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Short communication

Inductively coupled plasma mass spectrometry determination of metals in honeybee venom

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ABSTRACT

Inductively coupled plasma mass spectrometry (ICP-MS) technique was used to analyze the contamination of selected 20 metals in 32 samples of honeybee venom and to demonstrate differences in the content of these elements. Among the analyzed metal microelements (Al, Co, Cu, Zn, Mn, Mo, B, V, Sr and Ni), macroelements (Ca, Mg, K and Na) and toxic metals (As, Ba, Pb, Cd, Sb and Cr) were identified. The presented results showed that the metal levels in honeybee venom are much lower than the tolerable upper intake levels for the elements. Also the toxic metal contamination is much lower than the permissible levels for drugs established by the United States Pharmacopeia and the European Pharmacopeia. As opposed to the pharmacopeial tests for metals, a multi-element ICP-MS method has been developed. In order to confirm data obtained, the following steps and parameters were taken into account for the validation of the method: calibration verification, recovery, accuracy, precision, detection limit (LOD), quantitation limit (LOQ), spectral and matrix interference and comparison between ICP-MS and GFAAS (graphite furnace atomic absorption spectrometry) for Mn. All steps of validation proved the accuracy of the results.

This is most likely the first study in which the metal content in honeybee venom was evaluated by ICP-MS.

1. Introduction

Bee venom has interesting pharmacological properties [1] and it is used in treatment of conditions such as arthritis [2], rheumatism, pain [3,4], cancerous tumors [5,6] and skin diseases.

During the last century mainly due to the chromatographic methods [7], the majority of bee venom constituents were separated and identified, although there is not enough information about metal contamination in the honeybee venom in the literature. Metals catalyze both biochemical reactions and decomposition of biologically active compounds including chemicals from honeybee venom. Therefore, metal content monitoring of process intermediates and final drug substances, including natural products like bee venom, is an extremely important issue.

Physiological limits of toxic metals have been established by the environmental protection agency (EPA) [8]. For those metals whose determination efforts have been confirmed, the permissible limits were set by the Occupational Safety and Health Administration (OSHA) [9] and the National Institute for Occupational Safety and Health (NIOSH) [10].

The United States Pharmacopeia (USP) [11] and the European Pharmacopeia's (EP) [12] tests for heavy metals consist of precipitation of metal sulfides from an aqueous solution and visual comparison of the color of that preparation to that of simultaneously and similarly treated standard lead solution. These are tedious, cumbersome and time-consuming procedures suitable only for a few elements (Pb, Hg, Bi, As, Sb, Sn, Cd, Ag, Cu and Mo), which will typically respond to this test. Moreover, they are non-specific and insensitive methods which yield very low recoveries. Therefore, attempts must be made to look for better and newer techniques for multi-element analysis like inductively coupled plasma mass spectrometry (ICP-MS).

Metal contamination has been reported in traditional herbal remedies, dietary supplements [13] and other pharmaceutical materials [14], although there are insufficient data on metal content in honeybee venom. This is most likely the first study in which metal content in honeybee venom was evaluated by ICP-MS. Unlike pharmacopeial tests, this technique can be easily automated and hence it is a perfect tool for fast routine analysis.

The aim of this study was to measure the concentrations of twenty metals (^{39}K, ^{63}Cu, ^{66}Zn, ^{23}Na, ^{24}Mg, ^{42}Ca, ^{10}B, ^{27}Al, ^{51}V, ^{52}Cr,

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^{55}Mn, ^{59}Co, ^{60}Ni, ^{75}As, ^{88}Sr, ^{95}Mo, ^{111}Cd, ^{121}Sb, ^{134}Ba and ^{208}Pb) in samples of honeybee venom using ICP-MS.

2. Experimental

2.1. Reagents and materials

All solutions were prepared with de-ionized water obtained by passing distilled water through a Millipore Milli-Q—water purification system (Waters Corporation, Milford, MA, USA). Suprapure 65% nitric acid and 30% hydrogen peroxide used for the microwave digestion of bee venom were supplied by Merck (Darmstadt, Germany).

Samples of honeybee venom were collected from the apiary of the Department of Inorganic and Analytical Chemistry of Poznan University of Medical Sciences by stimulating the bees with electric current pulses. Venom collecting frames were placed in the upper body of the hive in the middle space of the super. The duration of venom-collecting event was 2 h during full activity of bees. Such schedule allowed to obtain the highest efficiency of bee venom production. 28 samples were obtained from 2002 to 2007 during the whole beekeeping seasons from May until September. In 2006 and 2007 bee venom was collected from two different lines of carnica bee race (*niemka*—I line, *singer*—II line). Additionally three samples were from 1991—Georgia and one Sigma Bee Venom sample was supplied by Sigma Chemicals Co. (St. Louis, MO, USA). The samples were stored until analysis at 5 °C in the darkness.

2.2. Preparation of standards and samples

Calibration standard solutions were prepared from 1.0 mg ml^{-1} single element standard solutions obtained from Merck (Darmstadt, Germany) and UltraScientific (Wesel, Germany) by dilution with de-ionized water. SLRS-4 (River Water Reference Material for Trace Metals) was supplied by the National Research Council, Canada. ERM-CA021a (Soft Drinking Water) was supplied by LGC (Teddington, UK).

The honeybee venom solutions were prepared by dilution of 5–10 mg of that product in 2% nitric acid (Suprapure 65% nitric acid diluted with ultra-pure de-ionized water) and filtered through a 0.45-μm cellulose nitrate membrane filters supplied by Agilent (USA). The concentration of these solution was from 1.0 to 3.0 g l^{-1}.

Table 1
ICP-MS operating conditions and measurement parameters

RF generator	27.12 (MHz)
H_2/He gas flow rate	2–4 (ml min^{-1})
Integration time	0.1–1.0 (s)
RF power	1500–1550 (W)
Sampling depth	8 (mm)
Carrier gas flow rate (Ar)	0.90 (l min^{-1})
Auxiliary (make up) gas flow rate (Ar)	0.15 (l min^{-1})
Sample uptake rate	0.1–0.5 (ml min^{-1})
Acquisition mode	analogue – K, Zn, Na pulse – the rest
Samples per peak	3
Number of replicates	5
Quadruple bias	−10 (V)
Isotopes	^{39}K, ^{63}Cu, ^{66}Zn, ^{23}Na, ^{24}Mg, ^{42}Ca, ^{10}B, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{75}As, ^{88}Sr, ^{95}Mo, ^{111}Cd, ^{121}Sb, ^{134}Ba, ^{208}Pb,

2.3. Instrumentation

2.3.1. ICPMS

The studies were carried out using Agilent 7500ce ORS ICP-MS system equipped with a micromist nebulizer, double pass spray chamber cooled with a Peltier's effect and quartz ICP torch. During the analysis the following procedure was utilized: optimization of the instrument, calibration with the standard solutions, analysis of the sample blank consisting of 2% ultra-pure nitric acid, analysis of the reference materials (after every 10 samples), analysis of the honeybee venom samples. The main elements (K, Na, Mg, Ca, Zn and Cu) and trace elements were analyzed separately. The operating conditions have been presented in Table 1.

2.3.2. Graphite furnace atomic absorption spectrometry (GFAAS)

The GFAAS determinations for ^{55}Mn were conducted using Varian SpestrAA20 plus atomic absorption spectrometer equipped with GTA-97 graphite tube. The method was based on PN-EN

Table 2
Calibration verification with reference materials: CRM SLRS-4 and CRM ERM-CA021a

Element	Isotope	Unit	CRM SLRS-4		Certified concentration	CRM ERM CA021a		Certified concentration
			Measured concentration	R.S.D. (%) (n = 5)		Measured concentration	R.S.D. (%) (n = 5)	
K	39	mg l^{-1}	–	–	–	1.23	2.4	1.16 ± 0.05
Cu	63	μg l^{-1}	–	–	–	2016	1.5	1975 ± 54
Zn	66	μg l^{-1}	–	–	–	530	1.5	514 ± 9
Na	23	mg l^{-1}	2.28	1.0	2.4 ± 0.2	–	–	–
Mg	24	mg l^{-1}	1.51	0.9	1.6 ± 0.1	–	–	–
Ca	42	mg l^{-1}	6.15	1.2	6.2 ± 0.2	–	–	–
B	10	μg l^{-1}	4.9	7.8	–	–	–	–
Al	27	μg l^{-1}	50	3.7	54 ± 4	–	–	–
V	51	μg l^{-1}	0.36	6.2	0.32 ± 0.03	–	–	–
Cr	52	μg l^{-1}	0.34	4.9	0.33 ± 0.02	–	–	–
Mn	55	μg l^{-1}	3.5	2.8	3.37 ± 0.18	–	–	–
Co	59	μg l^{-1}	0.04	–	0.033 ± 0.006	–	–	–
Ni	60	μg l^{-1}	0.74	5.0	0.67 ± 0.08	–	–	–
As	75	μg l^{-1}	0.73	5.3	0.68 ± 0.06	–	–	–
Sr	88	μg l^{-1}	28	2.5	26.3 ± 3.2	–	–	–
Mo	95	μg l^{-1}	0.39	7.6	0.21 ± 0.02	–	–	–
Cd	111	μg l^{-1}	0.016	–	0.012 ± 0.002	–	–	–
Sb	121	μg l^{-1}	0.26	4.2	0.23 ± 0.04	–	–	–
Ba	134	μg l^{-1}	12.6	2.7	12.2 ± 0.6	–	–	–
Pb	208	μg l^{-1}	0.082	9.9	0.086 ± 0.007	–	–	–

Table 3
Recoveries after fortification of honey bee venom samples

Element*Isotope*		Samples							
		Bulk sample—2005				June 2005			
		Analyzed concentration	Concentration after fortification	RS.D. (%) ($n=3$)	Recovery (%)	Analyzed concentration	Concentration after fortification	RS.D. (%) ($n=3$)	Recovery (%)
Concentration (mg g^{-1} D.M.[a]), fortification level = 1									
Na	23	0.52	1.48	1.6	97.4	0.34	1.34	1.8	99.5
Mg	24	0.66	1.59	1.0	95.4	0.56	1.53	0.9	97.8
Ca	42	1.80	2.71	1.1	95.7	1.25	2.33	1.4	103.7
Concentration (μg g^{-1} D.M.[a]), fortification level = 10									
B	10	39	48	1.8	98.4	21	32	2.1	103.2
Al	27	266	260	4.8	94.1	482	504	6.1	102.4
V	51	0.28	11.13	5.5	108.3	0.15	10.29	5.9	101.4
Cr	52	1.50	11.43	2.5	99.4	0.50	10.25	1.9	97.5
Mn	55	7.90	17.70	2.2	98.8	5.50	16.00	2.0	103.2
Co	59	0.15	10.08	6.2	99.3	0.08	9.82	5.5	97.3
Ni	60	0.80	10.34	2.4	95.8	0.70	10.14	2.1	94.7
As	75	0.11	10.21	1.8	100.9	0.08	10.34	1.9	102.6
Sr	88	2.10	11.80	3.9	97.2	3.00	12.80	4.2	98.2
Mo	95	2.42	13.68	1.8	110.1	6.00	17.60	1.5	109.9
Cd	111	0.23	9.68	4.1	94.6	0.85	10.76	3.4	99.1
Sb	121	0.21	10.05	3.9	98.5	0.08	10.46	3.2	103.8
Ba	134	6.49	16.10	2.3	97.5	6.30	16.90	1.9	103.2
Pb	208	6.57	15.90	0.9	96.1	5.90	16.50	2.1	103.6
		August 2006 bee line II				September 2006 bee line I			
Concentration (mg g^{-1} D.M.[a]), fortification level = 50									
K	39	0.47	51.90	1.1	103	0.51	51.00	0.9	101
Concentration (mg g^{-1} D.M.[a]), fortification level = 0.5									
Cu	63	0.12	0.63	0.8	102	0.22	0.77	1.1	107
Zn	66	0.15	0.67	0.7	102	0.20	0.73	1.2	104

[a] D.M.—dry mass.

ISO 15586:2003, which includes principles and procedures for the determination of low concentrations of elements. Graphite tube without an integrated L'vov platform was applied. Analysis was carried out without background correction. Palladium was used as a chemical modifier. Absorbance was measured at 279.5 nm with a slit width of 0.2 nm. 25 μl of sample were injected. An ashing temperature was kept at 700 °C, and an atomization temperature at 2400 °C. Detection limit for Mn (LOD): 2.0 μg g^{-1} D.M. and quantitation limit (LOQ): 5.0 μg g^{-1} D.M.

3. Results

Apart from routine analysis of all samples, some additional experiments were performed to confirm data obtained.

Table 4
LOD and LOQ and precision study for metal analysis by ICP-MS method

Element	Isotope	LOD	LOQ	Precision study (Sample—August 2002)	
				Concentration	R.S.D. (%) ($n=5$)
Concentration (mg g^{-1} D.M.)					
K	39	0.01	0.04	3.70	1.3
Na	23	0.05	0.17	0.24	1.2
Mg	24	0.02	0.07	0.24	1
Ca	42	0.10	0.32	0.80	3.4
Concentration (μg g^{-1} D.M.)					
Cu	63	0.5	1.4	1.10	0.5
Zn	66	0.3	1.0	1.10	0.8
B	10	2.0	10.0	6.00	3.5
Al	27	0.6	2.0	23.00	1.8
V	51	0.1	0.5	0.06	5.5
Cr	52	0.08	0.20	1.12	1.2
Mn	55	0.9	2.4	2.60	1.7
Co	59	0.05	0.20	0.04	–
Ni	60	0.2	0.7	1.20	2.1
As	75	0.07	0.20	0.05	2.9
Sr	88	0.5	2.0	1.70	4.8
Mo	95	0.3	1.0	1.97	1.4
Cd	111	0.02	0.06	0.29	2.8
Sb	121	0.05	0.20	0.08	3.9
Ba	134	0.6	1.3	4.10	3.2
Pb	208	0.1	0.3	6.10	0.7

Table 5
Concentration of metals before and after mineralization of samples

Element	Isotope	Samples							
		Bulk sample—2005				June 2005			
		Concentration before mineralization	R.S.D. (%) ($n=5$)	Concentration after mineralization	R.S.D. (%) ($n=5$)	Concentration before mineralization	R.S.D. (%) ($n=5$)	Concentration after mineralization	R.S.D. (%) ($n=5$)
Concentration ($mg\,g^{-1}$ D.M.)									
K	39	5.03	1.9	4.7	1.3	3.80	5.3	3.5	2.1
Cu	63	0.86	1.8	0.63	0.8	0.98	4.9	1	1.7
Zn	66	1.17	2.2	0.92	1.3	1.09	6.9	1.2	3.1
Na	23	0.30	2.3	0.24	5.5	0.18	0.8	0.11	9
Mg	24	0.34	3.1	0.3	3.2	0.24	1.6	0.19	7.4
Ca	42	0.74	3.6	0.8	8.1	0.38	4.4	0.42	1.1
Concentration ($\mu g\,g^{-1}$ D.M.)									
B	10	23.00	3.4	18	2	7.67	7.9	7	9
Al	27	34.33	1.3	120	2.1	22.67	3.5	161	7.1
V	51	0.14	5.4	0.13	6.9	0.03	8.2	0.05	2.5
Cr	52	0.79	1.9	0.68	3.2	0.13	2.6	0.17	8.1
Mn	55	4.50	2.5	3.5	2.3	1.60	3.9	1.8	5.5
Co	59	0.08	-	0.07	–	0.03	14.7	0.03	5.8
Ni	60	1.66	4.9	0.36	8	0.19	4.2	0.23	7.3
As	75	0.05	9.4	0.05	7.5	0.02	–	0.03	6.3
Sr	88	1.03	4.7	0.9	7.1	0.77	6.2	1	5
Mo	95	0.57	3.7	1.09	2	0.91	5.4	2	8.3
Cd	111	0.18	2.4	0.1	9.9	0.05	6.1	0.28	8.7
Sb	121	0.04	5.5	0.09	2.2	0.03	6.8	0.03	9.1
Ba	134	2.60	5.4	2.9	2	0.94	3.6	2.1	8.3
Pb	208	4.40	0.9	3	1.4	1.91	3.3	2	8.2

The verification of calibration was done by analyzing two reference materials: SLRS-4 (River Water Reference Material for Trace Metals) and ERM-CA021a (Soft Drinking Water). Their composition and concentration was comparable to metal content in honeybee venom samples. The results given in Table 2 show a very good agreement with certified values established for reference materials and concentrations analyzed in this study.

Recovery was evaluated analyzing four samples before and after fortification with standard solutions. Obtained recovery data shown in Table 3 were on acceptable levels (from 95 to 110%) [15].

Accuracy of the method was proved by analyzing two reference materials: SLRS-4 and ERM-CA021a. The data obtained are highly comparable with certified values (Table 2). In addition, recoveries demonstrated in Table 3 were excellent and that shows very good accuracy of the applied method.

To study the precision of the method, relative standard deviation (R.S.D.) among the replicate results from the same sample was evaluated. The results shown in Table 4 indicate, that R.S.D. values from five replicates ($n=5$) in the majority of results were much lower than 5, which proves an excellent precision of the method [15].

Detection limit (LOD) and quantitation limit (LOQ) for ICP-MS method (Table 4) was determined as 3σ and 10σ, respectively (σ—standard deviation of the blank, $n=20$).

To eliminate the spectral interference internal standards were added on line (^{45}Sc, ^{89}Y, ^{159}Tb) and reaction mode was applied for

Table 6
Metal contamination of selected samples of honey bee venom determined by ICP-MS method ($\mu g\,g^{-1}$ D.M.)

Element	Isotope	Samples							
		May 2007 bee line I	August 2007 bee line I	May 2007 bee line II	August 2007 bee line II	May 2005	May 2002	Georgia 1991	Sigma
K	39	4740	3367	3950	2708	5300	5500	2563	3933
Cu	63	526	453	682	494	470	90	748	15
Zn	66	875	791	827	895	775	450	439	457
Na	23	190	141	194	170	175	500	174	227
Mg	24	290	188	289	189	360	310	127	233
Ca	42	760	430	639	651	550	400	246	327
B	10	19	9	10	11	13.50	14.00	20.39	7.33
Al	27	276	59	157	116	20.50	15.00	29.37	15.80
V	51	0.08	0.03	0.04	0.08	0.03	0.04	0.09	0.03
Cr	52	0.37	0.25	0.25	0.79	0.14	0.30	0.43	5.73
Mn	55	3.5	1.3	2.4	3.4	4.50	2.10	2.97	5.43
Co	59	0.07	0.02	0.05	0.08	0.05	0.03	0.05	0.15
Ni	60	0.35	0.10	4.57	0.29	0.19	1.00	0.55	19.67
As	75	0.05	0.02	0.04	0.06	0.03	0.06	0.04	0.30
Sr	88	1.39	0.51	1.26	1.11	1.00	1.00	0.68	0.47
Mo	95	1.4	1.4	1.2	1.7	1.33	1.03	1.24	1.75
Cd	111	0.05	0.02	0.04	0.03	0.06	0.06	0.11	0.07
Sb	121	0.0	0.0	0.0	0.1	0.03	0.02	2.68	0.05
Ba	134	1.7	0.6	1.5	3.3	1.10	0.82	2.43	1.48
Pb	208	5.6	0.6	1.4	2.1	1.40	2.85	2.51	3.57

the following metals: H_2–V, He–K, Cu, Zn, Na, Mg, Ca, Cr, Mn, Co, Ni and As. Without reaction mode were analyzed: B, Al, Sr, Mo, Cd, Sb, Ba, Pb and U.

The absence of matrix effects was proved by very good recoveries after fortification shown in Table 3. Moreover, two samples were analyzed before and after digestion. Each sample analysis was carried out in five replicates ($n = 5$). The samples were digested in microwave oven—StartD (Milestone) during 20 min at 180 °C. Before digestion, 100 mg of honeybee venom was placed into PFA vessel and mixed with 6 ml of concentrated nitric acid and 2 ml of 30% hydrogen peroxide. After cooling, the digested samples were diluted with de-ionized water to obtain the concentration of honeybee venom: 1.0–3.0 g l^{-1}. The results before and after mineralization given in Table 5 are comparable, which shows that there are no significant matrix effects.

In order to compare the findings from two techniques, the content of ^{55}Mn was evaluated in seven samples using ICP-MS and GFAAS. The obtained data were on comparable and satisfactorily levels of analyzed element. The ICP-MS results were a bit higher than that from GFAAS, which may be due to complexity of the matrix and/or to the uncertainty of both methods.

4. Discussion

ICP-MS technique allows to analyze not only a few elements like pharmacopeial tests, but almost all elements across the periodic table with extremely low detection limits. Nevertheless there are no reference materials with certified values of metal contents in honeybee venom. It is very difficult to find relevant reference material with similar concentrations of metals to metal levels in bee venom. Two reference materials: SLRS-4 (River Water Reference Material for Trace Metals) and ERM-CA021a (Soft Drinking Water) possess quite similar composition and concentration levels of metals to the analyzed samples. Employing these reference materials, analysis of 20 elements was possible with adequate certainty. Moreover, the metals chosen to this investigation play the most important role in the human organisms and they are pharmaceutically important elements in relation to the other less common elements.

To check the significant differences between independent variables (the content of analyzed metals), the data from analysis of 28 venom samples were subjected to statistical analysis—factorial ANOVA. Three factors were considered to the calculations: the line of bees, month and year of venom collection. Statistical analysis showed that there are year-to-year significant differences in the content of Cu, Na, Ca, Al., V, Mn, Co, As, Sr, Mo, Ba and Pb. There were month-to-month differences for K and year and month differences for Mg. The content of Zn in bee venom depended on line of the bees and year of venom collection. No significant differences were recorded for B, Cr, Ni, Cd and Sb. The seasonal differences in the content of K and Mg are due to the agricultural influence (fertilization). Most fertilizers are given in the spring and therefore the content of K and Mg decrease during the beekeeping season.

Results for bee venom supplied from Sigma and Georgia (Table 6) were on the same concentration levels like the samples obtained by the authors. Therefore, this is strong evidence that the investigated batches are representative for all honeybee venom samples.

By the rules of the USP and EP heavy metal limits in different drugs are between 0.001 and 0.003% depending on the dosage form and kind of drug. Therefore, the content of toxic metals (As, Ba, Pb, Cd, Sb and Cr) in analyzed honeybee venom samples were much lower than the permissible levels for drug substances.

According to the tolerable upper intake levels (UL) for the elements established by the Food and Nutrition Board of the Institute of Medicine of the National Academies [16], the content of metals in honeybee venom set in this study does not exceed the limits. The metal contamination of bee venom analyzed in this survey was much lower for most metals than UL, even if bee venom is considered to be taken orally. Bee venom can be used as an anti-inflammation and anti-rheumatic agent in different dosage forms like ointments, creams and injections. The drugs with bee venom contain usually from 0.01 to 1% of this product. Therefore, a relative small amount of analyzed elements will not influence directly the human organism.

Metal contamination of honeybee venom should be taken into account, when the venom is considered to be used in medicine. In spite of quite low levels of metal concentrations in this product, it is obvious that even trace amounts of metals may control, trigger or stop the biochemical reactions in the living organisms [17] and modulate pharmacological activity of bee venom.

5. Conclusions

ICP-MS is a very useful method which can be used for determination of metals in honeybee venom. This study should contribute to the future investigations on the interactions between contained elements in bee venom and the rest of its constituents (biologically active components—peptides, enzymes). Therefore, it is necessary to possess a wide knowledge about metal content in the honeybee venom, which can be potentially used as a drug.

References

[1] J.S. Dong, W.L. Jae, H.L. Young, S.S. Ho, K.L. Chong, T.H. Jin, Pharmacol. Ther. 115 (2007) 246–270.
[2] H.J. Park, S.H. Lee, D.J. Son, K.W. Oh, K.H. Kim, H.S. Song, Arthritis Rheum. 50 (2004) 3504–3515.
[3] Y.B. Kwon, T.W. Ham, H.W. Kim, D.H. Roh, S.Y. Yoon, H. Han, J. Pharmacol. Biochem. Behav. 80 (2005) 181–187.
[4] H.W. Kim, Y.B. Kwon, T.B. Ham, D.H. Roh, S.Y. Yoon, H.J. Lee, J. Vet. Med. Sci. 65 (2003) 349–355.
[5] T. Putz, R. Ramoner, H. Gander, A. Rahm, G. Bartsch, M. Thurnher, Cancer Immunol. Immunother. 55 (2006) 1374–1383.
[6] P.J. Russell, D. Hewish, T. Carter, K. Sterling-Levis, K. Ow, M. Hattarki, Cancer Immunol. Immunother. 53 (2004) 411–421.
[7] V. Packova, K. Stulik, P.T. Hau, J. Jelinek, I. Vins, D. Sykora, J. Chromatogr. A 700 (1995) 187–193.
[8] Environmental Protection Agency. IRIS database for risk assessment. www.epa.gov/iris/index.html (accessed February 19, 2008).
[9] Occupational Safety and Health Administration. Technical links to safety and health topics. www.osha.gov/SLTC/index.html (accessed February 19, 2008).
[10] National Institute for Occupational Safety and Health. Occupational health guidelines for chemical hazards. www.cdc.gov/niosh/81-123.html (accessed February 19, 2008).
[11] The United States Pharmacopeia, The National Formulary, XXIV, United States Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD, 2000.
[12] European Pharmacopoeia, 4th ed., Council of Europe, Strasbourg Cedex, 2001.
[13] A.A. Grippo, B. Hamilton, R. Hannigan, B.I. Gurley, Am. J. Health-Syst. Pharm. 63 (2006) 635–645.
[14] T. Wang, J. Wu, R. Hartman, X. Jia, R.S. Egan, J. Pharm. Biomed. Anal. 23 (2000) 867–890.
[15] I. Taverniers, M. De Loose, E. Van Bockstaele, TrAC 23 (2004) 535–552.
[16] Food and Nutrition Board, Institute of Medicine of the National Academies www.iom.edu/Object.File/Master/21/372/0.pdf (accessed February 19, 2008).
[17] J.J.R. Fraústo da Silva, R.J.P. Williams, The Biological Chemistry of the Elements: The Inorganic Chemistry of Life, Oxford University Press Inc., New York, 2001.