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Observation
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DETERMINATION OF HEAVY METALS IN BIOLOGICAL MATERIAL WITH CHELEX 100 RESIN

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Own results of the determination of several metals in urine are presented. The samples were pretreated using the wet digestion procedure and concentrated by passing through a column filled with the chelating resin Chelex 100. After elution with diluted nitric acid, the same sample was used for the determination of lead, cadmium, copper, zinc and nickel. The metals eluted by this procedure can be determined with any instrumental technique available. For this study the method of atomic absorption spectrophotometry (AAS) was chosen. The best results were obtained at pH 6.0. Under the above mentioned conditions full chelation and elution from the column were achieved for all the metals determined. Being highly reproducible the method is applicable in everyday practice. Heavy metal content in biological material (urine, blood, hair, tissues) is an important parameter of human exposure in the working and living environment. Usually, it is very low, except in accidental or long-lasting exposures when even untoward clinical manifestations may become apparent.

From the analytical point of view the determination of low concentrations of heavy metals presents no problem. By means of modern analytical techniques, like AAS, anoding stripping voltammetry, pulse polarography, atomic fluorescence spectroscopy and others determinations are possible in the range from 10^{-10} to 10^{-12} g. However, in order to achieve reproducible and accurate results, it is necessary to use large sample volumes. This can lead to a great number of physical and spectral interferences because of the nature of biological material (salt redundant matrix).

To avoid possible problems we used a large sample volume (50 ml), and the chelating resin Chelex 100.* In the past this resin was used to concentrate

* Chelex 100 — chelating resin is a styrene divinyl benzene copolymer containing imminodiacetate functional groups. It is considered to be a weakly acidic resin. Bond strength is of the order of 15—25 kilocal/mol. It can operate in basic, neutral and weak acid solutions of pH 4 or higher. At a very low pH it acts as an anion exchanger.

trace elements from water (1, 2) and lead from biological samples (3). Final quantitative determination of heavy metals (Pb, Cd, Cu, Zn and Ni) can be done by any available instrumental technique. In this study the flame AAS was chosen.

METHOD AND MATERIAL

Native samples of urine were rather unhomogenous, because of the sediment and redundance of salts and albumin. Passing them through the column packed with Chelex 100 was therefore impossible. Chelex 100 resin was digested 3 times at room temperature with excess 5 N nitric acid and washed with redistilled water. A 1.5-cm diameter exchange column was filled with it to a depth of 6 cm. Passing through the column was achieved by diluting the sample, but the recovery of metals was not quantitative, probably because the metal — organic complex bond was much stronger than the metal chelating complex bond.

The samples were pretreated with the wet digestion procedure using strong oxidative agents (concentrated nitric acid and 30% hydrogen peroxide). The digestion was performed in a 100 ml conical flask on a hot plate at low temperature. To determine optimum conditions and to obtain the most accurate results the known aliquots of standard solutions were added to the digested sample and passed through the column by changing the pH values. The optimum results were obtained at pH 6.0, and all further determinations were done at this pH value, with a flow rate not exceeding 5 ml/min.

Aliquots of 2, 4, 6, 10, 20, 30, 40 and 50 μg lead, cadmium, copper, zinc and nickel were added to 50 ml urine samples. The digested samples were passed through the column packed with the chelating resin, eluted with two portions of 20 ml 5 N nitric acid and water, and the metals were determined from the same solution by AAS (Varian AA — 5, in acetylene-air flame).

To test the reproducibility of the method, ten samples of 50 ml aliquots of the same normal urine were treated as described above.

RESULTS AND DISCUSSION

The method described in literature involves extraction of a single metal (lead) complex into an organic solvent (4). Using Chelex 100 resin, extraction and concentration of five metals were achieved. At the same time a considerable amount of sodium and potassium, which interfere because of their light scattering effect, was also removed. Table 1 shows the results of our investigation.

It can be concluded that in our experimental conditions of chelation and elution, the recovery of heavy metals was quantitative (mean: Pb 100.2%, Cd 98.5%, Cu 103.9%, Zn 95.6% and Ni 100.7%). Column efficiency in a range of low concentrations was very high, except for zinc. This was probably because of high zinc concentrations in the matrix of biological material.

The reproducibility of metal determinations is given in Table 2.

Table 1.

Recovery of lead, cadmium, copper, zinc and nickel added to normal urine

Metals	Pb		Cd		Cu		Zn		Ni	
Added in μg	Found	Recovery %	Found	Recovery %	Found	Recovery %	Found	Recovery %	Found	Recovery %
—	0.74	—	0.06	—	0.97	—	18.55	—	0.28	—
2.00	2.74	100.0	2.06	100.0	2.96	99.5	20.05	75.0	2.28	100.0
4.00	4.74	100.0	4.06	100.0	5.36	109.7	21.55	75.0	4.68	110.0
6.00	6.74	100.0	6.06	100.0	7.39	107.0	24.55	100.0	6.28	100.0
10.00	11.50	107.6	10.00	99.4	12.00	110.3	29.58	110.3	10.00	97.2
20.00	21.50	103.7	19.70	99.7	22.00	105.1	39.58	105.1	21.00	103.6
30.00	30.00	97.5	28.50	94.8	30.50	98.4	48.08	98.4	31.00	102.4
40.00	40.00	98.1	38.00	94.8	40.50	98.8	58.08	98.8	41.00	101.8
50.00	48,24	95.0	49.56	99.0	52.13	102.3	69.71	102.3	45.60	90.6

Table 2.

Reproducibility of lead, cadmium, copper, zinc and nickel determination
(N = 10)

Metals	Added μg	Recovery μg $\overline{X} \pm SD$	CV %
Pb	50.00	47.50 ± 1.08	2.3
Cd	50.00	49.50 ± 0.85	1.7
Cu	50.00	51.16 ± 0.53	1.0
Zn	50.00	51.27 ± 2.67	5.2
Ni	50.00	44.00 ± 6.66	15.1

The described laboratory procedure for urine, with complete digestion of organic matter is likely to yield equally reliable results with different biological samples. This was partly confirmed in our work, but the matter needs to be further examined.

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Sažetak

ODREĐIVANJE METALA U BIOLOŠKOM MATERIJALU POMOĆU SMOLE CHELEX — 100

U ovom radu prikazani su rezultati dobijeni određivanjem metala u urinu. Uzorci su pretpripremljeni postupkom mokre digestije a zatim koncentrisani propuštanjem kroz kolonu ispunjenu jonoizmenjivačkom smolom. Nakon eluiranja razblaženom azotnom kiselinom iz istog rastvora izvršeno je određivanje Pb, Cd, Cu, Zn i Ni. Metali eluirani na ovaj način mogu se odrediti bilo kojom raspoloživom instrumentalnom tehnikom. U ovom radu opredelili smo se za plamenu atomsko asporpcionu spektrometriju. Najbolji rezultati određivanja Pb, Cd, Cu, Zn i Ni u mineralizovanom uzorku urina dobijeni su pri pH vrednosti 6,0. Pod ovim uslovima postignuta je kvantitativna helatizacija i eluiranje sa kolone za sve navedene metale. S obzirom na dobru reproduktivnost metode (koeficijent varijacije 15,1%) ista se može preporučiti za rutinsko određivanje metala u biološkom materijalu.

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