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Short communication

Separation and identification of metals in human bones, placenta and milk and in air by adsorption and ion-exchange thin-layer chromatography

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Abstract

A TLC method for the separation of heavy metals and their complexes with dithizone, 4-(2-pyridylazo)resorcinol and ethylenediaminetetraacetic acid was devised and conditions for the solid-phase extraction of heavy metal ions in human bones, placenta and milk after microwave mineralization and in air after alkaline absorption were elaborated.

Keywords: Milk; Complexation; Placenta; Bones; Environmental analysis; Air analysis; Metals

1. Introduction

In Poland there is currently great interest in investigations focused on the toxic influence of heavy metals on the human organism in ecologically endangered regions. The Silesian Technical University is located in Upper Silesia, the most polluted region of Poland, and carries out the major part of research work concerning the determination of heavy metal concentrations in various tissues and biological samples, mainly for medical and ecological purposes. Many time-consuming and expensive procedures have been used for these analyses, e.g., atomic absorption spectrometry, polarography, stripping voltammetry, inductively coupled plasma emission spectrometry and x-ray fluorescence spectrometry, but the frequent lack of significant concentrations of heavy metals in many of the biological samples studied causes problems.

In this paper we propose a method for concentrating samples of bones, placenta and milk after microwave mineralization and samples of air by solid-phase extraction, followed by TLC analysis. We applied the proposed TLC method with cellulose, Florisil and ion exchangers in analyses for metal ion and metal dithizonates and complexes with 4-(2-pyridylazo)resorcinol (PAR) and ethylenediaminetetraacetic acid (EDTA). The results obtained can be used as an indication of whether the biological material should be submitted for further investigation and whether the expected results are cost effective.

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2. Experimental

2.1. Microwave digestion

An MLS 1200 MEGA microwave digestion system (Millestone, Italy) was used.

2.2. Thin-layer chromatography

TLC was performed on 20 × 20 cm glass plates precoated with 0.25 mm layers of cellulose, Florisil, Polygram Ionex-25 SA-Na or Polygram Ionex-25 SB-Ac (Merck, Darmstadt, Germany).

Standard solutions (1 mg/cm^3) of Zn, Mn, Cd, Pb, Cu, Ag, Hg, Co, Ni, Fe, Cr, Sn and Bi (Titrisol grade from Merck) were prepared by dissolution in acidified water and 0.005 cm^3 aliquots were spotted on the plates. Also 0.005 cm^3 volumes of solutions of complexes were placed on the plates. All plates were developed to a height of 15 cm in saturated tanks (Shandon).

The metal dithizonates and complexes with PAR and EDTA were coloured, and therefore no staining was needed. Metal ions were revealed by treatment with a 0.25% solution of PAR in methanol.

2.3. Solid-phase extraction

Preparation of metal complexes

To the solutions containing metal ions and adjusted to a suitable pH value, 0.1 cm^3 of PAR (0.5 g in 200 cm^3 of methanol) (pH 10), dithizone (1 mg in 100 cm^3 of CCl_4) (pH 2) or EDTA (2.5 g in 100 cm^3 of water) (pH 4–5) was added. Complexes were prepared according to Marczenko's method [1].

Preconcentration of complexes on the column

Columns with an octyl (C_8) packing were activated with methanol before preconcentration and subsequently washed with water. Then 50 cm^3 of solution containing 5 μg of metals were introduced into the column. Solutions were passed through the columns under a reduced pressure of 85–90 hPa. The complexes collected on the column were eluted with 5 cm^3 of methanol. Solutions of the complexes were spotted on the plates.

Preconcentration of metal ions on the column (without prior complexing)

Columns with CN or NH_2 packings were activated with 10 cm^3 of 1 *M* HNO_3 before preconcentration and then washed with water. Volumes of 50 cm^3 of solution containing 5 or 10 μg of metals were introduced into the column. Solutions were passed through the columns under a reduced pressure of ca. 90 hPa. The metals collected on the column were eluted with 5 cm^3 of 0.1 *M* HNO_3, then the complexes were formed and spotted on the plates.

Preconcentration of metal ions from biological samples (C_8 column)

A 0.5-g amount of human bones or placenta or 1 cm^3 of human milk were mineralized in 5 cm^3 of concentrated HNO_3 by microwave digestion. The acidic solutions were transferred into 10 cm^3 standard flasks and diluted to volume with water. In the samples, complexes with PAR were formed, and the samples were treated according to the procedure given above.

Preconcentration of metal ions from samples of air (CN and NH_2 columns)

The air was passed through 5 cm^3 of 0.8% Na_2CO_3 solution. The content of heavy metal was measured simultaneously with the analysis of phenols in alkaline medium. Solution was acidifed to pH ≈ 5 and preconcentrated as described above.

3. Results and discussion

Chromatographic systems suitable for the separation of heavy metals or their complexes were developed (Tables 1 and 2). The chromatographic systems described are suitable for the analysis of various ions. The best R_F values for various mixtures of metals are distinguished in italic type. Co and Ni, which are usually present together, can be separated in the form of complexes with large differences in R_F values.

Table 1
R_F values for metal ions (standard solution)

Metal ion	Cellulose			Ionex-25 SA-Na				Ionex-25 SB-Ac	
	a	b	c	d	e	f	g	g′	h
Zn	*0.88*	0.86	*0.41*	*0.90*	*0.49*	*0.67*	*0.73*	*0.68*	*0.60*
Mn	*0.76*	*0.79*	0.30	*0.46*	*0.37*	0.63	*0.68*	*0.92*	*0.78*
Cd	0.85	0.85	*0.63*	0.62		*0.90*	*0.80*		
Pb	*0.62*	*0.55*	*0.27*			*0.76*	0.75	*0.56*	0.50
Cu	*0.93*	*0.87*	0.10	*0.77*	*0.43*	*0.65*	0.75	0.89	*0.50*
Ag			0.63				0.73		
Hg			0.40				0.56	*0.11*	0.00
Co	0.83	0.85	0.56	0.55	0.34	0.62	0.73	0.92	*0.85*
Ni	0.85	0.83	*0.54*	*0.54*	0.37	0.61	0.74	0.89	0.83
Fe	*0.80*	*0.71*	0.25	0.41	*0.34*	*0.50*	*0.57*	*0.86*	*0.39*
Cr			0.36		*0.14*		*0.27*	*0.00*	*0.00*
Sn	*0.55*	0.60	0.64		*0.72*	*0.97*	0.79	0.04	0.08
Bi	0.50	*0.46*	*0.00*		*0.60*	0.90	0.76	0.09	0.07

Solvent systems: a = 2 *M* HNO_3–methanol(1:1); b = 2 *M* HNO_3–methanol (1:2); c = 0.1 *M* HNO_3–*n*-propanol (1:2); d = 6 *M* HCl–methanol (1:1); e = 3 *M* HCl; f = 2 *M* HNO_3, then 1 *M* HCl, then 2 *M* HNO_3; g = 2 *M* HNO_3 (developed twice); g′ = 2 *M* HNO_3; h = 0.1 *M* HNO_3.

Table 2
R_F values for metal complexes (standard solution)

Complexes with	Dithizone				PAR				EDTA	
	Cellulose	Florisil			Cellulose			Ionex-25 SA-Na	Cellulose	
	a	b	c	d	e	f	g	h	i	e
Zn	0.76	0.72	0.49	0.79	*0.36*	0.16	0.00	*0.56*		
Mn	0.78	0.02	0.46	0.73	*0.76*	0.00	0.89	0.72		
Cd	0.77	0.00	0.47	*0.79*	0.73	*0.76*	*0.76*	*0.44*		
Pb	0.79	*0.00*	*0.10*	*0.13*	0.76	0.87	0.87	0.70		
Cu	*0.83*	0.00	*0.00*	*0.00*	*0.53*	*0.60*	*0.60*	*0.00*	0.48	0.57
Ag	*0.00*	0.00	0.00	0.00						
Hg	0.06	*0.74*	*0.48*	*0.78*						
Sn	0.49	*0.13*	0.17	0.45						
Co	*0.45*	*0.30*	*0.19*	*0.48*	0.79	*0.69*	*0.69*	*0.62*	*0.09*	*0.12*
Ni	*0.00*	*0.43*	*0.25*	*0.58*	0.81	*0.89*	*0.89*	*0.73*	*0.51*	*0.64*
Bi	*0.39*	*0.36*	0.20	0.46						
Cr					0.79	*0.82*	*0.00*	0.66	0.14	0.16

Solvent systems: a = acetone–water (2:1); b = toluene–chloroform (50:1) (developed twice); c = toluene–chloroform (50:5); d = toluene–chloroform (50:5) (developed twice); e = methanol; f = acetone; g = methanol–acetone (2:1); h = methanol–acetone–0.1 *M* HNO_3 (35:5:1); i = acetone, then methanol.

The possibility of preconcentrating metal ions and their complexes using a solid-phase extraction method was examined. It was found that when forming metal chelates preconcentration should be carried out on a C_8 column. For metal ions, CN and NH_2 columns are recommended. In both cases 95% recoveries of metals and complexes were obtained. When the metal content in the sample is 5 μg or less, the complexes or metals should be preconcentrated. Another approach is to preconcentrate metal ions, which are subsequently transformed into complexes and separated on the plates in these forms.

If the concentration of metals in the sample is high, they can be separated using TLC, and the solution of PAR is the most appropriate for detection.

The proposed chromatographic systems were used to analyse human bones, placenta and milk that had first undergone microwave digestion. Then the complexes with PAR were formed and preconcentration on the C_8 column was performed using the solid-phase extraction method. It was found that the major part of the samples of biological materials taken from the Upper Silesian industrial area contained toxic elements (Pb, Cd, Cr). In the analysis of human tissues, very good separations of metals were obtained using cellulose [acetone, methanol–acetone (2:1)] or Ionex-25 SA-Na [methanol–acetone–0.1 *M* HNO_3 (35:5:1)]. Metals that have physiological activity (Cu, Fe, Zn) also separated. Some air samples also contained heavy metals. The air samples were analysed using the same chromatographic system as above and in addition complexes with dithizone on Florisil [toluene–chloroform (50:5), developed twice] were analysed.

Amounts of metals have been determined by atomic absorption spectrometry only in the samples that contained heavy metals [2–5].

In conclusion, the proposed chromatographic system can be used for the qualitative analysis of samples when the influence of environmental pollutants is to be studied, and for various multicomponent ion mixtures. Preconcentration using solid-phase extraction is useful both to prepare samples for chromatographic analysis and for analysis using other methods, e.g., atomic absorption spectrometry, voltamperometry or spectrophotometry. After heavy metals have been eluted from the column, the collected solutions are without a matrix, which usually causes problems in the analysis.

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