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MULTI-ELEMENT ANALYSIS OF TRACE METALS IN SOME ENVIRONMENTAL SAMPLES BY SOLVENT EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF METAL CHELATE COMPLEXES*

EBI B. EDWARD-INATIMI**
School of Chemistry, Thames Polytechnic, Wellington Street, London SE18 6PF (Great Britain)
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SUMMARY

The use of high-performance liquid chromatography (HPLC), after preliminary solvent extraction, for the complete separation of trace amounts of metal chelates prior to their non-selective determination was investigated. A UV detector set to the most intense absorbance peak of the reagent was used as a universal detector for all of the metal chelates at approximately the same sensitivity. Sulphur-containing reagents such as dithizone and diethyldithiocarbamate were used owing to their high absorptivities and because they tend to form strong complexes with toxic metalloids.

The absorption mode of HPLC was used, because metal chelates are typically very soluble in non-polar solvents such as chloroform. The high distribution ratios of the complexes in such solvents made possible the use of small volumes for extraction and hence obviated the need for evaporation prior to direct HPLC injection.

The method was used for the analysis of trade effluents and compared with atomic-absorption spectrometry, and using a rapid wet-ashing procedure with nitric acid–hydrogen peroxide has been applied to standard kale and standard fish meal for calibration and to aquatic biota.

INTRODUCTION

Owing to the toxicity of many trace metals, there is a need for multi-element analysis especially of trace metals in trade effluents, before disposal to the aquatic environment, and in the aquatic biota, in order to monitor their enrichment and possible return to the human food chain.

A fairly simple and routine multi-element method is required in order that such monitoring can be mandatory and effective in different countries. High-performance

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** Present address: Department of Chemistry, School of Science and Science Education, Federal University of Technology, P.M.B. 0248, Bauchi, Nigeria.

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liquid chromatography (HPLC), after preliminary solvent extraction, was therefore investigated for the complete separation of trace amounts of metal chelates prior to their non-selective determination. The overall selectivity of the organic reagents can be increased in this way up to the point at which they will be useful for the rapid routine fingerprinting of groups of trace metals, and comparable to the use of derivative gas liquid chromatography for organic compounds. If the separation is complete, an ultraviolet detector set to the most intense absorbance peak of the reagent itself can be used as a universal detector for all the metals at approximately the same sensitivity. Sulphur-containing reagents, *e.g.*, dithizone[1] and diethyldithiocarbamate (DDTC)[2], are chosen because of their high molar absorptivities and because they form strong complexes with toxic metals, such as lead and mercury.

There have been few reports of the use of HPLC for the separation of metal chelate complexes[3–10], especially for real samples. In this work HPLC was used in the absorption mode because metal chelates are typically very soluble in non-polar solvents, such as chloroform. This high solubility has proved to be an advantage because, with the high distribution ratios of the complexes in such solvents, it was possible to use relatively small volumes for the extraction. This practice avoids the need for a slow evaporation step prior to direct injection of the aliquot on to the HPLC column.

EXPERIMENTAL

Reagents

All reagents were of AnalaR grade unless stated otherwise.

Dithizone (H_2DZ) solution. Dissolve 0.05 ± 0.01 g of dithizone in 100 ± 1 ml of dry chloroform (prepare freshly every week and store in a refrigerator after use).

Diethylammonium diethyldithiocarbamate (DDDTC) solution. Dissolve 0.50 ± 0.01 g of DDDTC in 100 ± 1 ml of dry chloroform (prepare freshly every week and store in a refrigerator after use).

Buffer solution. Dissolve 45.2 g of ammonium chloride and 9.6 ml of concentrated ammonia solution (sp. gr. 0.880) in 1000 ml of water in a graduated flask. This solution should have a pH of 8.5. Dilute 10-fold before use.

Chloroform. Dry over a molecular sieve.

HPLC eluent. Dry Spectrograde benzene (Fisons Scientific Instruments, Loughborough, Great Britain) with a molecular sieve and degas before use.

Apparatus

The HPLC system chosen was a laboratory-built one similar to that described by Cox[12], based on a Haskell Chemical pump and a Cecil variable-wavelength UV detector. The column (stainless steel, 150 × 4.6 mm I.D.) was packed in the laboratory with Hypersil 5 μm diameter silica gel using the slurry packing procedure.

Sample tubes (20 × 50 mm) were used for the collection of organic phases after the solvent extraction of the samples. These tubes should be fitted with snap-on polyethylene lids. Other apparatus consisted of 400- and 600-ml graduated borosilicate (Pyrex) glass beakers and 500- and 1000-ml separating funnels fitted with ground-glass stoppers and taps (if possible Interflow polyethylene taps); 1_s^P silicone-treated phase-separating paper (Whatman Labsales, Springfield Mill, Maidstone, Great Britain) was used.

Procedure (including calibration)

Add an appropriate aliquot of the standard (see Note a below) or sample (Note b) solutions to a clean 600-ml graduated Pyrex glass beaker and adjust the pH of the solution to about 8 with either 2 *M* hydrochloric acid or 2 *M* sodium hydroxide solution. Add an appropriate amount of buffer to maintain the pH of the metal solution at 8.5 ± 0.1 (Note c) (use a pH meter). Dilute the solution to 300 ± 1 ml in a beaker or large measuring cylinder.

Transfer the solution into a clean 500-ml separation funnel, add 5.0 ± 0.05 ml of H_2DZ or DDDTC (Note d) solution in chloroform with a clean pipette as required, stopper the funnel, shake it vigorously for 3 min ± 15 sec and allow it to stand for 5 min ± 30 sec.

Separate the organic from the aqueous phase by filtration through the phase-separating paper. Run the organic phase via the phase-separating paper into a sample tube and fit the lid (Note e). The HPLC stage must be completed during the same working day.

Inject 2–10 μl of this chloroform solution of the separated metal chelates on to an already equilibrated chromatographic column by the stop-flow syringe mode of injection. Mark the injection point on the recorder trace.

Measure the peak heights and compare with calibration standards.

Blank determination. A blank must be run with each batch of determinations (*e.g.*, up to 10 samples) using the same batch of reagents as for the samples and also the sample eluent for the HPLC run. Carry out the above steps again, but replace the standard or sample solution with distilled water and make up to mark as for samples.

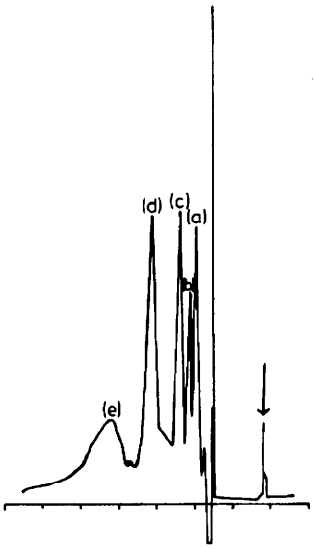
Calibration. Stock standard solutions (Note a) are used for calibrations. Duplicate calibration standards should be run with each batch of determinations (*e.g.*, up to 10 samples). Using an appropriate aliquot of the stock standard solution, carry out the above steps. The linearity of the calibration graph should be checked from time to time, by plotting peak height against concentration of sample solution in the aqueous phase (the volume ratio, V_{aq}/V_0, used is 100, *i.e.*, 500 ml of aqueous phase to 5 ml of organic phase).

Notes. (a) Stock standard solutions should be prepared by dissolving the appropriate metal or stoichiometric salt (AnalaR or 99.9% purity) at a concentration of 1000 ppm.

(b) Sample pre-treatment: for some solid samples, *e.g.*, standard kale (vegetable) or fish samples, a wet-ashing procedure, using nitric acid–hydrogen peroxide (perhydrol) (1:1) was adopted, in order to have the trace metals in solution before solvent extraction. Little or no pre-treatment is necessary for aqueous samples (*e.g.*, trade effluents), except in some instances where filtration is necessary.

(c) Do not use more than 100 ml of buffer solution, in order to avoid the introduction of errors.

(d) It was observed that some of the commercial diethyldithiocarbamic acids produce oxidation products that are liable to interfere in the determination of nickel and manganese dithiocarbamate, especially at lower levels. In order to remove these two interferents from the reagent, it is recommended that the reagent should be back-washed to remove oxidation products before being used for the preparation of metal complexes. The clean-up procedure used is as follows. Prepare a 10^{-4} *M* solution of recrystallized diethylammonium diethyldithiocarbamate (DDDTC) (BDH, Poole,



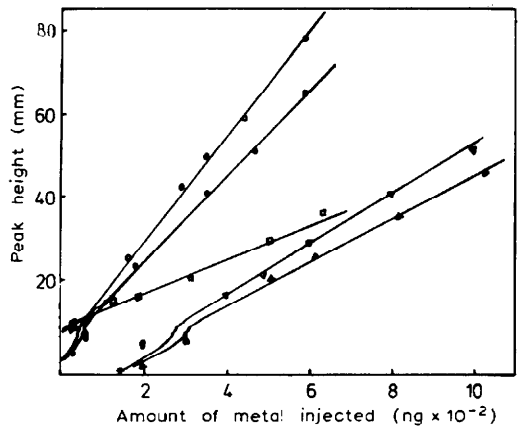


Fig. 1. Separation of a standard trace metal sample (extracted with dithizone solution). Divisions on baseline: 1 min per division. Peaks: dithizone complexes of (a) Hg, (b) Cu, (c) Ni, (d) Co and (e) Pb.
Fig. 2. Dependence of peak height on the amount of metal injected for various metal–dithizone complexes: ○, Ni; ●, Co; □, Cu; ▽, Hg; ▲, Pb.

Great Britain) in chloroform (BDH) in a 100-ml volumetric flask. Bubble nitrogen through 100 ml of distilled water in a 250-ml separating funnel for about 10 min. Add 10 ml of ammonia–ammonium chloride buffer (pH 8.5), followed by 10 ml of reagent solution in chloroform. Shake the chloroform and aqueous layers together for 2 min ± 5 sec, and allow the chloroform layer to separate. Repeat the extraction once more and discard the chloroform layers containing the oxidation products and any metal impurities present in the distilled water or in the reagent. Bubble nitrogen through the aqueous reagent solution until it is clear (about 20 min). This clear solution is ready for the preparation of metal complexes.

(e) All samples and standards should be processed to this stage before proceeding to the HPLC stage.

RESULTS AND DISCUSSION

Investigation of variables

The HPLC behaviour of the metal chelates of both ligands used in this work were studied on silica gel columns with Spectrograde benzene as mobile phase. The use of a Hypersil 5 μm column was found to be the most suitable for the metal chelates studied and this column was used for the remainder of the work. The separation of five metal dithizone complexes, *viz.*, $Hg(HDZ)_2$, $Cu(HDZ)_2$, $Ni(HDZ)_2$, $Co(HDZ)_2$ and $Pb(HDZ)_2$, extracted from a standard metal solution, is illustrated in Fig. 1. The separation of metal–DDTC complexes, also extracted from a standard metal sample solution, has been reported elsewhere[13]. The column efficiencies, resolution and other chromatographic properties are indicated in Tables I and II for the metal–dithizone and metal–DDTC chelates, respectively.

TABLE I

CHROMATOGRAPHIC PROPERTIES OF METAL–DITHIZONE CHELATES

Column, Hypersil (5 μm, 150 × 4.6 mm I.D.); eluent, Spectrograde benzene; flow-rate, 1.15 ml/min; pressure, 230 p.s.i. (15.6 bar); detector, UV wavelength 285 nm at 0.05 a.u.f.s.

*Parameter**	*Metal chelate*				
	Hg(HDZ)$_2$	*Cu(HDZ)$_2$*	*Ni(HDZ)$_2$*	*Co(HDZ)$_2$*	*Pb(HDZ)$_2$*
t_R (min)	2.05	2.25	2.60	3.35	5.20
V_R (ml)	2.36	2.59	3.00	3.85	5.98
k'	0.40	0.50	0.73	1.20	2.50
H(cm)	$6.4 \cdot 10^{-3}$	$5.3 \cdot 10^{-3}$	$4.0 \cdot 10^{-3}$	$9.0 \cdot 10^{-3}$	$9.0 \cdot 10^{-3}$
N	2344	2830	3750	1700	406
R_s (for successive peaks)	–	0.91	2.03	2.19	1.90
DL (ng)**	100	15	30	10	105

* t_R = Retention time of solute; k' = column capacity ratio; H = plate height (height equivalent to a theoretical plate); N = number of theoretical plates (plate number); R = resolution of peaks (see ref. 14 for definition). Coefficients of variation of retention times and peak heights for metal chelates were 0.5% and 1.5%, respectively (10 replicate measurements, sample amount 2 μg).

** DL = detection limit (2 × noise).

Quantitative studies were carried out on the metal chelates to determine the linearity of the detector response and the limits of detection. Fig. 2 shows the dependence of the peak height on the amount of metal injected for metal–dithizone chelates. There is no apparent reason for the order of the slopes for the various metals. However, the heavier metals, mercury and lead, give the smallest gradients of the metal–dithizone complexes. The gradient of the plot for the copper–dithizone complex is different from those of all the complexes, possibly because, with the extraction conditions used, a secondary $CuDZ_2$ complex was formed instead of the primary $Cu(HDZ)_2$ complex[1].

TABLE II

CHROMATOGRAPHIC PROPERTIES OF METAL–DDTC CHELATES

Column, Hypersil (5 μm, 150 × 4.6 mm I.D.); eluent, Spectrograde benzene; flow-rate, 1.34 ml/min; pressure, 288 p.s.i. (19.6 bar); detector, UV, wavelength 280 nm at 0.05 a.u.f.s.

*Parameter**	*Metal chelate*						
	CuDDTC	*NiDDTC*	*HgDDTC*	*PbDDTC*	*CoDDTC*	*MnDDTC*	*BiDDTC*
t_R (min)	1.85	2.25	2.50	3.05	3.45	4.10	6.95
V_R (ml)	2.45	3.00	3.35	4.10	4.62	5.49	9.31
k'	0.42	0.75	0.92	1.30	1.65	2.15	4.35
H (cm)	$7.0 \cdot 10^{-3}$	$5.2 \cdot 10^{-3}$	$6.1 \cdot 10^{-3}$	$3.5 \cdot 10^{-3}$	$5.1 \cdot 10^{-3}$	$4.1 \cdot 10^{-3}$	$6.8 \cdot 10^{-3}$
N	2100	3000	2500	520	3000	3750	2205
R_s (for successive peaks)	–	2.56	1.2	0.94	1.81	2.41	4.97
DL (ng)	25	5	45	200	5	10	50

* See Table I.

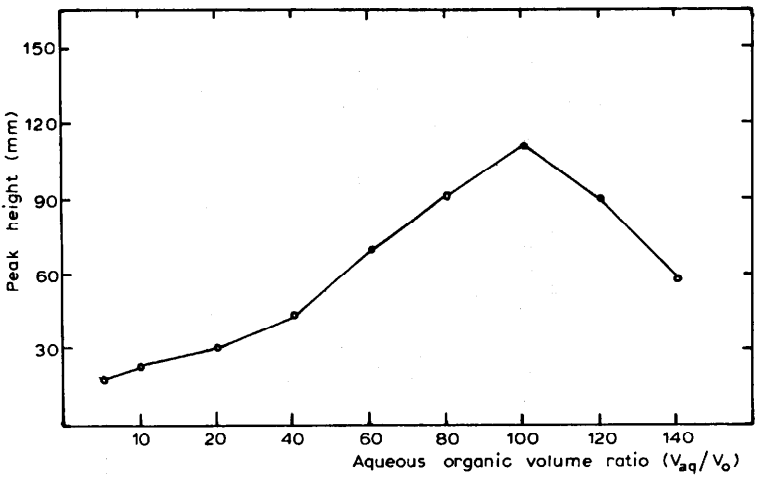


Fig. 3. Typical variation of peak height of a metal chelate with volume of buffered extraction medium.

The calibration graphs of peak height *versus* amount of metal injected are linear over the nanogram to microgram range. Calibration graphs were determined on the basis of 3–5 replicate determinations for 7–8 different concentration levels over the above range with a relative standard deviation of less than 5.0% for all the metals studied. Approximate detection limits for each metal using both ligands are given in Tables I and II. Samples for each concentration were carried through the entire extraction procedure. Replicate injections for each concentration were on a single organic extract. With the variable-wavelength detector set at 280 nm, the detection limits were in the range 5–200 ng (in the 5-μl aliquot injected), depending on the metal species. This is in the range 50–600 ppb in the original sample solution.

For the solvent extraction prior to HPLC injection of metal chelates, the effect of the volume of the buffered media (and hence the volume ratio of organic solvent to aqueous phase) was investigated. Fig. 3 shows the plot of peak height of a typical metal chelate [$Ni(HDZ)_2$] *versus* the volume of the buffered aqueous medium used in the extraction. A volume ratio of 100 (that is, an aqueous volume of 500 ml and an organic volume of 5 ml) gave optimum sensitivity of detection. However, many extractions have been made at aqueous to organic phase volume ratios of 60, 80 and 100 without a substantial decrease in sensitivity.

The optimum pH of extraction of the metal chelates that results in an optimum HPLC response was also investigated. The different metal chelates exhibit varying degrees of dependence of their extraction on the pH of the extraction medium. It was found that for the best multi-element extraction and HPLC determination, a pH of 8.5 gave the optimum response. Hence a buffer medium of pH 8.5 $\pm$ 0.1 was used for all extractions.

Applications

The method was applied to samples of importance in monitoring pollution in the aqueous environment.

Trade effluents. Several "consent-to-discharge" samples sent to one of the sewage treatment works laboratory in the London area were investigated. Twenty-

eight samples from 19 different manufacturing companies (non-ferrous effluents) were analysed. The HPLC method was compared with a recommended method based on atomic-absorption spectrometry (AAS) used in these laboratories for such analyses. The AAS method includes sample pre-treatment, which involves destroying complexing agents with concentrated nitric acid and heating, filtration and, with more complex samples, evaporating almost to dryness with addition of perchloric acid to destroy organics, before subjecting the samples to AAS.

Preliminary investigations showed that some of the samples contained some cyanide (non-ferrous effluents with cyanide). The metal–cyanide complexes were destroyed with an 8% (w/v) solution of formaldehyde[15]. Fig. 4 shows a typical chromatogram of one of the samples analysed. The formaldehyde treatment had no effect on the chromatograms of some of the samples (non-ferrous effluents with no cyanide). However, other samples showed a definite change before and after formaldehyde treatment. For example, with sample S-17 (Fig. 4), the peak height of the nickel peak increased from 120 to 170 mm for the untreated and treated samples, respectively. This gave concentrations of nickel of 2.02 μg/ml (untreated) and 2.28 μg/ml (treated), *i.e.*, an increase of about 13% relative to the untreated sample. This may be a route for determining (for the appropriate metals) the bound/unbound amounts of the metal in the samples (non-ferrous effluents with cyanide). The results for the samples analysed are summarized in Table III for the dithizone and DDDTC determinations, respectively. For the samples that showed a change in concentration of trace metal on formaldehyde treatment, only the value for the treated (higher) concentration is given in Table III. Peak identification here and in subsequent applications was carried out

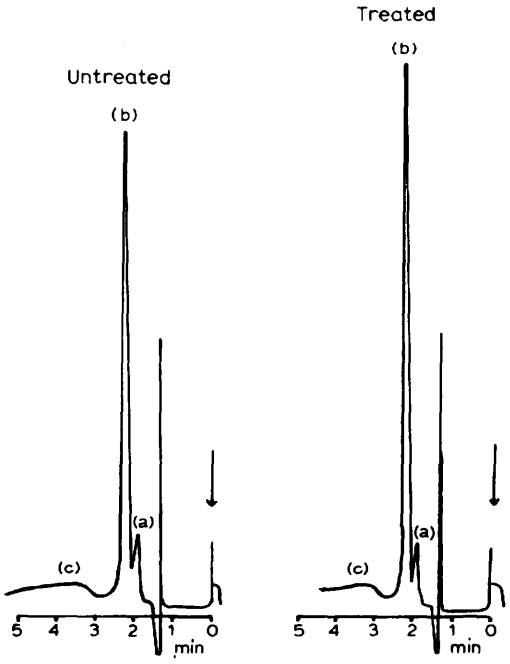


Fig. 4. Chromatogram of a polluted water sample for trade effluent disposal (extracted with dithizone solution). Peaks: dithizone complexes of (a) Cu, (b) Ni and (c) Pb.

TABLE III

DETERMINATION OF TRACE METALS IN POLLUTED WATER SAMPLES FOR TRADE EFFLUENTS DISPOSAL

BSTW + sample No.*	*Concentration of trace metals found (μg/ml)***								
	HPLC method with Dithizone extraction				*HPLC method with DDDTC extraction*				
	Hg	*Cu*	*Ni*	*Pb*	*Hg*	*Cu*	*Ni*	*Pb*	*Mn*
S-1***	2.40	4.20 (4.00)	0.16 (nd)	–	2.50	4.80 (4.00)	0.20 (nd)	2.78 (2.73)	18.40 (18.50)
S-2	–	1.05 (1.00)	–	–					
S-3***	–	0.27 (0.25)	0.40 (0.40)	0.62 (0.60)	–	0.30 (0.25)	0.42 (0.40)	0.60 (0.60)	2.65 (2.60)
S-4A	–	0.16 (0.17)	1.60 (1.50)	0.56 (0.60)	–	– (0.17)	1.60 (1.50)	0.60 (0.60)	5.80 (5.80)
S-6A***	–	1.56 (1.50)	1.55 (1.35)	0.29 (0.30)					
S-8	–	0.25 (0.23)	0.29 (0.30)	0.61 (0.61)					
S-10***	–	0.25 (0.20)	0.45 (0.40)	1.51 (1.55)					

* BSTW = Becton Sewage Treatment Works (Thames Water Authority, London, Great Britain).

** Values in parentheses obtained using a slower method, involving standard additions in the AAS method; nd = not determined.

*** Samples were found to contain cyanide.

by reference to the retention volumes (V_R) of standard metal chelate peaks under similar chromatographic conditions. This was confirmed by spiking the sample solution with a measured amount of a standard solution of the metal in question before the extraction step. Recovery of the metal chelates after the extraction step was investigated and found to be satisfactory. Typical recovery using nickel added to sample S-6A is as shown in Table IV.

TABLE IV

STANDARD ADDITIONS OF NICKEL TO SAMPLE S-A

Ni added (μg/ml)	*Ni found (μg/ml)*	*Recovery (%)*
5.00	4.97	99.3
4.20	4.21	100.3
3.65	3.47	95.0
3.25	3.13	98.2
2.89	2.86	98.8
2.78	2.79	100.1
2.75	2.61	95.0
2.12	2.14	101.1
	Mean:	98.2
	C.V.*:	2.5%

* Coefficient of variation.

Sequential injection, identification and quantification of samples and standards were carried out, and a comparison was made between samples and standards. Quantification was based on five replicate determinations, with a relative standard deviation of less than 5% for the whole determination, including the extraction and HPLC steps. Owing to the less extensive sample treatment involved, the HPLC method can be carried out in 20–30 min, whereas the AAS method takes over 90 min on average.

A preliminary investigation was undertaken, applying the method as a means of monitoring trace metals in the aquatic environment. Water samples were taken from the River Thames at sampling points stretching from Richmond, which is inland, to the Barrow deep, which is at the estuary of the river in the North Sea.

With very little sample preparation, except filtration where necessary, we are able to obtain trace metal profiles and determined the trace metals present. Fig. 5 shows the trace metal profiles of the various sampling points and Table V gives the values of the various trace metals found. Fig. 5 shows that the concentrations of the various trace metals vary considerably, depending on the sampling location. For example, the fate of the nickel and manganese peaks may be used as an indication of the human activities around that particular section of the river, for example sewage works and even drainage from precipitation. Owing to the simplicity of the method, with further development it may be useful as a means of cataloguing the trace metal profiles in various parts of a river, especially if it runs through industrial areas as the Thames does.

Serious consideration was given, especially with the aquatic samples, to poten-

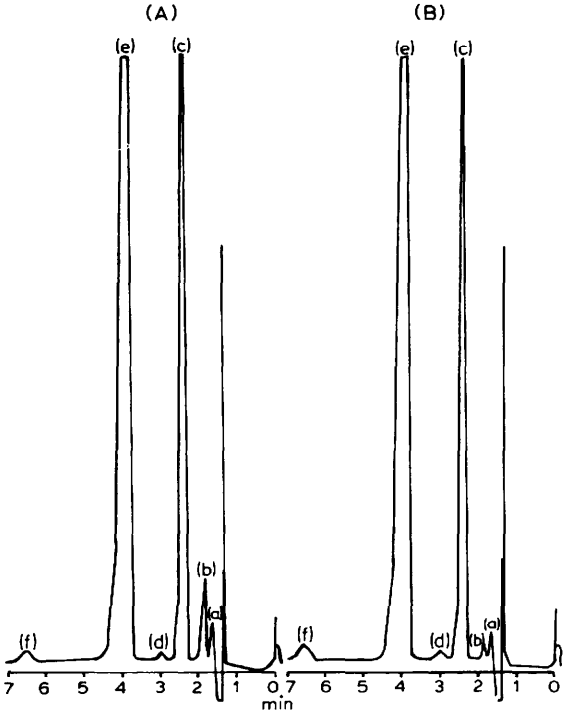


Fig. 5.

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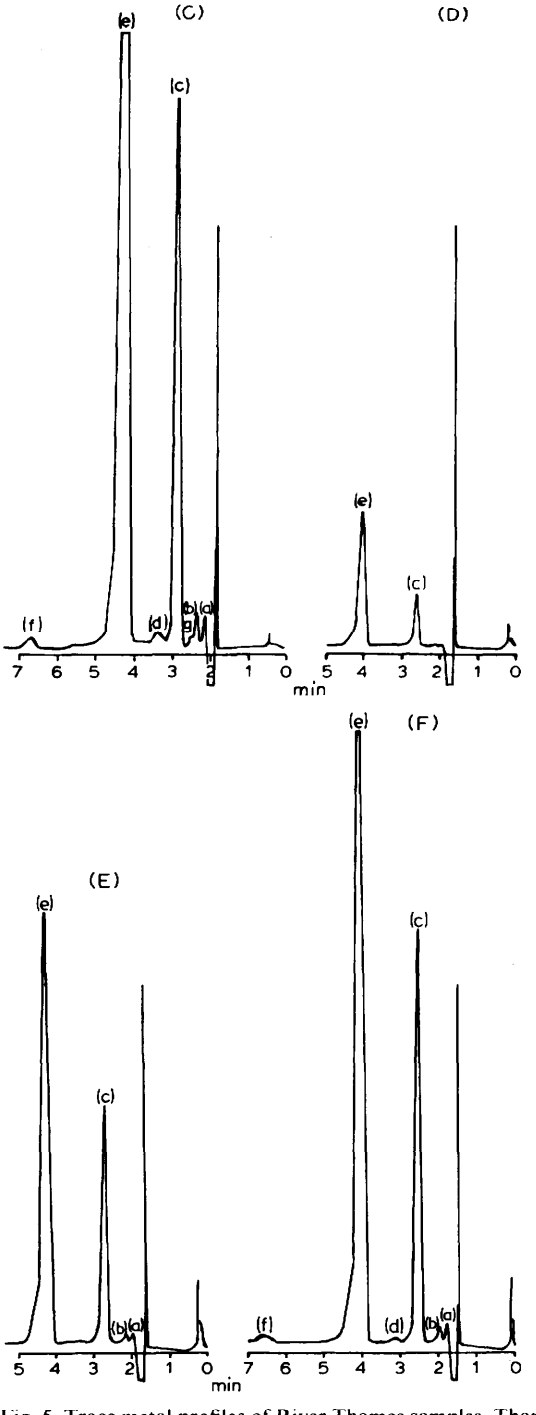

Fig. 5. Trace metal profiles of River Thames samples. Thames sampled at (A) Barrow deep, (B) Southend, (C) Gravesend, (D) Crossners, (E) London Bridge and (F) Richmond (*i.e.*, moving inland from A to E). Peaks: DDDTC complexes of (a) Cd, (b) Cu, (c) Ni, (d) Pb, (e) Mn, (f) Bi and (g) Hg.

TABLE V

ANALYSIS OF TRACE METALS IN RIVER THAMES WATER SAMPLES

Column, Hypersil (5 μm, 150 × 4.6 mm I.D.); eluent, Spectrograde benzene; flow-rate, 1.26 ml/min; pressure, 287 p.s.i. (19.5 bar); detector, UV, wavelength 280 nm at 0.5 a.u.f.s. Extraction was at a buffer pH of 8.5 with 0.5% (w/v) DDDTC in chloroform.

Sampling location	*Concentration of trace metal found (μg/ml)*				
	Cu	*Hg*	*Ni*	*Pb*	*Mn*
"Barrow 7"	0.20	–*	0.03	0.06	1.70
Southend	0.06	–	0.03	0.06	1.52
Gravesend	0.09	Trace	0.02	0.05	1.72
Crossness	0.03	–	0.04	–	0.20
London Bridge	0.05	–	0.03	0.04	0.49
Richmond	0.06	–	0.03	0.04	0.90

* Dashes indicate not detected.

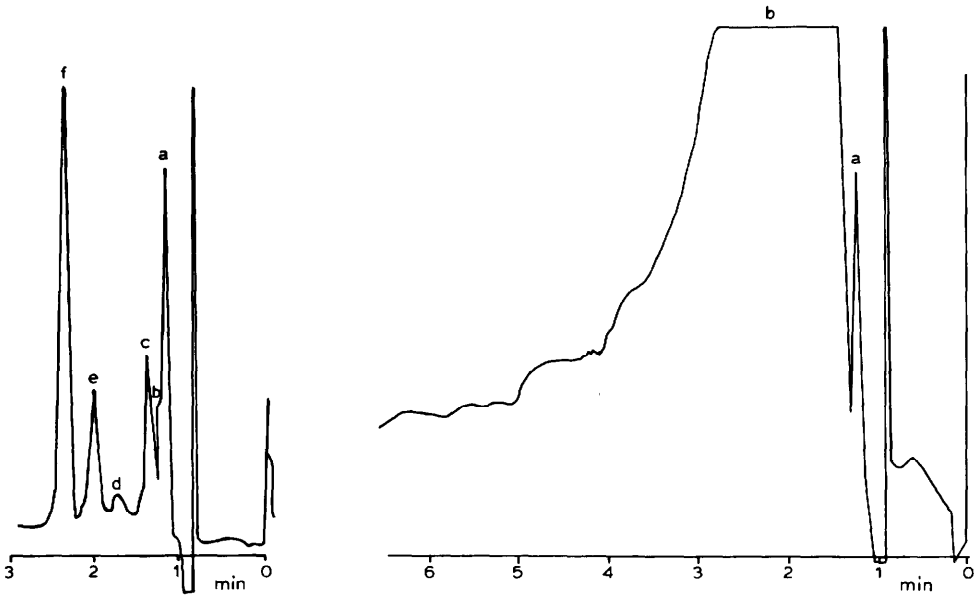

Fig. 6. Chromatogram of standard metal dithiocarbamates of known concentrations with 50 ppm each of lead and zinc added. Peaks: (a) CuDDTC, 0.2 ppm, V_R = 1.84 ml; (b) ZnDDTC, 50 ppm, V_R = 1.96 ml; (c) NiDDTC, 2.5 ppm, V_R = 2.16 ml; (d) PbDDTC, 50 ppm, V_R = 2.57 ml; (e) CoDDTC, 0.7 ppm, V_R = 3.00 ml; (f) MnDDTC, 11.0 ppm, V_R = 3.67 ml.

Fig. 7. Chromatogram of standard metal dithiocarbamates of known concentrations with 200 ppm each of lead and zinc added. Peaks: (a) CuDDTC; (b) ZnDDTC and PbDDTC interfere with all other peaks at higher concentrations.

TABLE VI

DETERMINATION OF TRACE METALS IN STANDARD KALE

Extractions were carried out at pH 8.5. The samples of the material were dried for 20 h at 90°C and subsequently cooled in a desiccator, for the determination of dry weight (the results are in μg/g dry weight = ppm).

Trace metal	*Concentration (ppm)*	
	*HPLC**	*Best value*** (including act, spe, vol, pol, X-ray, aas, col, fla, etc.)****
Cu	5.10, 5.00, 5.05	5.0
Ni	2.50, 2.55, 2.49	2.6
Pb	3.15, 3.15, 3.10	3.2
Mn	15.00, 15.05, 15.05	14.9
Bi	Trace	–

* Replicate determinations.

** "Best value" obtained from ref. 17.

*** Act = activation analysis; spe = spectrometry; vol = volumetric; pol = polarography; aas = atomic-absorption spectroscopy; fla = flame photometry; col = colorimetry.

tial interferences from other metals in the determination of the reported metals, as the pH of extraction was chosen to favour the maximum number of metals extracted in any particular sample. However, the separation of the metals is then left to the normal-phase HPLC column and the solvent system chosen. In preliminary work with the aquatic samples, no interferences with the various peaks were observed. This may be due to the levels at which the determinations were carried out.

When this question of interference was investigated further, it was observed that at higher concentrations of trace metals, some interferences occurred. Using DDDTC, iron interfered with the determination of manganese. This raises the possi-

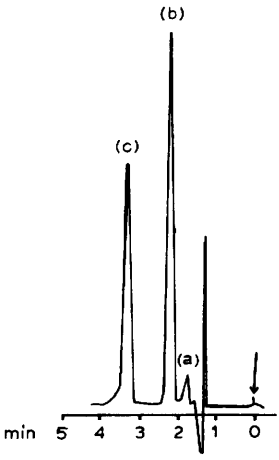

Fig. 8. Fish sample (wet digested) extracted at pH 8.5 with DDDTC solution in chloroform. Peaks: DDTC complexes of (a) Cu, (b) Ni and (c) Mn.

bility (as the iron peak is also sharp and symmetrical) of the approximate determination of both metals together (*i.e.*, total manganese and iron). In order to determine them separately the iron can be masked using 10% (w/v) of citric acid. It was also observed that at levels above 50 ppm each of lead and zinc, interference with the determination of other metals was observed (see Figs. 6 and 7). This may partly be due to the fact that at such levels, the lead or zinc dithiocarbamate peaks are broad and not symmetrical. However, it must be pointed out that with samples in which up to 50 ppm each of lead and zinc had been added to the sample before extraction and determination, no serious interference was observed and results obtained agreed with those obtained by AAS.

Memon[16] extended the use of the above method by using it for the determination of trace metals in soil samples. He compared his results obtained by HPLC with those obtained on the same samples using an inductively coupled plasma (ICP) and there was good agreement between the results of the two methods.

Standard kale and aquatic biota. As a means of monitoring biological enrichment of trace metals, the method was applied to standard kale (made up of dried leaves of *Brassica oleracea*) and also to biota from the aquatic environment. Both sets of samples were wet-digested with nitric acid–hydrogen peroxide (perhydrol) (1:1). Table VI gives the results for trace metals determined by the HPLC method; these are in reasonable agreement with those determined (best values) by other analytical methods reported in the literature.

For the aquatic biota, Fig. 8 shows a typical chromatogram obtained from a plaice sample. Table VII shows the values of the trace metals concentrated in the aquatic biota investigated. There is good agreement with the results obtained by AAS. These results demonstrate the biological accumulation in different species, as all biota was taken from the same area of the river around the same time of year (April 1980). The main values should help to establish datum lines for these species in a particular area of the marine environment.

TABLE VII

DETERMINATION OF TRACE METALS IN MARINE SAMPLES

Chromatographic conditions similar to those reported above. Extractions at pH 8.5.

Sample	*Metal*	*Concentration (ppm)*	
		AAS	*HPLC*
Plaice	Cu	0.38	0.41
	Ni	0.80	0.85
	Mn	26.10	26.30
Flounder	Cu	0.86	0.88
	Ni	0.95	0.93
	Mn	20.00	20.05
Shrimps	Cu	4.43	4.50
	Ni	1.35	1.30
	Mn	30.00	30.10

With the recent interest in the analysis of fish and other marine samples for trace metals (an FAO Workshop in May/June 1979 held in the Philippines was devoted to this problem), further development of this method as a multi-element trace analysis method may be useful.

In conclusion, as little or no sample pre-treatment is necessary, with the speed with which trace metal profiles can be obtained from both aqueous and solid samples and as the skills needed to apply the method on a routine basis (for a large sample throughout laboratory) are not too demanding, this method with further development should become useful as a cheap and rapid trace multi-element analytical method.

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