International Journal of Mass Spectrometry 307 (2011) 137–141

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Evaluation of metal distributions in small samples of mouse brain lesions (hematoma) by inductively coupled plasma mass spectrometry after sampling by laser microdissection (LMD)

Marcia F. Mesko[a,*], Dagmar Salber[b], Dirce Pozebon[c], Valderi L. Dressler[d], J. Sabine Becker[e]

[a] Instituto de Química e Geociências, Universidade Federal de Pelotas, Pelotas, RS, Brazil
[b] Institute of Neuroscience and Medicine, INM-4, Research Center Jülich, 52425 Jülich, Germany
[c] Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[e] Central Division of Analytical Chemistry, Research Center Jülich, 52425 Jülich, Germany

ARTICLE INFO

Article history:
Received 1 December 2010
Received in revised form 27 April 2011
Accepted 27 April 2011
Available online 5 May 2011

Keywords:
Biological tissues
Metal determinations
Laser microdissection (LMD)
ICP-MS

ABSTRACT

Laser microdissection (LMD) used for sample preparation was combined off-line with inductively coupled plasma mass spectrometry (ICP-MS) to evaluate metal distributions in mouse brain with hematoma lesion. Small amounts (from 0.014 to 0.338 mg) of tissue were sampled from selected regions of the brain, with a hematoma lesion and also from regions without hematoma. The obtained samples were decomposed in closed vessel in a microwave oven and a micronebulizer in conjuction with a desolvation system was used for introducing the solution of the sample into the plasma. Accuracy was evaluated using certified reference materials (bovine liver and mussel tissue), whilst the agreement between the concentrations found with those certified was better than 85%. The limits of detection (LODs) of Cu, Fe and Zn for the small mouse brain tissue samples were 12.4, 12.5 and 9.6 $\mu g\,g^{-1}$, respectively. The LODs of K and Na were 1.07 and 0.24 $mg\,g^{-1}$, respectively. The distribution of K, Na, Cu, Fe and Zn in the selected regions of the mouse brain was evaluated. It was observed that the Fe, Na and Zn concentrations were approximately 2–10 times higher in the hematoma region (inside and around the hematoma) than in the control (region without hematoma). The LMD system demonstrated to be useful for sampling small amounts of biological tissue from regions of interest for further analysis by ICP-MS.

1. Introduction

Metal ions are vital components in several biological processes and also essential for brain function. Complex mechanisms of biological processes are often influenced by metal ions and their deficit or surplus could explain the appearance of metal-associated diseases such as Alzheimer and Parkinson. Therefore, the characterization of biological samples with respect to the distribution of essential and toxic metals and metalloids, imaging or mapping, are important in life science research [1–3]. There is a growing interest in quantitatively accessing the total metal concentration in small structures in native tissue *in situ* [4,5].

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used for quantitative imaging of elements in biological tissues [6–8]. However, the spatial resolution achieved with commercially available LA systems (for example with solid-state 266 and 213 nm Nd-YAG lasers) is restricted to approximately 5–10 μm due to limitations in the laser optics. LMD is a microscope-based technique using a high-precision laser beam applied *via* the microscope objective to selectively isolate specific cell types, individual cells, or cell organelles from embedded, frozen or fresh tissue sections. This is possible due to the observation optics used, in combination with the option of cutting out previously identified and defined areas of a sample by means of a highly focused laser beam guided precisely over the sample. The sample is mounted on a special membrane slide (e.g., 1.4 μm thickness) or directly on a microscope (glass) slide and placed on the table of the LMD apparatus [4]. LMD has been used in life science fields like drug discovery, pathology, forensic, medical diagnostics, food and environmental analysis as well as in medical research [9–11]. An additional advantage of LMD is the preservation of the selected sample area [12]. Recently, LMD-ICP-MS was proposed for bioimaging of metals in small biological specimens [13]. It involved a new using mode of the LMD apparatus as laser ablation system with high spatial resolution with ICP-MS for sensitive analysis of selected elements in small tissue [4,13].

* Corresponding author. Tel.: +55 55 9158 8157; fax: +55 53 3275 7354.
E-mail address: marciamesko@yahoo.com.br (M.F. Mesko).
URL: http://www.brainmet.com (J.S. Becker).

1387-3806/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2011.04.009

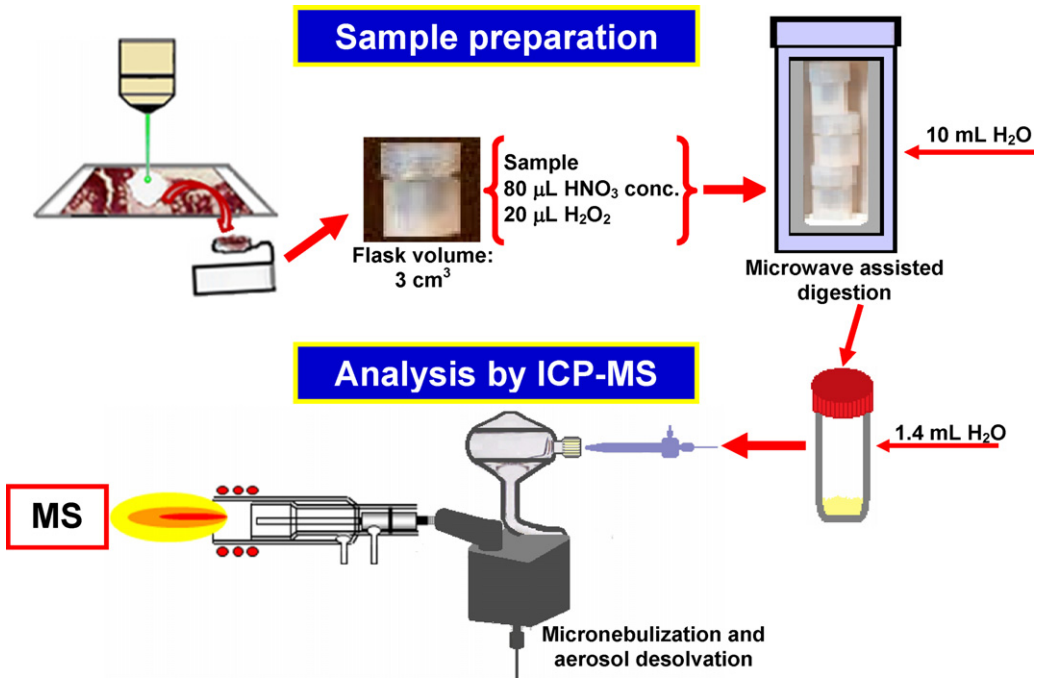

Fig. 1. Arrangement for mouse brain sampling by LMD (adapted from Ref. [26]) and scheme of the sample preparation procedure.

Trace element determinations in small amounts of biological samples require special sample preparation and measurement techniques [14,15], especially if the analyte concentration is very low. The feasibility of micronebulization in conjunction with a desolvating system (used for sample introduction into the plasma) and ICP-MS (for element detection) has already been demonstrated [15–18]. An effective sample decomposition of the biological tissue is strongly recommended, which can be troublesome when small amount of sample is available [19,20]. Currently, microwave-assisted decomposition in closed vessels can be considered as the state-of-art for sample decomposition for trace elements determination, which has been successfully applied to a variety of samples [19,21]. In addition, the employment of special vessels can be useful when the sample amount is limited [6,14,22].

LMD-ICP-MS has been proposed for bioimaging of metals in small biological tissues but there is still little application related to this issue and additional studies are needed. The aim of this work was to evaluate metal distributions in mouse brain lesion (hematoma) using LMD-ICP-MS. LMD is used for micro-sampling and ICP-MS was used for K, Na, Cu, Fe and Zn quantification.

2. Experimental

2.1. Instrumentation

A laser-microdissection (LMD 6500, Leica, Wetzlar, Germany) was used for brain tissue sampling (Fig. 1). The LMD 6500 has a diode-pumped solid state laser operated at a wavelength of 355 nm. The repetition rate, pulse period and energy were 80 Hz, 1 ns and 50 µJ, respectively.

A microwave oven (Microwave Accelerated Reaction Systems, MARS-5, CEM Microwave Technology Ltd., USA) equipped with PFA vessels with internal volume of 3 mL [23] was used for sample decomposition.

A high efficiency nebulizer ESI APEX-Q (ESI, Omaha, NE, USA), equipped with a PFA microconcentric nebulizer, a heated cyclonic spray chamber and a Peltier-cooled multipass condenser was coupled to an ICP-MS spectrometer (XSeries 2 from Thermo Scientific, Bremen, Germany) for element determination. In the APEX-Q system the aerosol generated by pneumatic nebulization was introduced into a cyclonic spray chamber heated at 140 °C and than transported to the condenser where the temperature was set at −5 °C [16]. Tubing and connectors provided by the instrument manufacturer were used. The operating conditions of ICP-MS (Table 1) were optimized in order to obtain maximum ion intensity, minimum oxide ion and double charged ion intensities, following the manufacturer instructions.

2.2. Reagents and solutions

High purity deionised water (18.2 MΩ cm) obtained from a Milli-Q (Millipore Corp., Bedford, USA) system was used throughout the work. Hydrogen peroxide and nitric acid (from Merck) were used after further purification by sub-boiling distillation.

A multi-element calibration solution was prepared in 2% (v/v) HNO_3 from serial dilutions of monoelement standard solutions (Merck CertiPrep). Calibration solutions ranging from 10 to 80 µg L^{-1} were prepared in 2% (v/v) HNO_3. At least 5 calibration solutions were used to compose the calibration curves.

Table 1
Operating conditions for Na, K, Cu, Fe and Zn determination by ICP-MS.

Parameter	
RF power (W)	1400
Plasma gas flow rate (L min^{-1})	14.0
Auxiliary gas flow rate (L min^{-1})	1.2
Nebulizer gas flow rate (L min^{-1})	0.9
Solution uptake rate (µL min^{-1})	330
Measurement mode	Peak hopping
Sweeps/reading	5
Readings/replicate	3
Replicates	3
Isotope (m/z)	^{23}Na, ^{39}K, ^{56}Fe, ^{57}Fe, ^{63}Cu, ^{64}Zn, ^{66}Zn, ^{65}Cu
Sampler and skimmer cones	Ni

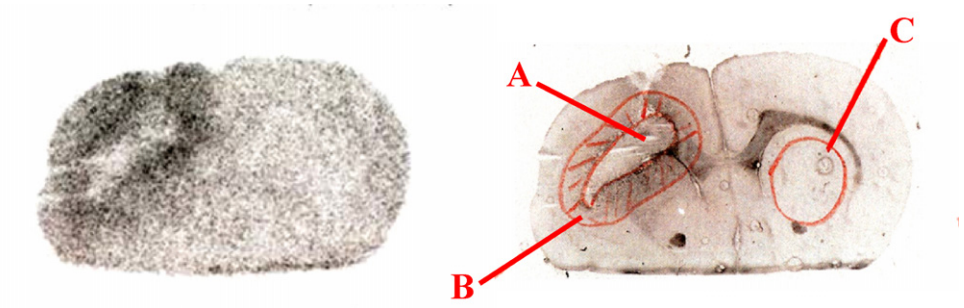

Fig. 2. Images of the hematoma in mouse brain by cell nuclei staining with toluidine. Selected regions sampled by LMD: (A) inside of the hematoma, (B) around of the hematoma and (C) control tissue.

2.3. Samples and laser microdissection sampling

Samples were taken from selected regions (Fig. 2) of mouse brain with hematoma lesions using LMD. The hematoma in the mouse brain was identified by cell nuclei staining with toluidine blue. Samples were identified as A (inside the hematoma), B (around the hematoma) and C (control regions – without hematoma). These regions (A, B, and C) were analyzed in three neighboring sections referred to as slice 01, slice 02, slice 03.

Bovine liver (NIST-1577b) from the National Institute of Standards & Technology (NIST) and mussel tissue (BCR-668) from the Community Bureau of Reference (BCR) were analyzed for analytical procedure validation.

2.4. Microwave-assisted decomposition

The brain and certified samples were decomposed following the procedure reported in reference [16]. The sample amount of brain tissue was in the range of 0.014–0.338 mg, whereas that of the certified sample was about 0.200 mg. The brain and certified samples were carefully weighed and transferred to microwave oven-vessels to which 0.08 mL of HNO_3 and 0.02 mL of 30% (m/m) H_2O_2 were added. The vessels were closed and placed inside major vessels (with internal volume of 75 mL) used for conventional digestion in microwave oven. Ten milliliters of water were added to each major vessel, assuring that the water level was just below the inserted small vessels. For microwave-assisted sample decomposition, the following heating program was used: 150 W for 10 min, cooling for 2 min, 300 W for 15 min and cooling for 30 min. Then, the obtained solutions were transferred to polypropylene vials and the volume completed to 1.5 ml by water addition. The scheme of the sample preparation procedure is shown in Fig. 1.

3. Results and discussion

Due to the small amount of brain tissue samples obtained, it was necessary to develop a special sample preparation procedure. A small vessel was used for sample decomposition, involving small amount of reagents and low dilution. The LODs obtained for Cu, Fe and Zn in the mouse brain samples (LOD = 3*s*, *s* is the standard deviation of 10 consecutive runs of the sample blank) using ICP-MS and solution nebulization were 12.4, 12.5 and 9.6 $\mu g\,g^{-1}$, respectively. The LODs of K and Na were 1.07 and 0.24 $mg\,g^{-1}$, respectively. Taking into account the very small amount of sample (between 14 and 338 μg) used, the results obtained were considered satisfactory. Concerning the accuracy of the procedure, it was observed that the K, Na, Cu, Fe and Zn concentrations measured were in agreement with the certified values (recoveries ranging from 85 to 90%, as shown in Table 2).

According to Table 2, the Fe concentration (measured *via* the isotopes ^{56}Fe and ^{57}Fe) was in good agreement with the certified value (approximately 88%). Despite the strong interference of ArO^+

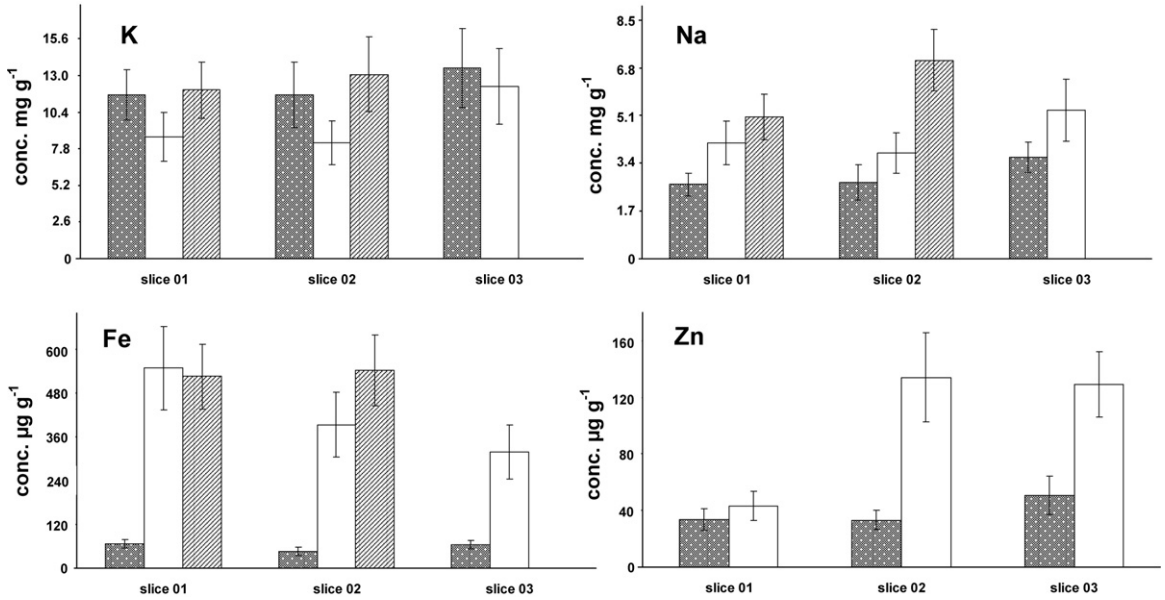

Fig. 3. Evaluation of K, Na, Fe and Zn distribution in three neighboring sections across the hematoma. (▩) A (inside of the hematoma); (□) B (around of the hematoma) and (▨) C (control tissue).

Table 2
Concentrations of Cu, Fe, K, Na and Zn found in mouse brain tissue with hematoma lesion, and in certified samples. LMD was used for brain tissue sampling ($n = 3$).

Sample (mass, mg)	Element concentration					
		K ($mg\,g^{-1}$)	Na ($mg\,g^{-1}$)	Cu ($\mu g\,g^{-1}$)	Fe ($\mu g\,g^{-1}$)	Zn ($\mu g\,g^{-1}$)
Slice 01 (0.014–0.161)	Minimum	8.65 ± 1.80	2.66 ± 0.40	<LOD	67.1 ± 10.6	33.8 ± 7.8
	Maximum	11.96 ± 1.99	5.05 ± 0.81	<LOD	549 ± 115	43.2 ± 10.4
Slice 02 (0.022–0.338)	Minimum	8.21 ± 1.56	2.72 ± 0.626	<LOD	47.2 ± 11.6	33.3 ± 7.0
	Maximum	13.07 ± 2.64	7.07 ± 1.09	<LOD	544 ± 97	134 ± 32
Slice 03 (0.094–0.177)	Minimum	12.21 ± 2.67	3.62 ± 0.54	<LOD	64.5 ± 11.2	50.9 ± 13.7
	Maximum	13.54 ± 2.80	5.29 ± 1.12	<LOD	318 ± 73	129 ± 23
BCR 668[a]	Detemined	nd	nd	nd	103 ± 18	60.3 ± 9.3
	Informed	–	–	–	84.7–93.5	70.7 ± 0.4
NIST 1577b[b]	Detemined	8.95 ± 2.55	2.07 ± 0.59	137 ± 29	160 ± 35	nd
	Certified	9.94 ± 0.02	2.42 ± 0.06	160 ± 8	184 ± 15	–

nd = not determined.
[a] Informed values.
[b] Certified values.

on $^{56}Fe^{+}$, the LOD of ^{56}Fe was better than that of ^{57}Fe. This result demonstrated the good performance of the nebulizer/desolvation system used in reducing oxide formation.

Metals such as K, Na, Cu, Fe and Zn are essential for growing and brain function as well as cellular processes. Thus, it is expected that the distribution of these metals is modified in brain region impacted by hematoma lesion. The concentrations of K, Na, Fe and Zn determined in three different slices of mouse brain tissue with and without hematoma lesion are summarized in Table 2. It is possible to observe in this table that the relative standard deviation (RSD) for all analytes was between 15 and 28%, which can be considered reasonable in view of the very small sample mass used.

The distribution of K, Na, Fe and Zn in the analyzed regions (A, B and C) of the brain is shown in Fig. 3. It can be noted that the concentrations of Fe, Na and Zn were increased in the region with hematoma. The Fe, Na and Zn concentrations in the hematoma lesion tissue (inside and around of the hematoma) were about 2–10 times higher than in the control (tissue without hematoma). The Na concentration is higher in the tissue inside the hematoma lesion. Zinc could not be determined in one sample (from region A) because the amount of sample from this region was lower than 0.022 mg. As a consequence, the Zn concentration in the obtained solution was lower than the LOD. With respect to K, the concentration of the element ranged from 8.2 to 13.5 $mg\,g^{-1}$. However, it was not possible to distinguish the variation of K concentration amongst regions with and without hematoma lesion. The concentration of Cu in the analyzed brain tissue samples was lower than the LOD (12.4 $\mu g\,g^{-1}$). These results are in agreement with the copper distribution image shown in Fig. 4, obtained by LA-ICP-MS [25].

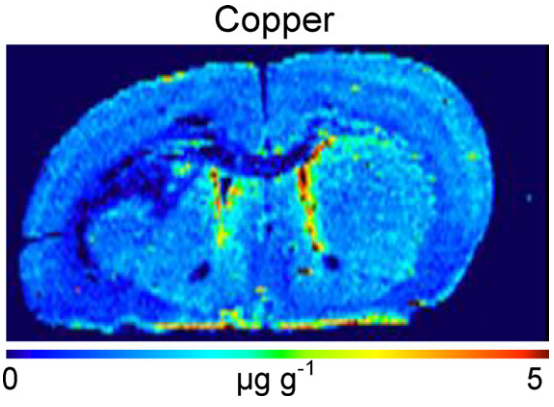

Fig. 4. Imaging of copper distribution in brain hematoma lesion [25].

The concentration of Fe increased in the hematoma lesion when compared with the control tissue. However, it was not possible to determine a significant difference between A and B regions, mainly because the amount of sample available was too low. It is important to point out that the Fe concentration found in brain tissue from region C was in good agreement with that found in previous works [6,24].

4. Conclusions

The LMD system was in general suitable for sampling of mouse brain tissues with hematoma lesions prior to metal determinations by ICP-MS. The results obtained for K, Na, Cu, Fe, and Zn showed the possibility of measuring metals in very small amounts of biological tissues. Moreover, LMD offers maximum preservation of the sample from contamination and high definition of the area for sampling. Therefore, the proposed combination of LMD and ICP-MS is a powerful tool for evaluation of metal distributions in brains with hematoma lesions.

Acknowledgement

The authors thank Thermo Fisher Scientific for instrumental support of BrainMet (BrainMet – Bioimaging of Metals and Metallomics) laboratory at Research Centre Juelich (www.brainmet.com).

References

[1] J.S. Becker, Inorganic Mass Spectrometry: Principles and Applications, John Wiley and Sons, Chichester, 2007.
[2] A. Sigel, H. Sigel, R.K.O. Sigel, Neurodegenerative Diseases and Metal Ions, John Willey, Chichester, 2006.
[3] R. Lobinski, J.S. Becker, H. Haraguchi, B. Sarkar, Pure Appl. Chem. 82 (2010) 493.
[4] J.S. Becker, S. Niehren, A. Matusch, B. Wu, H.F. Hsieh, U. Kumtabtim, M. Hamester, A. Plaschke-Schlütter, D. Salber, Int. J. Mass Spectrom. 294 (2010) 1–6.
[5] R.W. Hutchinson, A.G. Cox, C.W. McLeod, P.S. Marshall, A. Harper, E.L. Dawson, D.R. Howlett, Anal. Biochem. 346 (2005) 225–233.
[6] J.S. Becker, D. Salber, Trends Anal. Chem. 29 (2010) 966–979.
[7] J.S. Becker, M. Zoriy, A. Matusch, D. Salber, C. Palm, J.Su. Becker, Mass Spectrom. Rev. 29 (2010) 156–175.
[8] J.S. Becker, A. Matusch, C. Palm, D. Salber, K. Morton, J.Su. Becker, Metallomics 2 (2010) 104–111.
[9] L.M. Gjerdrum, S. Hamilton-Dutoit, Methods Mol. Biol. 293 (2005) 139–149.
[10] Y.C. Liu, J.S. Wu, G.Q. Yan, R.P. Hou, D.X. Zhuang, L.P. Chen, Q. Pang, J.H. Zhu, Proteome Sci. 8 (2010) 2.
[11] Y. Chen, M. Gruidl, E. Remily-Wood, R.Z. Liu, S. Eschrich, M. Lloyd, A. Nasir, M.M. Bui, E. Huang, D. Shibata, T. Yeatman, J.M. Koomen, J. Proteome Res. 9 (2010) 4215–4227.
[12] D.M. Kube, C.D. Savci-Heijink, A.F. Lamblin, F. Kosari, G. Vasmatzis, J.C. Cheville, D.P. Connelly, G.G. Klee, BMC Mol. Biol. 8 (2007) 25.

[13] J.S. Becker, D. Salber, Method and device for carrying out a quantitative spatially resolved local distribution analysis of chemical elements and in-situ characterization of the ablated surface regions, Patent (8.4.2009) WO2010115394 A1 granted Dec. 2010.
[14] J. Dombovari, J.S. Becker, H.J. Dietze, Fresenius J. Anal. Chem. 367 (2000) 407.
[15] J.L. Todolí, J.M. Mermet, Spectrochim. Acta B 61 (2006) 239–283.
[16] V.L. Dressler, D. Pozebon, A. Matusch, J.S. Becker, Int. J. Mass Spectrom. 266 (2007) 25–33.
[17] S. D'Ilio, N. Violante, M. Di Gregorio, O. Senofonte, F. Petrucci, Anal. Chim. Acta 579 (2006) 202–208.
[18] S. D'Ilio, N. Violante, S. Caimi, M. Di Gregorio, F. Petrucci, O. Senofonte, Anal. Chim. Acta 573–574 (2006) 432–438.
[19] M.A.Z. Arruda (Ed.), Trends in Sample Preparation, Nova Science Publishers, Hauppauge, 2006.
[20] E.M.M. Flores, J.S. Barin, M.F. Mesko, G. Knapp, Spectrochim. Acta B 62 (2007) 1051–1064.
[21] M. Wasilewska, W. Goessler, M. Zischka, B. Maichin, G. Knapp, J. Anal. At. Spectrom. 17 (2002) 1121–1125.
[22] E.M.M. Flores, A.P.F. Saidelles, J.S. Barin, S.R. Mortari, A.F. Martins, J. Anal. At. Spectrom. 16 (2001) 1419–1423.
[23] CEM Corporation, http://www.cem.com/page108.html.
[24] D. Pozebon, D.L. Valderi, M.F. Mesko, A. Matusch, J.S. Becker, J. Anal. At. Spectrom. 25 (2010) 1739–1744.
[25] J.S. Becker, Int. J. Mass Spectrom. 289 (2010) 65–75.
[26] Leica-microsystems, http://www.leica-microsystems.com/fileadmin/downloads/Leica%20LMD7000/Brochures/Leica_LMD6500-7000-Brochure_en.pdf.