Hg(II) complex is total and there is an enhanced detector response. In addition, the slower chart speed which applies for Fig. 6b tends to conceal the full extent of the peak widths and the interval between the peaks of the Cu(II) and Hg(II) chelates.

Subsequent developmental work was confined to work using only mobile phases consisting of methanol and water.

Optimum formation conditions for the Cr(III) chelate

Since the rate at which water molecules are substituted by the diethyldithiocarbamate anion is slow[17], the formation of the Cr(III) complex tends to be a very slow

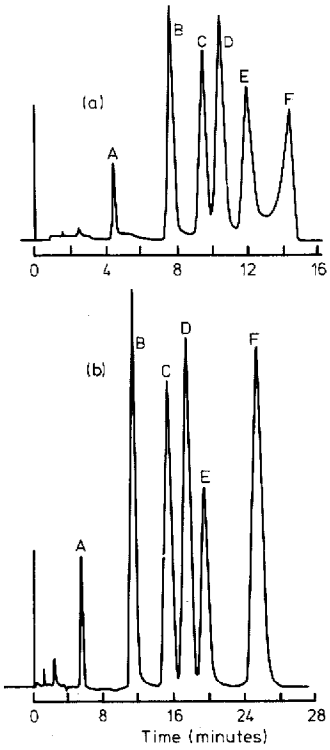

Fig. 6. Separation of mixtures of metal diethyldithiocarbamates on column 5 with mobile phases; (a) methanol–acetonitrile–water (65:5:30, v/v/v), chart speed 0.5 cm/min, and (b) methanol–water (66:34, v/v), chart speed 0.25 cm/min. Peaks: A = disulphide; B = Ni(II) 30 ng; C = Co(III) 10 ng; D = Cr(III) 15 ng; E = Cu(II) 30 ng; F = Hg(II) 100 ng.

process. Complex formation may be increased by reducing the stability of the hydrated ion (by adding ethanol to the aqueous solution) or by raising the reaction temperature[15,41]. For example, Gleu and Schwab[41] formed this complex at 70°C, using an acetate buffer at pH 5. However, the inertness of the Cr(III) ion affects its reactivity and in some cases the reaction mixture has been allowed to stand overnight[17,32]. Since such a procedure is not acceptable for routine analysis, optimal conditions for the formation of this complex were studied more closely and included reaction (using 20 μg Cr^{3+}) at room and elevated temperatures and in the presence of several reducing agents.

The most favourable conditions for complex formation were found to be reaction at pH 5.8 and at 80°C for 20 min. In later work, because of the decomposition of some chelates [especially the Ni(II) and Cu(II) chelates], these conditions were altered to the milder one of heating at 60°C for 15 min. Further to this work, the effect of pH on the formation of this complex was also examined more closely at pH 5.8 (acetate buffer), pH 8.0 (boric acid–sodium hydroxide buffer) and pH 9.5 (ammonia–ammonium chloride buffer). The experimental procedure was otherwise unchanged.

Fig. 7 shows the chromatograms of a mixture of the diethyldithiocarbamates formed under different experimental conditions using a mixture of the ions in aque-

ous solution. Clearly those applying to conditions (a) and (b) are unfavourable for complex formation. Here, it appears that the pH is too low in (a) and the reaction too slow in (b), whereas in (c), (d) and (e), the height of the Cr(III) peak increases progressively. No serious decomposition of other metal chelates is indicated, although there is a small reduction in the peak height of the Cu(II) complex. From these results, the final modification chosen for the analytical procedure involved reaction at pH 9.5 and 60°C for 15 min. For the chromatographic separation, column 5 was used with a mobile phase of methanol–water (66:34, v/v).

Linearity of calibrations plots and detection limits

Fig. 8 shows the calibration plots prepared from the chromatographic data obtained after reaction of known amounts of mixtures of the five metal ions with the ligand, following the procedure discussed above. All plots show good linearity for nanogram amounts of the metals injected into the column although, probably because of adsorption of the compounds on the column, only that for the Co(III) chelate passes through the origin. Because of the poorer sensitivity of the Hg(II) complex, this is shown separately from the other metals, nevertheless, all correlation coefficients fell in the range 0.9967 to 0.9996. The linear range of each of the five

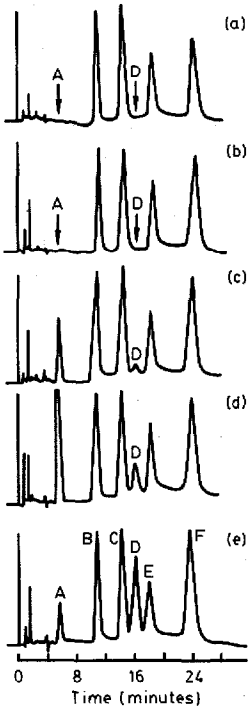

Fig. 7. Chromatograms of mixed metal diethyldithiocarbamates formed under various reaction conditions. Individual chromatograms are for reaction at 60°C for 15 min, unless stated otherwise, with: (a) no pH control (approx. pH 2); (b) pH 9.5, reaction at room temperature; (c) pH 5.8, (d) pH 8.0, (e) pH 9.5. Peaks: A = disulphide; B = Ni(II) 20 ng; C = Co(III) 10 ng; D = Cr(III) 20 ng; E = Cu(II) 20 ng; F = Hg(II) 100 ng.

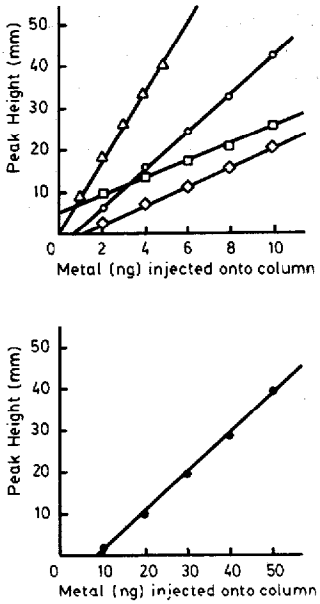

Fig. 8. Calibration plots for five metal diethyldithiocarbamates determined simultaneously by HPLC. Identity is: ○ = Ni(II); △ = Co(III); □ = Cr(III); ◇ = Cu(II); ● = Hg(II). Conditions as in Experimental section.

metals extended over two orders of magnitude. In fact, the linear ranges extended as far as 300 to 500 ng for all the elements except Co(III). In the latter case, the narrower range is probably related to the Co(III) complex having the lowest detection limit.

Detection limits for these metals and Se(IV), at 254 nm (and based on a signal-to-noise ratio of *ca.* 2) are shown in Table III with, for convenience, other data presented in graphical form in Fig. 8. Of the values listed, the detection limit for the Hg(II) chelate agrees closely with the value given by Inoue *et al.*[42], that is, 8.8 ng.

Recovery efficiency

A study of the recovery efficiency for the ions of interest [*viz.* Ni(II), Co(II), Cr(III), Cu(II) and Hg(II)] when in a mixture was undertaken to assess the extent of any inter-element effect. Other elements such as those forming the less stable chelates

TABLE III

LINEAR CALIBRATION RANGES AND DETECTION LIMITS OF METAL IONS

Metal ions	*Linearity range (ng)*	*Detection limits (ng)*	*Working curve range (ng)*
Ni(II)	200–450	0.2	0–10
Co(II)	100–150	0.1	0–5
Cr(III)	200–300	1.0	0–10
Cu(II)	200–400	0.8	0–10
Hg(II)	10–500	8	10–50
Se(II)	–	8	–

TABLE IV

DATA SHOWING THE EFFICIENCY OF THE RECOVERY OF METAL IONS FROM SOLUTION

R.S.D. = Relative standard deviation.

Metal ions	*Amount taken (ng)*	*Amount found (ng)*	*Recovery (%)*	*R.S.D. (%)*
Ni(II)	8.0	7.6 ± 0.2	95.3	2.1
Co(III)	4.0	3.9 ± 0.1	97.0	2.5
Cr(III)	8.0	7.6 ± 0.2	95.0	2.1
Cu(II)	8.0	7.5 ± 0.1	93.4	1.8
Hg(II)	40.0	39.4 ± 0.8	98.6	2.0

were deliberately excluded in this work but, of course, would be required in any more extensive development of this procedure.

The recovery data collected in Table IV show that there is no serious interference among these elements and that the recoveries, of acceptable precision, were not less than 93%. The lowest recovery of all elements was that for Cu(II), probably as a result of its thermal decomposition under the experimental conditions employed. Although more time-consuming, the obvious solution to this problem is to determine Cu(II) at room temperature, followed by Cr(III) at the elevated temperature and pH.

Precision

The precision in peak height measurement, as determined for a mixture of the five metals is shown in Table V. Two levels of concentration of the chelates are indicated. For the lower concentration of 2–50 ng of metal per injection, the corresponding relative standard deviations ranged from 0.87 to 2.8%. For the higher concentration of 10–200 ng of metal per injection, the relative standard deviation

TABLE V

DATA SHOWING THE PRECISION OF PEAK HEIGHT MEASUREMENTS FOR A MIXTURE OF METAL DIETHYLDITHIOCARBAMATES

Metal ion	*Level*[a]	*Peak height*[b] *(mm)*	*Mean*	*Standard deviation*	*R.S.D. (%)*
Ni(II)	0.2 ppm (2 ng)	22, 22, 22, 22, 22	22.1	0.41	1.84
	2.0 ppm (20 ng)	102, 102, 101, 99, 101, 101	101	1.1	1.09
Co(III)	0.2 ppm (2 ng)	37, 37, 38, 38, 38, 38	37.7	0.52	1.37
	1.0 ppm (10 ng)	90, 90, 89, 89, 90, 90	89.7	0.52	0.58
Cr(III)	4.0 ppm (40 ng)	59, 59, 59, 59, 60, 60	59.3	0.52	0.87
	20 ppm (200 ng)	84, 84, 85, 84, 85, 87	84.8	1.17	1.38
Cu(II)	0.8 ppm (8 ng)	28, 28, 27, 28, 28, 28	27.8	0.41	1.47
	2.0 ppm (20 ng)	79, 78, 77, 80, 81, 80	79.2	1.47	1.85
Hg(II)	5.0 ppm (50 ng)	43, 40, 42, 42, 41, 43	41.8	1.17	2.8
	20 ppm (200 ng)	129, 130, 126, 128, 120, 122	125.8	4.02	3.2

[a] Detector sensitivity: 0.01 a.u.f.s. for lower levels and 0.02 a.u.f.s. for higher levels.

[b] Measured at 254 nm, with other conditions as in Fig. 6b.

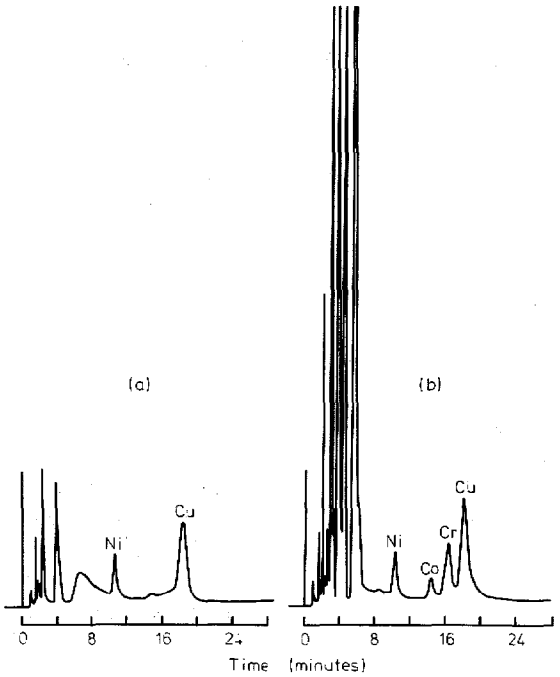

Fig. 9. Chromatograms of chelates extracted from (a) reagent blank and (b) brewer's yeast SRM 1569. Separation on column 5 with mobile phase methanol–water (66:34, v/v) and chart speed 0.25 cm/min.

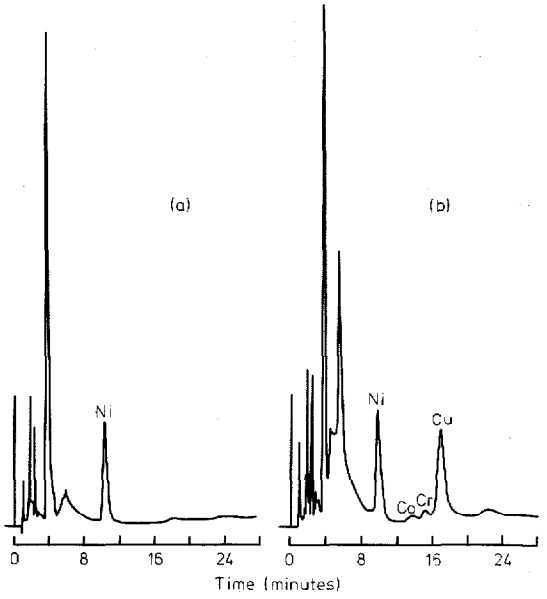

Fig. 10. Chromatograms of chelates extracted from (a) reagent blank and (b) orchard leaves SRM 1571. Conditions as for Fig. 9.

TABLE VI

RESULTS FOR THE ANALYSIS OF BIOLOGICAL MATERIALS SRM 1569 AND SRM 1571

Standard	*Element*	*Certified value (μg/g)*	*Amount found (μg/g)*	*Mean (μg/g)*
SRM 1569	Cr(III)	2.12 ± 0.05	2.007, 2.087	2.05 ± 0.06
SRM 1571	Ni(II)	1.3 ± 0.2	1.22, 1.27, 1.44	1.31 ± 0.13
	Co(III)	0.2	0.15, 0.16, 0.20	0.17 ± 0.02
	Cr(III)	2.3	1.99, 2.34	2.19 ± 0.25
	Cu(II)	12 ± 1	12.97, 13.48	13.2 ± 0.4
	Hg(II)	0.155 ± 0.015	–	–

varied from 0.58 to 3.2%. In both sets of data, the poorest precision applied to measurements of the Hg(II) complex.

It is anticipated that the precision of these measurement would be improved using an auto-injector coupled with an integrator for the measurement of peak height or area.

Analytical data

Following the analytical procedure outlined in the Experimental section involved no difficulties. Fig. 9 is a typical chromatogram obtained for the brewer's yeast and shows peaks due to Ni(II), Co(III), Cr(III) and Cu(II). Similarly, Fig. 10 shows the presence, mainly, of Ni(II) and Cu(II) in the sample of orchard leaves, together with traces of Co(III) and Cr(III). The analytical results, summarized in Table VI, are in good agreement with the certified values and indicate the potential of the method. However, two relatively minor problems remain to be resolved. One of these, as is obvious in the chromatograms for the two "blanks", is the need for acids of adequate purity, since all other reagents are (and were in this case) easily treated to remove traces of metals. The other is related to the appearance of a cluster of large peaks (with short retention times) seen in the chromatogram for the brewer's yeast. Although the reason for these peaks is not clear, it is probable that a more vigorous digestion procedure would remedy the problem. Without introducing any additional difficulty, the use of larger samples should also improve the overall precision attainable.

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