TECHNIQUES IN MARINE ENVIRONMENTAL SCIENCES

No. 1

Cadmium and lead: Determination in organic matrices with electrothermal furnace atomic absorption spectrophotometry

U. HARMS
Bundesforschungsanstalt für Fischerei
Labor für Radioökologie der Gewässer
Wüstland 2
D-2000 Hamburg 55
Bundesrepublik Deutschland

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

Palægade 2-4, DK-1261 Copenhagen K, Denmark

December 1987

ISSN 0903-2606

CADMIUM AND LEAD: DETERMINATION IN ORGANIC MATRICES WITH ELECTROTHERMAL FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY

1 OUTLINE OF METHOD

Experience has shown that atomic absorption analyses of lead and cadmium with pulsed-type graphite furnaces such as the Massmann design (Massmann, 1968) often are subject to various kinds of serious matrix interferences. Several authors, in particular L'vov (1976); Sturgeon and Chakrabarti (1977); Grégoire and Chakrabarti (1977); Van den Broek and de Galan (1977); Slavin and Manning (1979); Hageman et al. (1979); and L'vov and Ryabchuck (1982), have explained both by theoretical analyses and experimental measurements that many of the observed interference problems result in fact from the non-uniform temperature environment provided by the furnace.

A methodological approach apt to reduce deterioration of analyte response is the application of chemical separation techniques prior to the determination step. Extraction of metal ions in the form of a chelate by an organic solvent is one of the most suitable methods.

The solvent extraction procedure, described below, is based on the following principle: metal ions are coordinated with 1,5-diphenylthiocarbazone ("dithizone") to form metal chelates which are then transferred to an organic solvent (toluene). Several metals (Cu, Hg, Ag, Zn, Cd, Pb, and Bi) are generally extracted simultaneously (Iwantscheff, 1972). Back-extraction with diluted acid (hydrochloric acid) allows an improvement of the desired selectivity. Owing to a sufficiently large distribution coefficient (K_d value), smaller volumes of organic solvent than the original sample volume (sample solution) can be used. Thus, a considerable gain in concentration can be achieved in addition to the separation of the desired elements from the interfering sample matrix.

For levels of cadmium and lead often encountered in natural biological materials in the marine environment (i.e., for the levels at the ng/g level) contamination and losses represent significant sources of systematic errors (Zief and Mitchell, 1976; Tölg, 1972, 1974, 1979; Tschöpel et al., 1980; Stoeppler et al., 1979). Analyses at low concentrations within predictable error limits are only feasible if certain principles as postulated by Tölg (1979) are followed:

> "In the measurement of extreme trace quantities, the size and variability of the blank can be reduced by carrying out the essential operational steps in a reaction room in as rapid a sequence as possible and also with a minimum surface exposure to the reagents.
>
> "All vessels used must, as far as possible, be of materials (quartz, PTFE, graphite of high purity) that exclude any adsorptive or reactive effects (adsorption losses, contamination by desorption of impurities).

> "The optimum conditions for decomposition and further analytical steps are a favourable ratio of the sample amount to surface area and only a small excess of reagents which can easily be purified.
>
> "All possible contamination through the air in the laboratory should be minimized."

With special regard to these principles, a multi-stage analytical procedure has been worked out, which is illustrated in Figure 1.

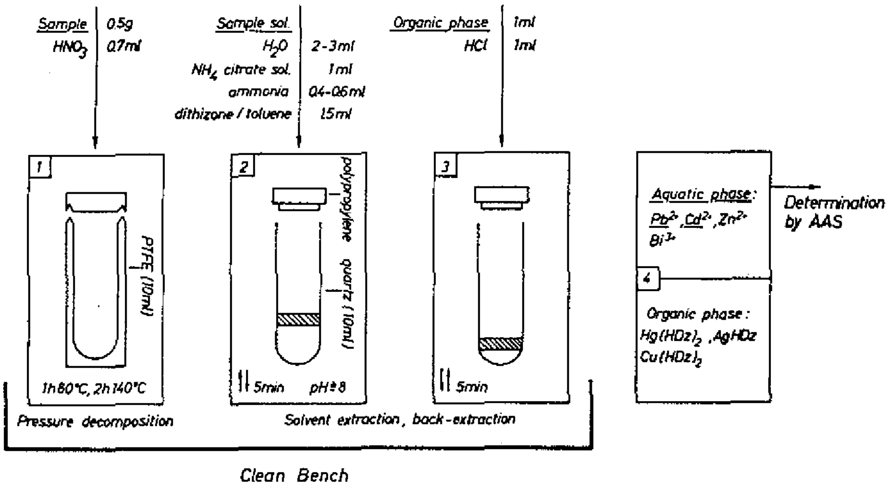

Figure 1.

2 RANGE

The method is apt to the determination of cadmium and lead in the trace range $< 10^{-8}$ to 10^{-6} moles/kg, i.e., at the ng/g level. The application of analytical data for a variety of purposes may require differing ranges of analytical certainty. Hence, the actual range for quantitative element determination depends on the obtained and tolerable error, respectively (compare also paragraph 3).

3 PRECISION

A control of the reproducibility of the method, based on replicate (n = 20) analyses of different fish liver samples, produced the following results (all data in ng/g).

Cadmium			Lead		
Element conc. determined	s.d.	r	Element conc. determined	s.d.	r
6	±1.2	3.5	32	±6	18
68	±5	15	180	±13	38
110	±7	21	380	±21	62

s.d. = standard deviation, r = s.d. x t(95) x $\sqrt{2}$.

4 INTERFERENCES

Nitrogene oxides produced in sample digestion form powerful oxidants which have deleterious effects on the chelating reagent (formation of non-complexing yellow to orange-red oxidation products). It is therefore necessary to use an adequate excess of dithizone in order to compensate reagent deficiency caused by oxidation.

There are considerable differences in the affinity between "dithizone" and various metal ions (Iwantscheff, 1972). As a consequence, kations forming strong dithizone chelate complexes tend to substitute (displace) kations from weak dithizone complexes.

However, kationic interference is probably not a major concern in a variety of natural biological materials in the marine environment. Most of these samples do not contain significant amounts of elements which inhibit the chelate complexing of the analytes we are concerned with.

Low extraction rates can be obtained when precipitates of alkaline earth phosphates act as trace collector (adsorption losses). Precipitation of phosphates is prevented by adding a masking reagent (citrate) to the sample solution.

5 EQUIPMENT

Clean bench which meets the requirements of US federal standard 209b, class 100.

Pressure decomposition equipment according to Kotz *et al*. (1972) with 10-ml PTFE (teflon) digestion vessels.[1]

Ten-ml quartz reaction vessels with polypropylene caps.[2]

Microlitre piston pipettes with disposable polypropylene pipette tips (colourless).[2]

---

[1] A variety of instruments specially designed for acid decomposition in closed PTFE vessels under pressure and high temperature (pressure decomposition) are describerd in the literature (e.g., Bernas, 1968; Holak *et al*., 1972; Kotz *et al*., 1972; Stoeppler and Backhaus, 1978).

The large range of systems available permits the handling of practically any sample quantity required for analysis in the laboratory. For the inexperienced investigator, however, it is important to note, that the properties of teflon can vary considerably. Contamination due to desorption of impurities from the inside vessel surface may particularly pose problems. Therefore, it must be strongly recommended that each PTFE digestion vessel which is used for trace cadmium and lead analysis be checked beforehand on its suitability for the purpose desired. In most instances, unused vessels can be properly cleaned through a repeated treatment with high-purity HNO_3 (10 mol/l) at 160^0C in the closed system.

[2] In order to minimize contamination, quartz reaction vessels and polypropylene pipette tips are to be washed repeatedly in HNO_3 (10 mol/l), further to be rinsed in double-distilled water, and to be dried at 60^0C. They should then be stored under dustproof conditions.

Atomic absorption spectrophotometer equipped with background corrector and a heated graphite furnace for electrothermal atomization.

6 REAGENTS

Nitric acid, 10 mol/l, high-purity grade,[3] diluted nitric acid, 1.0 mol/l.

Hydrochloric acid, 10 mol/l, high-purity grade,[3] diluted hydrochloric acid, 5 mol/l and 0.5 mol/l.

Ammonia, 13 mol/l, high-purity grade,[3] diluted ammonia, 2.6 mol/l.

Double-distilled water.

1,5-diphenylthiocarbazone ("dithizone"), $C_{13}H_{12}N_4S$, 256.33 g/mole, dithizone/toluene, 3×10^{-3} mol/l.[4]

di-Ammonium hydrogen citrate, $C_6H_{14}N_2O_7$, 226.19 g/mole, di-ammonium hydrogen citrate/diluted ammonia, 2 mol/l.[5]

7 SAMPLE PREPARATION, DISSECTION

When handling tissue samples directly (e.g., fish muscle or selected organs), the main source of error is contamination from air particles and metallic tools. Therefore, clean working conditions (clean bench) must be maintained during dissection and sample preparation. Properly cleaned dissecting instruments (glass- or quartz-knives) have to be used.

A precise description of suitable subsampling and handling procedures in trace analysis and of appropriate tools to be used is given in

---

[3] High-purity acids and ammonia are commercially available. However, in the case of storage over a long time, as a rule these products do not meet the standards which are required for extreme trace analysis because desorption of impurities will proceed from the container surface without control. The only way to be sure of the purity of reagents is to check them regularly or to clean them, possibly by subboiling distillation as described by Kuehner *et al*. (1972).

[4] The dithizone/toluene solution is purified immediately before use by replicate extraction with diluted (5 mol/l) hydrochloric acid. Dithizone disintegrates under the influence of light, forming yellow to orange-red oxidation products. Therefore, the solution should be handled in dimmed rooms and stored under light-tight conditions.

[5] Purification of citrate/ammonia solution is achieved by replicate extraction with dithizone/toluene.

Anon., 1979. Reference is also given to the previously mentioned treatise by Zief and Mitchell (1976). Dissection of tissues and weighing of subsamples should be performed as soon as possible after sampling to minimize error sources due to loss of moisture. If samples are frozen for transportation or storage, a loss of moisture can be avoided if the material to be analysed is semi-thawed before dissecting the tissue and weighing the subsamples. In the case of fish tissue analysis, additional precaution is necessary to minimize the transfer of heavy metals in the mucosal slime of the epidermis to the interior tissues during dissection (Chow et al., 1974).

8 CALIBRATION (standards for determining the instrumental response as function of the given analyte concentration)

Standard stock solutions of 1 g cadmium and lead per litre are prepared using diluted (1.0 mol/l) nitric acid. From the stock solutions, working standard solutions for calibration purposes - usually containing 10 to 100 ng Pb/ml and 1 to 10 ng Cd/ml - are prepared with diluted hydrochloric acid (0.5 mol/l) as solvent immediately before use.

9 REAGENT BLANK

The size and variability of the blank ($\bar{x}_{bl}+3\ s_{bl}$) introduced by reagents, laboratory air, and by desorption from vessel material amounted to 1 ng Cd/g and 5 ng Pb/g.

10/11 PRETREATMENT OF SAMPLE AND ANALYSIS OF SAMPLE

A suitable sample amount (0.5 g wet weight) is transferred into a teflon digestion vessel and the necessary quantity (0.7 ml) of high-purity nitric acid is carefully added. The vessel is placed into the stainless steel jacket, which is sealed with a screw cap.

The unit is slowly (ramptime 60 min) heated up to about 80^0C, controlled by a thermocouple, then heated with rapidly rising temperature up to 140^0C (ramptime 45 min). The system is held at this temperature for another 120 min. The screw cap is untightened when the unit has been cooled down to room temperature. **Caution! Fumes of nitrogen oxides will be expelled. Therefore, the use of a fume cupboard is absolutely necessary.**

The sample solution is transferred together with double-distilled water (2-3 ml) into a 10-ml quartz reaction vessel. After adding citrate/ammonia solution (1 ml),[6] the pH of the mixture is adjusted to

---

[6] Citrate/ammonia acts as a buffer and simultaneously prevents precipitation of phosphates. The actual amount of citrate/ammonia solution required should be tested in separate experiments. In most instances, 2 ml citrate/ammonia solution per gram of sample (wet weight) is sufficient.

about 8.5 by addition of ammonia (0.4-0.6 ml).[7] Solvent extraction is then performed with dithizone/toluene solution (1.5 ml).[8]

After vigorous shaking for 5 minutes, the phases are allowed to separate and a specific quantity (1 ml) of the supernatant organic phase is introduced into another quartz reaction vessel by means of a microlitre piston pipette.

Back-extraction follows by shaking for another 5 minutes with diluted (0.5 mol/l) hydrochloric acid (1 ml). The hydrochloric acid phase is submitted to quantitative determination of cadmium and lead by electrothermal atomic absorption spectrometry.

12 CALCULATION OF RESULTS

The cadmium or lead concentration C (ng/g) in the organic sample is calculated by the following equation:

$$C = \frac{(V_{01}/V_{02}) \times V_a \times c}{W} \text{ (ng/g)}.$$

V_{01} = volume of organic phase (ml) used for solvent extraction of the sample solution,

V_{02} = aliquot af V_{01} (ml) used for back-extraction,

V_a = volume of acid stripping solution (ml) used for back-extraction,

c = cadmium or lead concentration (ng/ml) measured in the acid stripping solution,

W = sample amount (g) used for pressure decomposition.

---

[7] The amount of ammonia required for neutralization and pH adjustment is dependent on the actual excess of acid after pressure decomposition. Small pH variations in the range between 8 and 9 do not significantly influence the extraction yield. If the pH exceeds the value of 9.5, the conditions of quantitative extraction cannot be fulfilled because dithizone, on account of its dissociation, passes to a large extent into the aqueous phase.

[8] An excess of chelating reagent is guaranteed if the organic phase has an intense green colour. A change in colour (brown or orange-red) indicates reagent deficiency. Solvent extraction with an increased amount of dithizone/toluene is required. Possibly several extracts can be combined.

13 REFERENCES

Anon. 1979. Report of the ICES Advisory Committee on Marine Pollution, 1978. Annex II, Appendix I. Coop. Res. Rep. Cons. int. Explor. Mer, No. 84.

Bernas, B. 1968. A new method for decomposition and comprehensive analysis of silicates by atomic absorption spectrometry. Anal. Chem., 40:1682-1686.

Chow, T.J., Patterson, C.C., and Settle, D. 1974. Occurrence of lead in tuna. Nature, 251:159-161.

Grégoire, D.C., and Chakrabarti, C.L. 1977. Atomization from a platform in graphite furnace atomic absorption spectrometry. Anal. Chem., 49:2018-2023.

Hageman, L.R., Nichols, J.A., Viswanadham, P., and Woodriff, R. 1979. Comparative interference study for atomic absorption lead determinations using a constant temperature vs. a pulsed-type atomizer. Anal. Chem., 51:1406-1412.

Holak, W., Krinitz, B., and Williams, J.C. 1972. Simple, rapid digestion technique for the determination of mercury in fish by flameless atomic absorption. J. Ass. Off. Anal. Chem., 55:741-742.

Iwantscheff, G. 1972. Das Dithizon und seine Anwendung in der Mikro- und Spurenanalyse, (second edition). Verlag Chemie, Weinheim, Federal Republic of Germany.

Kotz, L., Kaiser, G., Tschöpel, P., and Tölg, G. 1972. Aufschluss biologischer Matrices für die Bestimmung sehr niedriger Spurenelementgehalte bei begrenzter Einwaage mit Salpetersäure unter Druck in einem Teflongefäss. Fresenius Z. Anal. Chem., 260:207-209.

Kuehner, E.C., Alvarez, R., Paulsen, P.J., and Murphy, T.J. 1972. Production and analysis of special high-purity acids purified by sub-boiling distillation. Anal. Chem., 44:2050-2056.

L'vov, B. 1976. Electrothermal atomization - The way towards absolute methods of atomic absorption analysis. Invited paper presented at the 3rd FACSS Meeting and the 6th International Conference on Atomic Spectroscopy, Philadelphia, Pa., 15-19 november.

L'vov, B., and Ryabchuk, G.N. 1982. A new approach to the problem of atomization in electrothermal atomic absorption spectrometry. Spectrochim. Acta, 37B:673-684.

Massmann, H. 1968. Vergleich von Atomabsorption und Atomflurescenz in der Graphitküvette. Spectrochim. Acta, 23B:215-226.

Slavin, W., and Manning, D.C. 1979. Reduction of matrix interferences for lead determination with the L'vov platform and the graphite furnace. Anal. Chem., 51:261-265.

Stoeppler, M., and Backhaus, F. 1978. Pretreatment studies with biological and environmental materials. I. Systems for pressurized multisample decomposition. Fresenius Z. Anal. Chem., 291:116-120.

Stoeppler, M., Valenta, P., and Nürnberg, H.W. 1979. Application of independent methods and standard materials: An effective approach to reliable trace and ultratrace analysis of metals and metalloids in environmental and biological matrices. Fresenius Z. Anal. Chem., 297:22-34.

Sturgeon, R.E., and Chakrabarti, C.L. 1977. Mechanism of atom loss in graphite furnace atomic absorption spectrometry. Anal. Chem., 49:1100-1106.

Tölg, G. 1972. Extreme trace analysis of the elements I. Methods and problems of sample treatment, separation and enrichment. Talanta, 19:1489-1521.

Tölg, G. 1974. Recent problems and limitations in the analytical characterization of the high-purity material. Talanta, 21:327-345.

Tölg, G. 1979. Neue Wege zur analytischen Charakterisierung von Reinstoffen. Fresenius Z. Anal. Chem., 294:1-15

Tschöpel, P., Kotz, L., Schulz, W., Veber, M., and Tölg, G. 1980. Zur Ursache und Vermeidung systematischer Fehler bei Elementbestimmungen in wässrigen Lösungen im ng/ml- und pg/ml-Bereich. Fresenius Z. Anal. Chem., 203:1-14.

Van den Broek, W.G.T., and Galan, L. de. 1977. Supply and removal of sample vapor in graphite thermal atomizers. Anal. Chem., 49:2176-2186.

Zief, M., and Mitchell, J.W. 1976. Contamination control in trace analysis. Vol. 47. John Wiley, New York.