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CHEMOMETRIC EXAMINATION OF TRACE ELEMENTS ANALYSIS IN URINARY CALCULI

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Abstract: The content of Pb, Cd, Ni, Fe, Na and K was determined in sixteen samples of urinary calculi taken from patients in Macedonia. The trace elements were determined by atomic absorption spectrophotometry and by flame emission spectrometry. The chemometric examination of the calculi was done by factor analysis. This chemometric method has been extensively used for classification purposes while solving different multidimensional problems. The results obtained from the analysis revealed that the highest correlation exists between the concentrations of lead and potassium. No significant correlation among other analyzed elements was found. The examination of the first two principal components (with 67 % variance captured) calculated from the autoscaled data matrix showed a clear separation between the composition of the calculi taken from male and from female patients, especially in the case of the calculi consisting of the two oxalates (whewellite and weddelite) and those composed of carbonate apatite in mixture with oxalates.

Key words: trace elements; urinary calculi, AAS, chemometry

1. INTRODUCTION

The urinary calculi have been extensively studied in our laboratory [1–6]. In the late sixties, the first statistical examination of the composition of urinary calculi in Macedonia was done using infrared spectroscopy as a tool for determining the composition of the calculi [1, 2, 7]. More recently, as a part of the studies of human concrements in our laboratory [3–6, 8], it was possible to apply some more advanced chemometric techniques for determining the composition of the calculi [4–6] so that after some thirty years a new statistical examination of the qualitative composition of concrements taken from the patients from Macedonia [3] has been made.

In this work, we present the results of the chemometric treatment of data of trace elements analysis of sixteen urinary calculi by atomic absorption spectroscopy. Although there are literature data on the determination of trace elements in urinary calculi [9–16], we did not find any information about chemometric treatment of the data. In this work, the principal-component analysis [17, 18] (an extremely useful tool for the classification of samples according to some of their properties) was used to analyze the experimental data.

2. EXPERIMENTAL

Instrumentation

A Varian SpectrAA 640Z Zeeman atomic absorption spectrophotometer equipped with a Varian PSD-100 Autosampler was used for Pb, Cd and Ni (Table 1), a Thermo Elemental Solaar S4 flame atomic absorption spectrophotometer for the determination of Zn and Fe (Table 2) and a JENWAY model PFP flame emission spectrometer was used for the determination of Na and K. An Ethos Touch Control pressurized microwave digestion device (Milestone, Italy) with a rotor for 12 Teflon digestion vessels was used. To prevent explosions, the vessels are equipped with a pressure release system. A specially designed vessel which allows a temperature sensor and a pressure sensor to be connected and the progress of the digestion to be monitored, is substituted for one of the 12 vessels.

Procedure

Calculi samples weighing 0.5 – 1.0 g were placed in Teflon digestion vessels, 3 mL concentrated HNO_3 and 2 mL H_2O_2 (30 %, *m*/*V*) were added, the vessels were capped closed, tightened and placed in the rotor of the microwave oven. The digestion was carried out with the digestion programs: 800 W/15 min; 1000 W/25 min and a pressure of 20 bar. Finally the vessels were cooled, carefully opened and the digests quantitatively transferred to 25 mL calibrated flasks and filled up with redistilled water. Fe, Zn, Na and K were analyzed directly from these solutions.

Table 1

Instrumental parameters for trace element determination of Cd, Ni and Pb by Zeeman ETAAS

Parameter	Cd	Ni	Pb
Wavelength/nm	228.8	232.0	283.3
Spectral slit/nm	0.5	0.2	0.5
Lamp current/mA	4.0	4.0	5.0
Calibration mode	Absorbance, peak height		
Background correction	Zeeman		
DRY			
Temperature/°C	85; 95; 120	85; 95; 120	85; 95; 120
Ramp time/s	5; 40; 5	5; 40; 5	5; 40; 5
Hold time/s	10	10	10
PYROLISIS			
Temperature/°C	600	900	800
Ramp time/s	40	10	10
Hold time/s	30	40	30
ATOMIZATION			
Temperature/°C	1800	2400	2100
Ramp time/s	0.8	1.1	1
Hold time/s	2	2	2
CLEANING			
Temperature/°C	1800	2400	2100
Hold time/s	2	2	2
Gas	Argon		

Table 2

Instrumental parameters for determination of Fe and Zn by FAAS

Parameter	Fe	Zn
Wavelength/nm	248.3	213.9
Spectral slit/nm	0.2	1.0
Lamp current/mA	20	5

For Pb, Cd and Ni an extraction method [19] was applied to eliminate the influence of Ca and Mg. 5 mL of each sample solution were evaporated in a 100 mL glass beaker of to eliminate HNO_3 and H_2O_2. After that, 1 mL of HCl and 15 mL of redistilled water were added and the solution was transferred into a separatory funnel. 5 mL of ammonium citrate solution (50 % *m*/*V*) were added and the pH value was adjusted with NaOH (20 %, *m/V*) to 6. Then, 5 mL of sodium diethyldithiocarbamate (0.2 %, *m/V*) were added and the mixture was shaken for 1 min. After 15 min, 5 mL of methyl isobutyl ketone were added and the mixture was shaken for 2 min. In the organic layer, the contents of cobalt, copper, lead and nickel were determined by electrothermal atomic absorption spectrometry (ETAAS).

3. RESULTS AND DISCUSSION

In order to examine how many factors have an influence on the results, the data presented in Table 3 were subjected to principal component analysis (PCA). The central idea of PCA is to reduce the dimensionality of the data set, explaining the variance–covariance structure [17, 18]. This is achieved by linear transformation of the original data, usually into smaller number of uncorrelated significant principal components (PCs). Geometrically, the transformation represents the rotation of the original coordinate system in the direction of the maximum residual variance given by the first PC axis. The second PC, orthogonal to the first one, has the second maximum variance and so on. In this way, the projections preserving the maximum variance in the data sets can be visualized by computer.

In this work PCA was used in order do find possible grouping in the analyzed data. Before the data matrix was decomposed, the variables were autoscaled. The eigenvalues obtained by the decomposition of the data matrix are presented in Table 4.

Table 3

The content of Na, K, Fe, Zn, Pb, Ni and Cd in the investigated calculi samples

No.	No. of the calculi in [3]	Type of the calculi	Sex of the patients	Na	K	Fe	Zn	Pb	Ni	Cd
				mg/g	mg/g	µg/g	µg/g	µg/g	µg/g	ng/g
1	23	oc	Female	9.43	3.14	4.93	459.05	0.88	0.31	7.75
2	24	oc	Male	4.40	1.19	5.20	44.04	1.52	0.34	7.09
3	25	ox	Male	0.12	0	24.44	52.89	2.38	0.36	8.77
4	26	oc	Female	8.21	3.23	16.30	198.40	1.22	9.54	3.04
5	27	st	Female	1.32	1.66	1.79	829.4	2.05	0.20	3.27
6	34	oc	Male	8.97	1.45	9.24	745.21	2.25	0.56	8.36
7	36	ua	Male	–	0.20	5.52	6.82	5.48	0.90	26.80
8	38	oc	Female	1.53	0.06	17.60	924.39	3.46	0.36	6.14
9	39	oc	–	–	–	12.55	744.95	1.91	3.53	21.18
10	43	ox	Male	14.39	–	17.15	24.86	2.92	0.97	32.16
11	47	ox	Male	0.28	0.56	7.47	538.07	1.86	0.23	70.40
12	48	oc	Male	–	–	22.51	278.35	4.09	1.27	14.78
13	74	st	Male	14.97	0.16	15.61	107.08	2.93	0.64	9.61
14	–	–	–	0.80	1.77	5.20	152.51	2.69	0.24	5.75
15	–	–	–	26.77	0.32	10.26	59.06	1.88	0.42	3.51
16	–	–	–	–	4.06	10.19	22.64	1.45	0.32	9.45

Types of calculi: oc – oxalates and carbonate apatite, ox – calcium oxalates, st – struvite, ua – uric acid

Table 4

Eigenvalues (EV), percentage variance (PV) and cumulative percentage variance (CPV) calculated from preprocessed data matrix

№	EV	PV	CPV
1	4.038	43.65	43.65
2	2.159	23.34	66.99
3	1.329	14.36	81.35
4	0.759	8.20	89.55
5	0.691	7.47	97.02
6	0.248	2.68	99.69
7	0.028	0.31	100.00

Table 4 shows that almost 90 % of the variance could be explained by the first four principal components. This means that four factors could be considered as influencing our data. 2D and 3D examination of the scores matrix calculated by decomposition of the preprocessed data matrix did not present significant information about possible correlations among the variables (the concentrations of the analyzed elements). In our case, more informative was the correlation matrix presented in Table 5 which shows that the highest correlation among of concentrations of Pb and K is –0.608.

Table 5

Correlation matrix

	Fe	Zn	Pb	Ni	Cd	Na	K
Fe	1						
Zn	–0.158	1					
Pb	0.228	–0.118	1				
Ni	0.253	–0.025	–0.22	1			
Cd	–0.059	0.041	0.153	–0.131	1		
Na	0.026	–0.273	–0.221	0.032	–0.323	1	
K	–0.379	–0.074	–0.608	0.277	–0.209	–0.088	1

More significant information was obtained by plotting PC1 and PC2 from the loadings matrix (Fig. 1). From this figure (Fig 1a) it is possible to see the grouping of the calculi samples taken from the male patients in the left and the upper part of the figure, while the stones taken from female patients are grouped at the bottom. In Fig. 1b it can be noted that the phosphate containing calculi are mainly grouped in the lower part of the figure, while the rest of the calculi are grouped in the upper part.

The present analysis seems to show that the content of the trace elements depends on the composition of the calculi and the sex of the patients, although a similar examination obtained with a larger sample could lead to more reliable results. At present we are not able to determine which factors mainly influence the content of the analyzed trace elements in the concrements. In order to find information about this, a more detailed medical examination of the factors influencing the formation of the calculi and the incorporation of the trace elements in them should be considered.

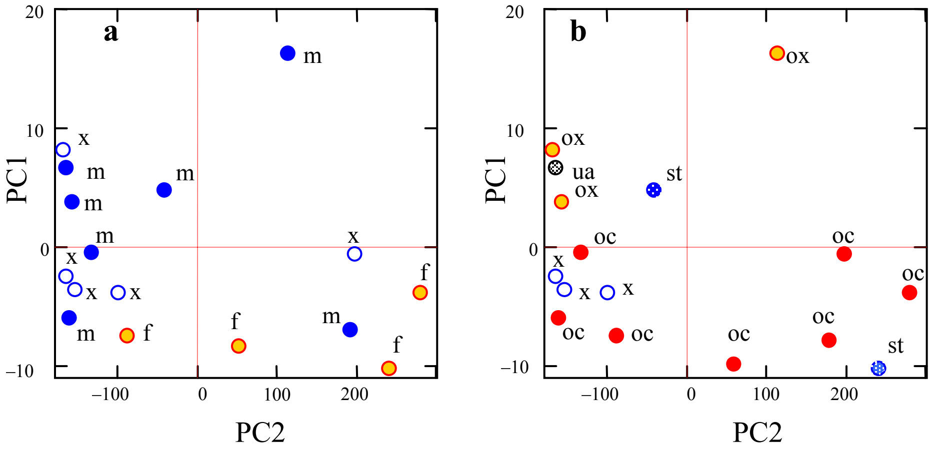

Fig. 1. Principal component analysis on 16 samples using 7 variables. a – labeled with the sex of the patients (m – male, f – female, x – unknown); b – labeled with the composition of the samples (oc – oxalates and carbonate apatite, ox – oxalates, st – struvite, ua – uric acid, x – unknown)

REFERENCES

[1] K. Stojanova, I. Petrov, B. Šoptrajanov. *Yugoslav. Phisiol. Pharmacol. Acta*, **5**, 137 (1969).

[2] К. Стојанова, И. Петров, Б. Шоптрајанов, *Макед. мед. преглед*, **24**, 71 (1969).

[3] И. Кузмановски, М. Трпковска, Б. Шоптрајанов, *Макед. мед. преглед*, **53**, 251 (1999).

[4] I. Kuzmanovski, M. Trpkovska, B. Šoptrajanov, V. Stefov, *Vib. Spectrosc.*, **19**, 249 (1999).

[5] I. Kuzmanovski, Z. Zografski, M. Trpkovska, B. Šoptrajanov, V. Stefov, *Fresenius' J. Anal. Chem.*, **370**, 919 (2001).

[6] I. Kuzmanovski, M. Trpkovska, B. Šoptrajanov, V. Stefov, *Anal. Chim. Acta*, **491**, 211 (2003).

[7] N. Q. Dao, M. Daudon, *Infrared and Raman Spectra of Calculi*, Elsevier, Paris, 1997.

[8] I. Kuzmanovski, M. Ristova, B. Šoptrajanov, V. Stefov, V. Popovski, *Talanta*, **62**, 813 (2004).

[9] I. Durak, A. Yasar, Z. Yurtarslan, M. Akpoyraz, S. Tasman, *Br. J. Urol.*, **62**, 203 (1988).

[10] J. B. Smith, C. Thevenon-Emeric, D. L. Smith, B. Green, *Anal. Biochem.*, **193**, 118 (1991).

[11] I. Durak, *Turk. J. Med. Sci.*, **17**, 93 (1993).

[12] S. Pevez, G. S. Pandey, *Environ. Monit. Assess.*, **32**, 93 (1993).

[13] K. Shirin, M. Qadiruddin, W.W.T. Manser, A.M. Syed, *Pak. J. Sci. Ind Res.*, **37**, 88 (1994).

[14] K. Kobarth, C. Koeberi, J. Hofbauer, *Urol. Res.*, **21**, 261 (1993).

[15] C. Koeberi, P. M. Bayer, K. Kobarth, *J. Radioanal. Nucl. Chem.*, **169**, 269 (1993).

[16] T. Umeyama, Y. Ogawa, *Nippon, Hinyokika Gakkai Zasshi*, **75**, 1038 (1984).

[17] R. A. Johnson, D. W. Wichern, *Applied Multivariate Statistical Analysis*, Prentice-Hall, New Jersey, 1982, p. 521.

[18] E. R. Malinowski, *Factor Analysis in Chemistry*, 2nd. Ed., Wiley, New York, 1991.

[19] T. Stafilov, D. Zendelovska, *Acta Chim. Slov.* **47**, 381 (2000).

Резиме

ХЕМОМЕТРИСКО ИСПИТУВАЊЕ НА ЕЛЕМЕНТИ ВО ТРАГИ ВО УРИНАРНИ КАЛКУЛУСИ

Во 16 уринарни калкулуси од пациенти од Македонија е определувана содржината на Pb, Cd, Ni, Fe, Na и K. Елементите во траги беа определувани со примена на атомска апсорпциона спектрометрија и со пламена емисиона спектрометрија. Извршена е хемометриска анализа на калкулусите со користење на факторната анализа. Овој хемометриски метод широко се применува за класификација на различни примероци, како и за решавање на други повеќедимензионални проблеми. Резултатите добиени со анализата на податоците покажуваат дека најголема корелација постои меѓу концентрациите на оловото и калиумот, додека меѓу концентрациите на другите елементи не беше најдена значителна корелација. Со разгледување на првите два карактеристични вектора пресметани од матрицата на автоскалирани податоци (овие вектори содржат 67% од варијанцата), може да се забележи раздвојување меѓу калкулусите кои потекнуваат од пациенти мажи и оние од пациенти жени. Освен тоа, забележано е групирање на калкулусите создадени само од оксалати, од една страна, и калкулусите создадени од смеса на оксалати и карбонатен апатит, од друга.

Клучни зборови: елементи во траги; уринарни калкулуси; ААС; хемометрија

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