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Determination of methylmercury in human hair by ethylation followed by headspace solid-phase microextraction–gas chromatography–cold-vapour atomic fluorescence spectrometry

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Abstract

An analytical procedure for the determination of methylmercury in human hair after acid digestion using aqueous ethylation, headspace solid-phase microextraction sampling and final gas chromatography–cold-vapour atomic fluorescence spectrometry detection is described. Acid digestion, extraction procedure and chromatographic conditions were optimised. An optimal linear range using standard mercury solutions was found and concentration detection limits for the mercury species, MeHg and Hg^{2+}, were about 50 and 80 ng/g, respectively, for 100 mg of human hair. The reproducibility of the developed analytical procedure assessed for hair samples with incurred MeHg was better than 18% ($n=5$). A certified reference material from the National Institute of Environmental Studies (Japan) was used for validation. Analysis of human hair collected from urban inhabitants was performed and the mean value of methylmercury content in hair samples was 0.764 ± 0.732 μg/g for the population tested. The developed analytical method is simple, fast and a suitable procedure for the monitoring and screening of human exposure to methylmercury.

Keywords: Cold-vapour atomic fluorescence spectrometry; Detection, GC; Headspace analysis; Extraction methods; Methylmercury; Organomercury compounds; Mercury; Metals

1. Introduction

Mercury has been well known as an environmental pollutant for several decades. In the 1950s, it was established that emissions of mercury to the environment could have serious effects on human health. On the hand, environmental cycling of mercury is extremely complicated involving a variety of physical and chemical processes that affect its toxicity and mobility. Critical species in this cycling are elemental Hg vapour (Hg^0), a common form in air, and methylated forms. Generally, human uptake mercury in two ways: as methylmercury (MeHg) from fish consumption, or by breathing vaporous mercury (Hg^0) emitted from various sources such as metallic mercury, dental amalgams, and ambient air. The potential toxicity effects from vaporous mercury are relatively rare. Therefore, methylmercury is the most toxic species of mercury found in the environment because it is able to enter the food chain, accumulating and contaminating humans. In addition, methylmercury, deteriorates the central nervous system, impairs hearing, speech, vision and gait, causes involuntary muscle movements, corrodes skin and mucous membranes, causes chewing and swallowing to become difficult, and in severe cases irreversibly damages areas of brain.

The most well documented cases of severe

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methylmercury poisoning are from Minamata Bay, Japan in 1956 (industrial release) and in Iraq in 1971 (wheat treated with a methylmercury fungicide). Those studies demonstrated that direct intake of fish or food contaminated by methylmercury from human-related activities has extreme damages on human health. In both cases, hundreds of people died, and thousands were affected, many with permanent damage.

Recent studies in the Brazilian Amazon demonstrate that the population had increased exposure to methylmercury because of their consumption of fish contaminated by upstream gold-mining activities [1–3]. For its determination, many studies have been carried out analyzing different human body fluids such as blood and urine [3–6] or tissues such as nail or hair [6–11].

Hair is a suitable indicator for the monitoring of human exposure to mercury that reflects organ mercury levels [12] as well as dietary intake [13]. A convincing relationship between the content of mercury in hair versus its content in blood has been reported in several studies [14–17]. Methylmercury is incorporated into scalp hair at the hair follicle in proportion to its content in blood. The hair-to-blood ratio in humans has been estimated as approximately 250:1 expressed as μg Hg/g hair to μg Hg/l blood. Estimates of threshold levels for neurotoxicity has been performed and considered in detail by the US Environmental Protection Agency (EPA) and the World Health Organization (WHO). The minimum threshold value by the WHO for methylmercury in hair is 50 μg/g, while US EPA estimates lower threshold value as 10 μg/g [18,19].

Conventional methods of methylmercury extraction involves multiple liquid–liquid extraction with hazardous organic solvents (i.e., benzene or toluene) and final determination by gas chromatography with electron-capture detection (GC–ECD) [20], GC with atomic fluorescence spectrometry (AFS) [21], purge-and-trap and atomic absorption spectrometry (AAS) [22] and microwave-induced plasma (MIP) [23]. The introduction of the use of sodium tetraethylborate, as an alkylation reagent, improves the determination procedure since it reduces the analytical time and eliminates the organic solvent extraction [24].

On the other hand, the solid-phase microextraction (SPME) procedure in headspace or aqueous-phase sampling for the analysis of mercury species has been reported previously in fish tissues and river water samples [24,25], in urine [26,27], biological tissues [28] or even in soils [29]. Most of these methods are based on ethylation with sodium tetraethylborate, although direct hydride derivatization of methyl- and ethylmercury chlorides in aqueous solution with KBH_4 has also been reported [30].

In the present study, we report an analytical procedure for the determination of MeHg and Hg^{2+} in human hair after acid digestion using aqueous ethylation with $NaBEt_4$, headspace SPME sampling and final GC–cold-vapour AFS (CVAFS) detection. This is, to our knowledge, the first application of SPME to the determination of methylmercury levels in human hair. The developed analytical procedure is much faster, simpler and does not require organic solvent consumption than the methods previously reported for human hair analysis. Furthermore, since MeHg and Hg^{2+} are completely derivatised to alkylated volatile species, this headspace SPME method does not present fibre damage or carryover. The entire analytical procedure was validated by analysing human hair certified reference material from the National Institute of Environmental Studies (NIES CRM No. 13), Environmental Agency of Japan, and applied to human hair samples from urban inhabitants.

2. Experimental

2.1. Reagents and materials

Methylmercury chloride (CH_3HgCl, 99%) and mercury dichloride ($HgCl_2$, 99.9995%) were purchased from Strem (Newburgport, MA, USA), phenylmercury chloride [$(C_6H_5)HgCl$, 97%] and diphenylmercury [$(C_6H_5)_2Hg$, >96%] were from Fluka (Buchs, Switzerland). Sodium tetraethylborate ($NaBEt_4$, 98%) was purchased from Galab (Geesthacht, Germany) and Strem. Analytical-grade sodium acetate (99%) and acetic acid (99.7%) were obtained from Sigma–Aldrich (Steinheim, Germany). Hydrochloric acid (25%) was obtained from Merck (Darmstadt, Germany).

Stock standards were prepared at 1000 mg/l (as Hg) in acetone. All standards were stored at 4 °C,

and working solutions were prepared weekly by diluting the stock solutions with acetone to a range of 0.5–500 μg/l as Hg. A fresh $NaBEt_4$ solution of 1% (w/v) was prepared daily in deionized water and stored at 4 °C. A buffer solution at pH 4.5 was prepared by mixing sodium acetate (0.2 *M*) and acetic acid (0.2 *M*). All other chemicals were at least of analytical grade.

2.2. Procedure

2.2.1. Sample preparation and acid digestion

In this study, sample preparation and digestion were conducted on a slightly modified methods developed by Akagi et al. [17] at the National Institute for Minamata Disease (NIMD) in Japan.

Hair samples, collected from local sources, were finely cut and placed in a 100-ml beaker and ultrasonically washed with non-ionic detergent solution. After vigorous rinsing with a large volume of deionized water, hair samples were rinsed with acetone. Finally, they were air-dried and weighed until the difference between two sequential masses was less than 5%. Therefore, results are expressed as amount of Hg in dry mass. Phenylmercury chloride (300 μl) was added at a spiking level of 200 ng as a recovery spike (surrogate).

The digestion procedure was carried out as follows, 2 ml of 2 *M* HCl was added to 50–100 mg of finely cut hair placed in a 5-ml Pyrex tube. The tube was capped non-tightly and then heated nearby 100 °C for 15 min.

2.2.2. SPME sampling

After the digest was cooled down to room temperature in an ice bath, a 1-ml aliquot was transferred to a 7-ml glass vial with 3 ml of acetate buffer solution (pH 4.5) containing a magnetic stirring bar. A 300-μl volume of diphenylmercury, as internal standard, was added to the sample. Once the vial was closed, the fibre was drawn into the needle of the holder and was used to drill the septum of the sample vial. Then, 300 μl of 1% aqueous $NaBEt_4$ solution was added and the fibre was lowered and situated into the headspace, about 0.5 cm above the aqueous phase. After 10 min of sampling time under strong stirring conditions, the fibre was retracted into the needle and immediately inserted into the GC injector port for thermal desorption. For calibration, 1 ml of mixed mercury standards (0.5–500 μg/l as Hg) was added in the vial instead of the digest aliquot. The headspace SPME procedure was calibrated with a series of MeHg and Hg^{2+} standards. Then, fresh standards were prepared weekly and were used to carry out calibration graphs. Procedural blanks were carried out for every set of samples.

2.3. Apparatus

2.3.1. SPME device

The SPME fibre holder for manual use and the silica fibre coated with 100 μm thickness of poly(dimethylsiloxane) (PDMS) were obtained from Supelco (Bellefonte, PA, USA). A 7-ml glass vial with PTFE-coated silicone rubber septa was used for headspace SPME. Experiments were performed with magnetic stirring (1200 rpm) to ensure the proper mixing of the sample solution and a 12×2 mm PTFE-coated magnetic stirring bar was used.

2.3.2. GC–CVAFS

The analysis was accomplished with a Carlo Erba FTV 4130 (Milan, Italy) gas chromatograph equipped with a Tekran Model 2500 Mercury Vapor Detector (Toronto, Canada). The CVAFS system is an extremely sensitive detector where the elemental mercury (Hg^0) atoms, in an inert carrier gas stream, are excited by a source of ultraviolet radiation. Excitation and fluorescence occurs at a wavelength of 253.5 nm.

A split/splitless injector was used in the splitless mode and maintained at 170 °C. A 40 s desorption time was used for all fibre injections. A 30 m×0.53 mm I.D. fused-silica column coated with a 1.5 μm film thickness of DB-5 (J&W Scientific, Folsom, CA, USA) was used as analytical column. Initial column temperature was held for 1 min at 50 °C, programmed at 10 °C/min to 250 °C, holding this temperature for 10 min. After the Hg forms were separated, the column effluent was connected to a pyrolytic reactor consisting of a 20 cm×2 mm I.D. quartz glass tube to convert the different mercury species to Hg^0 by thermal decomposition at 900 °C and then detected by CVAFS. Argon gas 5.0 grade at a gas flow of 4 ml/min was used as carrier gas and also as make up gas for the CVAFS detector. Finally,

data were acquired and processed by a Perkin-Elmer Nelson interfaced to a personal computer.

3. Results and discussion

3.1. Sample preparation

The most common methods to determine methylmercury levels in human hair are based on a digestion procedure by alkaline dissolution of human hair and extraction of MeHg into benzene from an acidic solution of potassium iodide [31]; alkaline digestion with cysteine and NaOH, H_2SO_4, and $CdCl_2/SnCl_2$ as reducing agent [32] where MeHg is determined as the difference between total and inorganic mercury; or acid digestion with 5 ml of 2 *M* HCl at 100 °C for 5 min and benzene extraction of an aliquot [17].

Therefore, most of the analytical procedures require several steps (digestion, extraction, reextraction), labour intensive (hours or even overnight digestion) and uses carcinogenic solvents such as benzene. The digestion procedure used in this study was selected according to the method from Akagi et al. with some modifications since it is simple, fast, with low reagent consumption and it provides a final sample digest able to be used by SPME methodology.

The main changes from Akagi et al.'s procedure were: (i) to reduce the volume of acid from 5 to 2 ml, (ii) to increase the amount of human hair from 10 to 100 mg, and (iii) to increase the heating time from 5 to 15 min.

Since ethylation should be performed in a pH range from 4 to 4.5, and the main aim of our procedure was to minimise the amount of all reactants, we need to reduce the acid aliquot volume to be buffered with a small volume (about 3 ml) of acetic acid/acetate buffer. Then, several volumes were tested and about 1 ml of acid aliquot was the maximum to achieve the required final pH suitable for the ethylation reaction.

A second modification was carried out to assure a final mercury concentration of the species above detection limit. Finally, heating time was extended to 15 min since in some cases the sample digestion was not complete when 5 min was applied.

3.2. SPME procedure and GC–CVAFS determination

As described before, the SPME procedure for the analysis of mercury species has been reported previously. In the present study, the MeHg and Hg^{2+} were derivatized to the volatile ethylmercury and diethylmercury species, respectively, by the use of $NaBEt_4$, and then headspace extracted by the PDMS fibre. Derivatization process improves greatly the partitioning of the species between the aqueous sample and the fibre.

As described by Cai and Bayona [24], in the headspace mode, the reaction and extraction equilibration would be reached in about 10 min for both compounds at room temperature. Increasing the extraction temperature, the amount extracted by the fibre is not increased, but in aqueous-phase sampling, the extraction equilibration was extended to 20 min considering the fact that diffusion of analytes is faster in the vapour phase than in the aqueous phase. No significant improvement in the amount extracted by the fibre was found when higher temperature was investigated (35 °C). Therefore, reaction and extraction could be accomplished at room temperature (20–25 °C).

The addition of saturated NaCl (salting out) does not increase the extraction efficiency of the methylated mercury species by the fibre, since the ethylation reaction could be inhibited in presence of high chloride concentration.

Temperature of the injection port is critical, since an inappropriate temperature could cause thermal decomposition of mercury species [21,24,25]. Then, the effect of temperature on the desorption of mercury species for the fibre was investigated. The desorption temperature has some effects on the peak areas of mercury species, especially for MeHgEt. As shown in Fig. 1, the peak area of MeHgEt smoothly decreases from 170 °C to 210 °C. These results may possibly indicate that MeHgEt is fairly decomposed to Hg^0 at high injector temperature. Consequently, the 170 °C desorption temperature was selected to assure an accurate determination of methylmercury species.

Desorption time was also investigated, however no differences were observed, all experiments were performed leaving the SPME fibre deployed in the

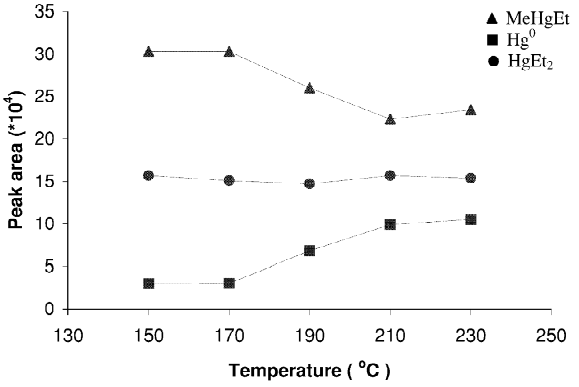

Fig. 1. Effect of injection port temperature on the desorption of MeHg.

injection port during the temperature programming to clean it.

Memory effect experiments were performed in order to determine any problem in the determination of mercury species by the SPME method. Then, after a typical exposure to a standard solution containing both mercury species (MeHg and Hg^{2+}) and their subsequent desorption, a second desorption was accomplished. In this experiment neither compound showed any carryover.

In another experiment, after initial desorption of the fibre, we ran a blank (no standard was added). In this case an apparent peak of $HgEt_2$ was detected (Fig. 2B). The origin of the $HgEt_2$ peak in the chromatogram will be discussed. The procedural blank was composed of anything but the ethylating reagent and the internal standard. As was discussed before by Cai and Bayona [24], the diethylmercury peak could appear due to carryover when absorbed in the first sampling and ethylated in the next sampling. Nevertheless that might occur in the aqueous-phase mode and certainly not in the headspace. The possibility that corresponds to an unknown compound of the ethylating reagent, which could coelute to the $HgEt_2$ peak in the chromatogram, was rejected as soon as was confirmed as $HgEt_2$ by GC–MS. In conclusion, this peak is related to the ethylated species of Hg^{2+} and presumably corresponds to some impurities of the diphenylmercury used as internal standard.

The linearity ranges for both species are from 0.1 to 100 μg/l as Hg for MeHg and 0.1 to 20 μg/l as Hg for Hg^{2+}. Fig. 2A shows a typical chromatogram for mercury chloride solutions. Quantification and recovery calculation were performed by the internal standard procedure using diphenylmercury and phenylmercury chloride, as internal standard and surrogate, respectively. In all cases, the linear regression coefficient of the calibration plots showed an acceptable lineal correlation (r^2>0.992). Linearity range for Hg^{2+} is narrower, compared with the others, probably due to the problems related to poor reagent blanks.

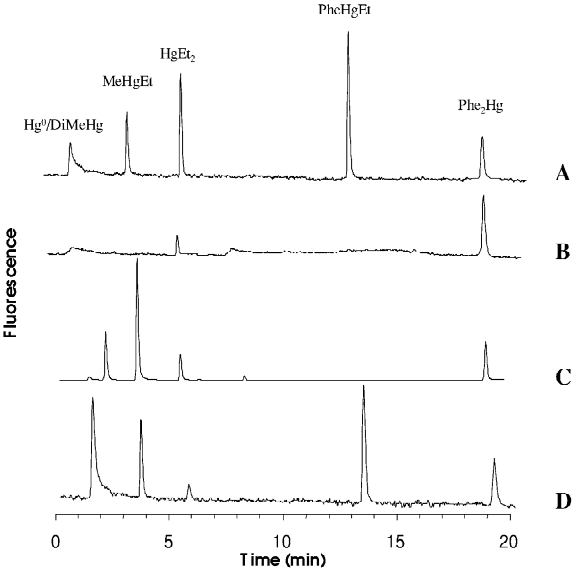

Fig. 2. (A) Selected chromatogram for a typical standard mercury chloride solutions. (B) Chromatogram obtained for a blank sample run. (C) Chromatogram obtained for NIES CRM No. 13. (D) Selected chromatogram for a typical MeHg and Hg^{2+} analysis from human hair sample.

The absolute detection limit is a function of the sample size that could be used in the experiment. In the present study, 4.6 ml of sample was used. For mercury hair analysis, 100 mg of hair was digested in 2 ml of HCl and, from this solution 1 ml was analysed. Absolute detection limits calculated as three times the baseline noise in real samples chromatograms are 2.5 ng for MeHg and 4.0 ng for Hg^{2+}. Concentration detection limits were 0.05 μg/g dry mass for MeHg and 0.08 μg/g dry mass for Hg^{2+}. The reproducibility was assessed for hair samples running 5 independent replicates the relative standard deviation (RSD) being less than 18%.

3.3. Method validation and application to real samples

Fig. 2 shows typical chromatograms obtained for standard mercury chloride solutions (A), and a procedural blank (B). As can be seen, during the analysis of mercury chloride standards, detectable peaks due to Hg^0 and dimethylmercury appeared. As was reported by Fisher et al. [33], compounds that may be formed during derivatization initiate dismutation reactions in solution that might be improved by optimizing the amount of $NaBEt_4$ employed for the specific analytical procedure. Nevertheless, actual optimized conditions provide an excellent separation and under no circumstances does this peak interfere to accomplish quantitative methylmercury determination.

The entire analytical procedure was validated by analysing human hair certified reference material from the National Institute of Environmental Studies (NIES CRM No. 13) for methylmercury in human hair. The MeHg concentration found in NIES CRM No. 3 was 3.6 ± 0.4 μg/g ($n=3$), which is close to the certified value (3.8 ± 0.4 μg/g) [34]. Fig. 2C shows the chromatogram of this certified reference material where methylmercury is the most prominent peak.

The analytical technique was applied to hair samples collected from local sources. As can be seen in Fig. 2D, the main compound found in human hair is methylmercury being detectable the $HgEt_2$ peak probably coming from the ethylation of inorganic labile mercury since some impurities of the products used (internal standard or/and surrogate). Table 1 gives some statistical parameters for the methylmercury concentrations in hair for all the cases analysed. Individual and overall mean values of the methylmercury content in the hair samples (0.764 ± 0.732 μg/g) were below the minimum values are considered by the WHO (50 μg/g) and the EPA (10 μg/g) to possibly induce neurotoxicity in humans. Further studies should be carried out in order to find out if MeHg could impair health effects to our study area.

Table 1
Results of methylmercury contents in human hair samples analysed from urban inhabitants and the NIES CRM No. 13

Parameter	MeHg value (μg/g)
Mean ($n=5$)	0.764
Minimum value	0.164
Maximum value	2.034
Standard deviation	0.732
NIES CRM No. 13 Human hair	
Certified value	3.8 ± 0.4
Measured values ($n=3$)	3.6 ± 0.4

In summary, quantitative determination of MeHg from human hair could be achieved using the proposed methodology, performing acid digestion, in situ derivatization with $NaBEt_4$ followed by headspace SPME and final GC–CVAFS detection.

This developed analytical method is simple, fast, reliable, solvent-free, low-priced, reproducible and suitable for the monitoring and screening of human exposure to mercury species.

References

[1] J. Lebel, D. Mergler, F. Branches, M. Lucotte, M. Amorim, F. Larribe, J. Dolbec, Environ. Res. 79 (1998) 20.
[2] P. Granjean, R. White, A. Nielsen, D. Cleary, D. Oliveira Santos, Environ. Health Perspect. 197 (1999) 587.
[3] J. Dolbec, D. Mergler, C.J. Sousa Passos, S. Sousa de Morais, J. Lebel, Int. Arch. Occup. Environ. Health 73 (2000) 195.
[4] S.M. Talebi, F. Tavakoli-Ghinani, M. Ahmadi-Golsephidi, Biotechnol. Environ. Sci. 1 (1999) 73.
[5] L. Liang, C. Evens, S. Lazoff, J.S. Woods, E. Cernichiari, M. Horvat, M.D. Martin, T.J. DeRouen, J. Anal. Toxicol. 24 (2000) 328.
[6] H. Akagi, H. Nishimura, in: T. Suzuki, N. Imura, T.W. Clarkson (Eds.), Advances in Mercury Toxicology, Plenum Press, New York, 1991, p. 53.
[7] M. Dermelj, M. Horvat, A.R. Byrne, P. Stegnar, Chemosphere 16 (1987) 877.
[8] K. Ayyadurai, J. Environ. Biol. 9 (1988) 281.
[9] O. Malm, F.J.P. Branches, H. Akagi, M.B. Castro, W.C. Pfeiffer, M. Harada, W.R. Bastos, H. Kato, Sci. Total Environ. 175 (1995) 141.
[10] N.B. Al-Majed, M.R. Preston, Environ. Pollut. 109 (2000) 239.
[11] D. Babi, M. Vasjari, V. Celo, M. Koroveshi, Sci. Total Environ. 259 (2000) 55.
[12] T. Suzuki, T. Hongo, J. Yoshinaga, H. Imai, M. Nakazawa, N. Matsuo, H. Akagi, Arch. Environ. Health 48 (1993) 221.
[13] D. Airey, Sci. Total Environ. 31 (1983) 157.
[14] T.W. Clarkson, Environ. Health Perspect. 100 (1992) 31.
[15] W.C. Pfeifer, L.D. Lacerda, W. Salomons, O. Malm, Environ. Rev. 1 (1993) 26.

[16] E. Cernichiari, R. Brewer, J.G. Myers, D.O. Marsh, L.W. Lapham, C. Cox, C. Shamlaye, F. Conrad, M. Berlin, P.W. Davidson, T.W. Clarkson, Neurotoxicology 16 (1995) 705.
[17] H. Akagi, O. Malm, F.J.P. Branches, Y. Kinjo, Y. Kashima, J.R.D. Guimaraes, R.D. Oliveira, K. Haraguchi, W.C. Pfeiffer, Y. Takizawa, H. Kato, Water Air Soil Pollut. 80 (1995) 85.
[18] WHO, ICPS: International Programme on Chemical Safety. Environmental Health Criteria 101: Methylmercury, World Health Organization, Geneva, 1990.
[19] EPA (US Environmental Protection Agency), Mercury Study Report To Congress, An Assessment of Exposure To Mercury in the United States, Vol. IV, EPA, Office of Air Quality Planning and Standards and Office of Research and Development, 1997, EPA/452/R-97-006.
[20] E. Bulska, D.C. Baxter, W. Frech, Anal. Chim. Acta 249 (1991) 545.
[21] L. Liang, M. Horvat, N.S. Bloom, Talanta 41 (1994) 371.
[22] R. Fischer, S. Rapsomanikis, M.O. Andreae, Anal. Chem. 65 (1993) 763.
[23] A.M. Carro-Díaz, R.A. Lorenzo-Ferreira, R. Cela-Torrijos, J. Chromatogr. A 683 (1994) 245.
[24] Y. Cai, J.M. Bayona, J. Chromatogr. A 696 (1995) 113.
[25] Y. Cai, S. Monsalud, K.G. Furton, R. Jaffe, R.D. Jones, Appl. Organomet. Chem. 12 (1998) 565.
[26] L. Dunemann, H. Hajimiragha, J. Begerow, Fresenius' J. Anal. Chem. 363 (1999) 466.
[27] M. Guidotti, M. Vitali, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999, p. 229.
[28] Z. Mester, J. Lam, R. Sturgeon, J. Pawliszyn, J. Anal. At. Spectrom. 15 (2000) 837.
[29] A. Beichert, S. Padberg, B.W. Wenclawiak, Appl. Organomet. Chem. 14 (2000) 493.
[30] B. He, G. Jiang, Z. Ni, J. Anal. At. Spectrom. 13 (1998) 1141.
[31] K. Kratzer, P. Benes, V. Spevackova, Int. J. Environ. Anal. Chem. 57 (1994) 91.
[32] A.C. Barbosa, A.M. Garcia, J.R. De Souza, Water Air Soil Pollut. 1 (1997) 97.
[33] R. Fisher, S. Rapsomanikis, M.O. Andreae, Anal. Chem. 65 (1993) 763.
[34] J. Yoshinaga, M. Morita, K. Okamoto, Fresenius J. Anal. Chem. 357 (1997) 279.