Available online at www.sciencedirect.com
ScienceDirect
Microchemical Journal 89 (2008) 1 – 6
MICROCHEMICAL JOURNAL
www.elsevier.com/locate/microc

Trace aluminium determination in biological samples after microwave digestion followed by solid phase extraction with L-methionine on controlled pore glass

Pablo H. Pacheco [a,b], Raúl A. Gil [a,c], Patricia Smichowski [c,d], Griselda Polla [e], Luis D. Martinez [a,c,*]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Chacabuco and Pedernera, 5700 San Luis, Argentina
[b] Fondo para la investigación científica y tecnológica (FONCYT), Avenida Córdoba 831 Piso 6 (C1054AAH) Ciudad de Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rivadavia 1917, CP C1033 AAJ, Ciudad de Buenos Aires, Argentina
[d] Comisión Nacional de Energía Atómica, Unidad de Actividad Química, Avenida. General Paz 1499, B1650KNA—San Martín, Argentina
[e] Comisión Nacional de Energía Atómica, Unidad de Actividad Física, Avenida General Paz 1499, B1650KNA—San Martín, Argentina

Received 5 October 2007; accepted 6 October 2007
Available online 16 October 2007

Abstract

Aluminium is an element found in biological samples at low concentrations and aluminum-related neuropathological diseases in human beings have been reported. For this reason the determination of Al in this type of samples requires the development of enrichment methods capable of improving the instrumental detection for this analyte.

L-methionine immobilized on controlled pore glass (CPG) was tested for the retention of aluminium. This adsorbent material was packed in a conical minicolumn and connected to an on-line flow injection system. An inductively coupled plasma optical emission spectrometer associated to an ultrasonic nebulization system (USN-ICP OES) was used for aluminium detection. Al(III) was retained at pH 12.5 and removed from the column with 20% (v/v) HNO_3.

The precision of the preconcentration method was evaluated by passing a measured volume of aluminium standard solution (1 μg L^{-1}) through the minicolumn and repeating this procedure five times. The relative standard deviation (RSD) was 2.5%, calculated with the peak heights obtained. A total enhancement factor of 1600 was attained for a sample volume of 10 mL (10-fold for the ultrasonic nebulization system and 160-fold for the preconcentration methodology). The detection limit (DL), calculated as the amount of Al required to yield a net peak equal to three times the standard deviation of the blank solution, was 25 ng L^{-1}.

The proposed system was successfully applied to the determination of aluminium in urine, hair, and saliva samples, which were pretreated using a microwave digestion methodology with the introduction of a digestion program for saliva. The average aluminium levels found in urine, hair, and saliva samples were 5.5 μg L^{-1}, 19 μg g^{-1} and 93 μg L^{-1} respectively.

Keywords: L-methionine; Aluminium; Solid phase extraction; Biological samples

1. Introduction

Aluminium is a non-essential, toxic metal to which humans are frequently exposed by the use of Al-containing drugs, inhalation of atmospheric dust, food, drinks, etc. This element has been involved as a causative factor in several clinical and neuropathological diseases, particularly in patients with chronic renal failure. Elevated levels of Al have been implicated in the etiology

* Corresponding author. Department of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Chacabuco and Pedernera, 5700 San Luis, Argentina. Fax: +54 2652 430224.
E-mail address: ldm@unsl.edu.ar (L.D. Martinez).

0026-265X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2007.10.004

of Alzheimer's disease, Parkinson's disease, Parkinson–Guam's disease, amyotrophic lateral sclerosis, diabetes and cancer [1].

Aluminium is widespread throughout nature (8.3% in the earth's crust) and is found in air, water, plants, and consequently in food [2]. It is especially found at high levels in tea leaves. Aluminium may be included in food products by leaching from cookware or storage containers when the contents are acidic. Aluminium is also a component of many food additives and is incorporated to drinking water during water treatment [3].

Due to the ubiquitous character of aluminium, human beings are exposed to it; therefore, the analysis of biological samples acquires a great relevance for aluminium intoxication diagnostic. Urine samples are suitable as biological monitoring indices for assessing recent occupational exposure and increased exposure because a large proportion of ingested aluminium passes quickly through the body [4]. Hair samples have many advantages over other materials such as blood and bone because they are easily obtained, repeatedly available and require no special storage conditions. In addition, the slow growth of hair can provide retrospective information about the burden of Al in the body. Unlike blood, short-term variations in hair concentrations of analytes are averaged out, so that the values for Al in hair reflect its accumulation over long periods [5]. On the other hand, it is well known that aluminum has interesting anticariogenic properties and the release of it from dental material can be determined in saliva [6].

Nieboer et al. reviewed eight studies on aluminum concentrations in urine and reported that Al concentrations in healthy individuals typically ranged from 2.7 to 8.1 $\mu g\ L^{-1}$ [7]. Sighinolfi et al. reported Al concentration ranging from 25 to 102 $\mu g\ L^{-1}$ in human saliva [8]. Concentrations ranging from 0.1 to 36 $\mu g\ g^{-1}$ of aluminium have been found in hair samples [9].

The low aluminium levels in the mentioned samples are not compatible with the detection limit reached by inductively coupled plasma optical emission spectrometry (ICP-OES). In addition, Al determination in complex matrices such as biological samples is a difficult task.

SPE is an effective means for extending the detection limits of ICP-OES technique and solving problem referred to matrix interferences. However, in the batch mode, the operations are usually tedious and time consuming. Stringent control of the laboratory environment is also required to avoid sample contamination when determinations in the ng mL^{-1} range are being attempted. This situation has been significantly improved by coupling flow injection (FI) to ICP OES [10–12].

Several materials have been used as sorbent agents in solid phase extraction (SPE) methods, such as Chelex-100 resin [13], octadecyl silica disk, methyltrioctylammonium chloride-naphthalene and MCM-401 mesoporous adsorbent [14]. In addition, extraction/enrichment procedures such as cloud point methodologies have been developed for Al preconcentration [15,16].

Aminoacids and poly-aminoacids chains have also been used in continuous-flow SPE systems for the retention of metals [17–22]. These substrates appeared as an attractive alternative to other chemical adsorbent materials because of their high accumulation capacity and specificity, allowing preconcentration and/or speciation of different analytes [23]. However, to the best of our knowledge, these materials have not been employed in the retention and enrichment of Al.

The aim of the present work was the development of a method for the aluminium retention in different biological samples using a minicolumn packed with L-methionine immobilized on controlled pore glass as retaining agent. The samples analyzed were hair, urine, and saliva and they were pretreated by microwave digestion. Aluminium was eluted from the column with nitric acid. After that, the analyte was directly determined by FI-USN-ICP OES.

2. Experimental

2.1. Instrumentation

Measurements were performed with a sequential ICP spectrometer Baird ICP 2070 (Bedford, MA, USA.). The 1 m

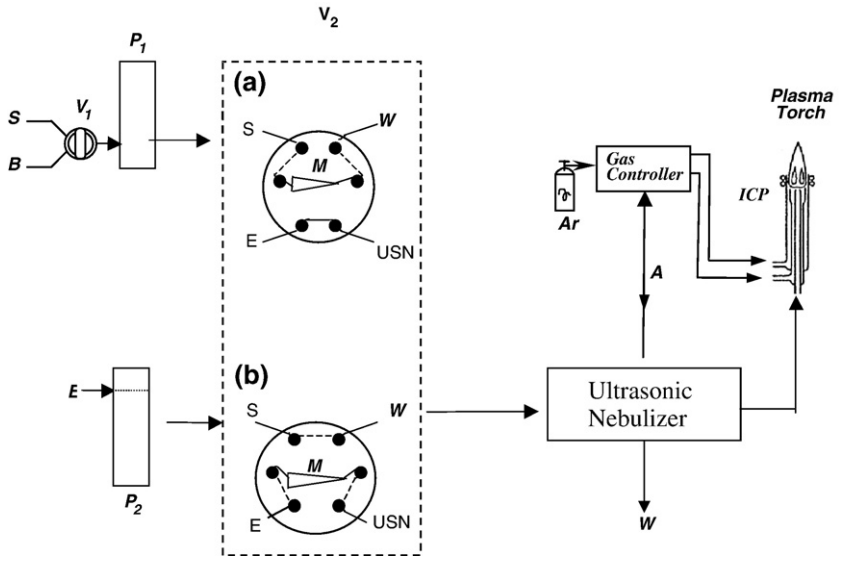

Fig. 1. Schematic diagram of the instrumental setup. S: sample (flow rate: 10 mL min^{-1}); B: buffer; E: eluent (flow rate: 1.0 mL min^{-1}); A: Ar (flow rate: 0.7 L min^{-1}); W: waste; P_1, P_2: peristaltic pumps; C: minicolumn packed with L-methionine-CPG; V: injection valve. Valve positions: (a) sample loading and (b) injection.

Table 1
USN-ICP instrumental parameters

ICP conditions	
RF generator power (kW)	1.0
Frequency of RF generator (MHz)	40.68
Plasma gas flow rate (L min^{-1})	8.5
Auxiliary gas flow rate (L min^{-1})	1.0
Carrier gas flow rate (mL min^{-1})	90
Observation height above load coil (mm)	15
Analytical line: Al (nm)	308.215
USN conditions	
Heater temperature (°C)	140
Condenser temperature (°C)	4.0
Carrier gas flow (mL min^{-1})	1.0

Czerny–Turner monochromator had a holographic grating with 1800 grooves mm^{-1}. The FI system used is shown in Fig. 1. An ultrasonic nebulizer, U-5000 AT [CETAC Technologies (Omaha, NE, USA)], with desolvation system was used. The ICP and USN operating conditions are listed in Table 1. A Minipulse 3 peristaltic pump Gilson (Villiers-Le-Bell, France) was used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) model 50, four-way and of 6 ports, 2 positions, rotary valve. The conical minicolumn was prepared by placing 60 mg of L-methionine-CPG into an empty conical tip using the dry packing method. The conical design of the minicolumn allowed a higher enrichment factor due to a diminution of the dispersion [24,25]. To avoid loss of filling when the sample solution passed through the conical minicolumn, a small amount of quartz wool was placed at both ends of the conical minicolumn. The column was then connected to a peristaltic pump with PTFE tubing to form the preconcentration system. Tygon type pump tubing (Ismatec, Cole Parmer, Vernon Hills, IL, USA) was employed to propel the sample, reagents and eluent. The Al 308.215 nm spectral line was used and measurements of FI system were expressed as peak-height emission.

Microwave digestion was performed with a domestic microwave oven (Philco, Ushuaia, Argentina) equipped with a magnetron of 2450 MHz; and with Milestone hermetically sealed 100 mL internal volume and 1 cm wall thickness polytetrafluoroethylene (PTFE) vessels.

2.2. Reagents

Unless otherwise stated, the chemicals used were of analytical grade and, therefore, no further purification was required.

Hence all glass and plastic ware were thoroughly washed with acid or EDTA solutions and then checked for their possible contributions of Al to the sample. Special attention was paid to avoid any dust on the samples because of the high content of Al in dust.

Aluminium working standard solutions were prepared by stepwise dilution from 1.00 mg mL^{-1} stock standard solution (Merck, Darmstadt, Germany) immediately before use. Ultrapure water (18 MΩ cm) was obtained from an EASY pure (RF Barnstedt, Iowa, USA).

L-methionine was obtained from Fluka A.G., (Switzerland). CPG (pore diameter 240 Å, mesh size 240–400), 8-aminopropiltriethoxysilane and glutaraldehyde were supplied by Sigma (St. Louis, USA).

2.3. Immobilization procedure

A 0.2 g portion of L-methionine was suspended in 15 mL of 0.1 mol L^{-1} phosphate buffer at pH 7.0. A detailed explanation of silanization of the CPG using 8-aminopropyltriethoxysilane and the use of the bifunctional property of glutaraldehyde to prepare the glutaraldehyde-treated CPG has been reported in Ref. [26]. Glutaraldehyde is the substance that allows L-methionine binding to CPG, acting as a cross linker agent. The glutaraldehyde-treated CPG was filtered and washed with ultrapure water. To the beaker containing the L-methionine solution, 1.0 g of the treated glass was added and N_2 was flushed for 15 min. The mixture was kept at 4 °C for 24 h under a N_2 atmosphere and then air-dried filtered.

2.4. Sample treatment

2.4.1. Hair

Samples of suboccipital hair from patients were cut into several pieces with stainless-steel scissors and placed in individual microwave polystyrene tubes [5]. The microwave digestion program is listed in Table 2. An aliquot of 0.2 g of human hair was treated with a mixture containing 3 mL HNO_3, 2 mL H_2O_2, and 5 mL of deionized water [27].

2.4.2. Urine

Urine samples were collected in 50 mL polypropylene tubes, and were analyzed on the day of collection. Samples aliquots of 4.0 mL of urine were digested with 2.0 mL of concentrated nitric acid [28]. The program used in microwave digestion is also shown in Table 2.

2.4.3. Saliva

In order to minimize the possibility of contamination with food debris or cigarette and airborne particles, the subjects were asked to thoroughly rinse their mouths three times, first with 1.5% citric acid solution (a salivation stimulant) and then twice

Table 2
Experimental conditions of microwave digestion for the different biological samples

Step	Hair		Saliva		Urine	
	Time (min)	Power (W)	Time (min)	Power (W)	Time (min)	Power (W)
1	3	250	3	150	10	550
2	2	0	2	0	10	350
3	5	250	3	350		
4	5	500	5	550		
5	5	500	2	0		
6	5	250				
7	2	0				
Total time (min)	27		15		20	

with deionized water [29]. No microwave digestion program for saliva could be found in literature. Samples were digested following the step program mentioned in Table 2; 3 mL of saliva were digested with 1 mL HNO_3 plus 1 mL H_2O_2.

2.5. Procedure

The flow injection system used for preconcentration, separation and subsequent determination of Al is shown in Fig. 1. Before loading, the column was conditioned at the desired pH with valve V_1 in position B. A volume of sample was then loaded on the conical-minicolumn (M) at flow rate of 10 mL min^{-1} with valve V_1 in position S and valve V_2 in load position (a). Finally, valve V_2 was switched to the injection position (b) and the aluminium retained was eluted with a 20% (v/v) nitric acid solution. After that, the eluate was introduced into the USN unit and subsequently pumped to the ICP torch. The operation conditions were established and the determination was carried out.

3. Results and discussion

3.1. Optimization of separation conditions

Aluminium retention conditions and sample loading flow rate were studied off-line. In all cases, Al concentration was determined by USN-ICP OES. Recoveries were calculated against the theoretical concentration.

3.1.1. Effect of pH

Aliquots of 10 mL of aqueous solution containing Al(III) buffered at different pH values were loaded on the column at a flow rate of 2 mL min^{-1}. In order to optimize the sorption conditions for the retention of Al on L-methionine-CPG, the intensity of Al signal was monitored by USN-ICP OES as a function of the pH of the solution that passed through the conical minicolumn. Fig. 2 shows that the optimal pH value for Al retention was in the range between 11.0 and 13.0. For further experiments, pH 12.5 was selected. A sodium hydroxide solution was used to reach the optimum pH.

The sorption capacity of CPG for Al retention, without L-methionine, including silanization and glutaraldehyde-treatment, was tested and no retention was observed; therefore, it was concluded that only L-methionine was responsible for Al retention.

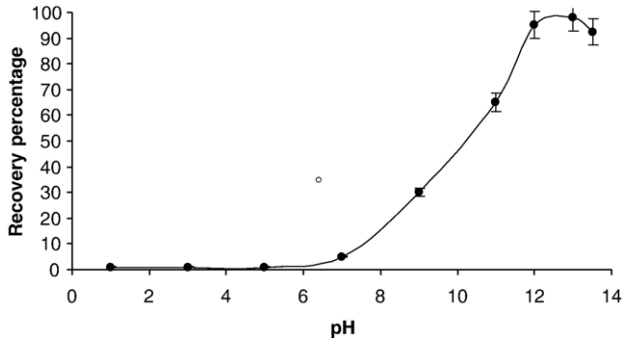

Fig. 2. Dependence of Al(III) retention on pH of loading solutions. Volume of sample: 25 mL; Al concentration: 0.05 mg L^{-1}; HNO_3 concentration: 20.0% (v/v).

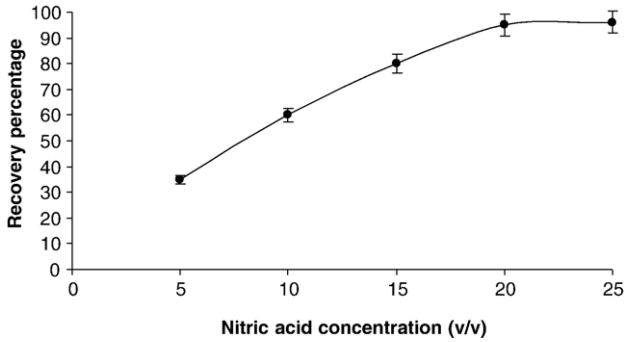

Fig. 3. Dependence of Al(III) recovery on eluent concentration. Volume of sample: 10 mL; pH: 12.5; Al concentration: 0.1 μg L^{-1}.

3.1.2. Eluents

To achieve the maximum aluminium desorption efficiency from the sorbent material, different eluents at several concentration values were tested. HNO_3 demonstrated to be the best alternative. A concentration of 20% (v/v) reached the optimal analyte elution, yielding in an aluminium recovery (%) close to 100, as can be seen in Fig. 3.

3.1.3. Sample flow rate

This is a very important parameter to optimize, since this is one of the steps that control the time of analysis. We verified that with flow rates up to 15.0 mL min^{-1} there was no effect on the analyte recovery (98–100%). Higher flow rates were avoided because the recovery decreased and the back pressure generated could damage the FI system. To avoid filling overpacking a 10 mL min^{-1} flow rate was chosen for further experiments.

3.2. Effect of coexisting ions

The effects of common coexisting ions were investigated and the results are given in Table 3. The tests were made at the concentration levels at which they may occur in the studied samples (urine, saliva and hair) to avoid possible interferences in Al preconcentration/determination, mainly in the sample loading step. Results demonstrate that L-methionine-CPG has selectivity for Al, despite the variety of foreign ions present in the studied samples.

Table 3
Tolerance limits of coexisting ions for determination of Al

Foreign ions	Tolerance limit (μg mL^{-1})
K^+, Na^+	1000
Mg^{+2}	500
Ca^{+2}	100
Zn^{2+}, Cu^{+2}, Fe^{3+}	50
NO_3^-	1000
SO_4^{-2}	500
CO_3^{-2}	1000
Cl^-	1000

Table 4
Determination of aluminium in biological samples

Aliquots	Base value (μg L^{-1})	Quantity of Al(III) added (μg L^{-1})	Al found (μg L^{-1})	Recovery [a] (%)
Urine				
1	5.5	0	5.5±0.3	–
2	5.5	5	10.6±0.2	102.0
3	5.5	10	15.1±0.1	96.0
4	5.5	20	25.2±0.4	98.5
Saliva				
1	93	0	93±0.1	–
2	93	10	102.8±0.2	98.0
3	93	20	113.2±0.1	101.0
4	93	50	144.1±0.3	102.2

Aliquots	Base value (μg g^{-1})	Quantity of Al(III) added (μg g^{-1})	Al found (μg g^{-1})	Recovery [a] (%)
Hair				
1	19	0	19±0.3	–
2	19	5	23.8±0.4	96.0
3	19	10	29.2±0.2	102.0
4	19	20	38.8±0.3	99.0

[a] 100 [(found-base)/added].

3.3. Performance of the proposed system

The detection limit (DL), calculated on the basis of the 3σ criterion, and with the preconcentration of a 10 mL sample volume, turned out to be 25 ng L^{-1}. The precision calculated as the relative standard deviation (RSD) for five replicate determinations of a solution containing 1.0 μg L^{-1} of Al(III) was 2.5%. When compared to the conventional ICP OES mode with pneumatic nebulization, a total enhancement factor of 1600-fold was obtained (160-fold for the preconcentration system and 10-fold for the USN). Linearity was attained from levels close to the detection limit up to at least 2000 μg L^{-1}, the coefficient of correlation was 0.9988.

The overall time required for the preconcentration of 10 mL of sample (1 min, at a flow rate of 10 mL min^{-1}), elution (0.16 min, at a flow rate of 1 mL min^{-1}), washing and conditioning (0.4 min) was about 1.2 min, reaching a sample throughput of 50 h^{-1}.

3.4. Recovery study

When no standard reference materials with a certified content of aluminium are available, a recovery study can be considered as a validation alternative [30]. The proposed method was evaluated by analyzing urine, saliva and hair samples. The recovery study was applied to three portions of sample and the mean Al concentration of each sample was taken as a base value. Then, increasing quantities of Al (5.0, 10.0 and 20.0 μg L^{-1} in hair and urine aliquots; and 10.0, 20.0, and 50.0 μg L^{-1} in saliva aliquots) were added to the other aliquots and the same procedure was followed. As shown in Table 4, the recovery values were between 96 and 102.2% for Al. The results were compared with the *t*-test and no significant differences were observed at 95% confidence level. The recovery studies for the other samples showed similar performance.

3.5. Applications

The developed method was applied to the determination of Al in different biological samples such as hair, urine and saliva. The analytical results for aluminium determination are listed in Table 4. The results obtained for Al in saliva, using the proposed step program for microwave digestion (Table 2), are in good agreement with those mentioned in literature [8].

4. Conclusions

On the basis of the findings of this study, L-methionine immobilized on CPG constituted a suitable substrate for solid phase extraction of aluminium in complex matrices such as biological samples. The on-line combination of USN-ICP OES associated to microwave digestion allowed aluminium determination in biological samples at ng L^{-1} levels. The determination procedure shows quantitative extraction, good reproducibility and accuracy.

L-methionine demonstrated great capacity retention for aluminium (100%), without complexing agent. A fast kinetic of adsorption/desorption process, allowed a high enhancement factor (1600).

To the best of our knowledge this is the first time that L-methionine is employed as retaining agent for aluminium determination in urine, hair and saliva samples.

Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICTBID), Universidad Nacional de San Luis (Argentina) and Comisión Nacional de Energía Atómica (CNEA).

References

[1] S. Polizzi, E. Pira, M. Ferrara, M. Bugiani, A. Papaleo, R. Albrera, Neurotoxicology 23 (2002) 761–774.
[2] U. Tamer, N.O. Pekmez, A. Yildiz, Fresenius Environ. Bull. 11 (2002) 1087–1090.
[3] R. Cornelis, B. Heinzow, R.F.M. Herber, J. Molin Christensen, O.M. Paulsen, E. Sabbion, D.M. Templeton, Y. Thomasse, M. Vahter, O. Vesterberg, Pure Appl. Chem. 67 (1995) 1575–1608.
[4] Agency for Toxic Substances and Disease Registry, Draft Toxicological Profile for Aluminium, vol. 209, September 2006.
[5] P. Chappuls, L. Duhaux, F. Paelaggl, N.C. de Vemejoul, F. Rousselet, Clin. Chem. 34 (1988) 2253–2255.
[6] M.F. Hayacibara, G.M.B. Ambrosano, J.A. Cury, Oper. Dent. 29 (2004) 16–22.
[7] E. Nieboer, B.L. Gibson, A.D. Oxman, J.R. Kramer, Environ. Rev. 3 (1995) 29–81.
[8] G.P. Sighinolfi, C. Gorgoni, O. Bonori, E. Cantoni, M. Martelli, L. Simonetti, Mikrochim. Acta 3–4 (1989) 171–179.
[9] S. Caroli, A. Alimonti, E. Coni, E. Petrucci, O. Senofonte, N. Violante, Crit. Rev. Anal. Chem. 24 (1994) 363–398.
[10] R.A. Gil, S. Pasini-Cabello, A. Takara, P. Smichowski, R.A. Olsina, L.D. Martínez, Microchem. J. 86 (2007) 156–160.
[11] G.M. Farias, R.G. Wuilloud, S. Moyano, J.A. Gásquez, R.A. Olsina, L.D. Martinez, J. Anal. Toxicol. 26 (2002) 181–185.
[12] B. Fairman, A. Sanz-Medel, Fresenius' J. Anal. Chem. 355 (1996) 757–762.

[13] S. Knežević, R. Milačič, M. Veber, Fresenius' J. Anal. Chem. 362 (1998) 162–166.
[14] M.A. Zanjanchi, H. Noei, M. Moghimi, Talanta 70 (2006) 933–939.
[15] A.B. Tabrizi, Food Chem. 100 (2007) 1698–1703.
[16] L.L. Sombra, M.O. Luconi, L.P. Fernandez, R.A. Olsina, M.F. Silva, L.D. Martinez, J. Pharm. Biomed. Anal. 30 (2003) 1451–1458.
[17] H.A. Jurbergs, J.A. Holcombe, Anal. Chem. 69 (1997) 1893–1898.
[18] M. Howard, H.A. Jurbergs, J.A. Holcombe, J. Anal. At. Spectrom. 14 (1999) 1209–1214.
[19] E. Gutierrez, T.C. Miller, J.R. Gonzalez-Redondo, J.A. Holcombe, Environ. Sci. Technol. 33 (1999) 1664–1670.
[20] T.C. Miller, J.A. Holcombe, J. Hazard. Mater. 83 (2001) 219–236.
[21] M. Howard, H.A. Jurbergs, J.A. Holcombe, Anal. Chem. 70 (1998) 1604–1609.
[22] L. Malachowski, J.A. Holcombe, Anal. Chim. Acta 495 (2003) 151–163.
[23] P.H. Pacheco, R.A. Gil, P. Smichowski, G. Polla, L.D. Martinez, J. Anal. At. Spectrom. 22 (2007) 305–309.
[24] Z.L. Fang, Flow Injection Preconcentration and Separation, VCH Publishers, Inc., New York, 1993.
[25] G.M. Farias, S. Cerutti, J.A. Gásquez, R.A. Olsina, L.D. Martinez, At. Spectr. 24 (2003) 213–217.
[26] H.A.M. Elmahadi, G.M. Greenway, J. Anal. At. Spectrom. 6 (1991) 643–646.
[27] R.S. Jung, S.R. Yang, J.K. Han, G.H. Kang, G.H. Lee, Anal. Sci. 17 (2001) 999–1002.
[28] S.C. Chen, M.Y. Shiue, M.H. Yang, Fresenius' J. Anal. Chem. 357 (1997) 1192–1197.
[29] M.O. Luconi, R.A. Olsina, L.P. Fernández, M.F. Silva, J. Hazard. Mater. 128 (2006) 240–246.
[30] E. Prichard, G.M. MacKay, J. Points, Trace Analysis: A Structures Approach to Obtaining Reliable Results, The Royal Society of Chemistry, United Kingdom, 1996, p. 27.