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Antimony speciation in environmental samples by interfacing capillary electrophoresis on-line to an inductively coupled plasma mass spectrometer

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Abstract

Antimony is a widely distributed trace element of ecotoxicological interest. A pathway via bioalkylation of inorganic Sb species is discussed in the literature, resulting in organically bound Sb species. Therefore, Sb speciation becomes increasingly a matter of interest for risk assessment in the environment. This contribution investigates the possibilities of CE on-line hyphenated to ICP–MS for Sb speciation. Two methods are employed, both highly resolving the species but only one preserving the species stability. The latter used Na_2HPO_4/ NaH_2PO_4, 20 m*M*, pH 5.6 as the background electrolyte and NaOH or acetic acid as stacking electrolyte 1 or stacking electrolyte 2, respectively. Detection limits of 0.1 μg/l–0.7 μg/l, depending on species, were achieved. When analysing liquid phases from fouling and sewage sludge up to eight antimony species were detected. Sb (V) as well as methylated Sb species were found.

Keywords: Speciation; Sample stacking; Sewage sludge; Inductively coupled plasma mass spectrometry; Antimony

1. Introduction

Antimony is a widely distributed trace element of toxicological interest found in the environment mainly as inorganic Sb (V) and Sb (III). However, organic antimony compounds are also described in environmental samples [1–6]. For the chemically similar arsenic, the formation of these compounds via bioalkylation is well documented [7]. A similar pathway via bioalkylation of inorganic Sb species is discussed, too, resulting in organically bound Sb species. The experimental proof, however, still remains to be obtained. One reason for the limited data about organic antimony compounds in the environment is possibly the lack of reliable methods for determining individual antimony species.

This contribution employs capillary electrophoresis (CE) for a highly resolving separation of Sb species. For element-selective Sb detection at low concentrations the capillary electrophoresis system has been interfaced on-line to an inductively coupled plasma mass spectrometer (ICP–MS). The hyphenation has been already performed in earlier investigations [8,9] and was set up in analogy to Ref. [10]. The first experiments for Sb speciation were started during the late hyphenation experiments for Se speciation. Therefore, first investigations used the ‘Se-speciation method’ described in Ref. [10] after slight modifications, which showed high resolving power. During method development it turned out that this method showed good results for e.g. Sb (V).

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However, other Sb species, such as trimethyl antimony dichloride [$(CH_3)_3SbCl_2$], were not stable under these conditions. Further investigations focused on a method derived from arsenic speciation [11] (with modifications). Arsenic is known to be chemically similar to Sb. This method turned out to cause no stability problems for Sb species. The separation potential was sufficient for separating four Sb standards. The detection limits were improved to 0.1 μg Sb/l, being suitable for application to environmental samples even at low concentrations. Liquid phase samples of fouling and sewage sludges were analysed.

2. Experimental

2.1. Chemicals and reagents

Standard stock solutions for Sb (III) (100 mg Sb/l) and Sb (V) (100 mg Sb/l) were prepared by dissolving the appropriate amount of potassium antimonyl tartrate trihydrate (Aldrich, A.C.S) and of potassium hexahydroxyantimonate (Fluka, p.a.) in Milli-Q H_2O. Trimethyl-antimony dichloride [$(CH_3)_3SbCl_2$] was a donation of Prof. W. Cullen, Vancouver Canada, and synthesised there according to published methods [12,13]. A standard stock solution of this compound was prepared by dissolution in water. All stock solutions were stored in the dark at 4°C. Working standard solutions of lower concentrations were prepared daily by appropriate dilution with Milli-Q H_2O. A standard stock solution of $Sb(OH)_3$ was prepared daily by making a saturated solution of Sb_2O_3 (Aldrich, 99.999%) in Milli-Q H_2O. The solution was centrifuged and the supernatant solution was diluted with Milli-Q H_2O. The actual Sb concentration of this solution was determined by ICP–MS.

2.2. CE buffers

'Method A': Na_2CO_3 (50 m*M*, pH 11.6) was prepared by dissolving the appropriate amount in Milli-Q H_2O. This solution was used as 'stacking electrolyte', or, after dilution with Milli-Q H_2O as background electrolyte (20 m*M*). The 'stacking electrolyte 2' (termination) was prepared by mixing the background electrolyte with acetonitrile ($v+v=1+1$) [11,14].

'Method B': Due to stability problems with 'method A', a second method was developed, modifying the separation technique described in [11]. Na_2HPO_4/NaH_2PO_4, 20 m*M*, pH 5.6 was used as the background electrolyte and for sheathflow from the outlet electrode to capillary end within the nebuliser. NaOH (100 m*M*) served as 'stacking electrolyte 1', whereas acetic acid (1%, pH 2) was used as 'stacking electrolyte 2' (termination).

Acetonitrile, NaOH, acetic acid, Na_2CO_3, NaH_2PO_4 and Na_2HPO_4 were obtained from Merck (Darmstadt, Germany). Ar and Ar/H_2 were obtained from Messer Griesheim (Munich, Germany). The capillary was bought from CS-Chromatographie Service GmbH (Langerwehe, Germany).

2.3. Samples

First single standards and standard mixtures were used as samples for determining detection times, resolution and concentration detection limits (DL). Concentrations of standard solutions for calibration curves were: $c_{Sb}=0$, 0.5, 1, 10, 50 each μg/l. DL were determined according IUPAC recommendations (3σ criterion) using standard addition method.

Subsequently, environmental samples were analysed. A fouling sludge and a sewage sludge were sampled and kindly donated by Dr. E. Krupp.[1] Aliquots (1 ml) of each sludge were centrifuged at 25 800 *g*, 30 min 8°C and the supernatant used as a sample for CE–ICP–MS determination and total Sb determination by ICP–MS.

2.4. Capillary zone electrophoresis

A 'Biofocus 3000' capillary electrophoresis system (BioRad, Munich, Germany) was used as the CE device. The temperature was set to 20°C for sample/buffer carousels by air cooling and also 20°C for the total capillary by liquid cooling.

[1] Institut für Umweltanalytik und Geowissenschaften, Universität Essen, Germany.

2.5. Operation

Before each run, the capillary was purged with Milli-Q H_2O (180 s, 8 bar) and running buffer (180 s, 8 bar).

The separation methods used different buffers and stacking electrolytes. The stacking procedures were based only on conductivity ('method A') or on conductivity and pH stacking ('method B'). Table 1 shows an overview about CE conditions.

Based on former experiences, separation was differentiated from the detection step [8,9]: After 10 min, separation inlet buffer was forced into the capillary (pressure driven, 8 bar, 130 s), moving separated species to ICP–MS. This resulted in a flow of ca. 1.5 μl/min.

This 'two-step procedure' is derived from capillary isoelectric focusing [15–17]. After application of pressure at the capillary inlet, the separated species move to ICP–MS. The pattern of separated molecules is not compromised and is monitored without interference at the end of capillary.

2.6. Interfacing and nebulisation

For interfacing CE with ICP-MS a 'user assembled cartridge'(BioRad, Munich), equipped with a uncoated capillary (150 cm×50 μm ID) was employed. This cartridge allows a CE–ICP-MS coupling with the liquid coolant bypassing the UV detection window and leaving the cartridge within a tubing coaxially around the capillary up to the nebuliser. Thus, a temperature control along the whole capillary is possible.

The CE nebuliser was laboratory made and especially designed for the requirements of CE–ICP-MS interface [8,10]. Special care was focused on an exact and optimised positioning of the capillary end. The CE capillary was fixed to a nut by a screw and silicone seal. When turning the nut, it was moving closer to the body of the nebuliser (or vice versa), thus, also moving the CE capillary. A reliable closing of the CE electrical circuit during nebulisation was provided by a coaxial sheath flow (10 μl/h buffer) around the CE capillary. This sheath flow filled a buffer reservoir at the end of the nebuliser, with the outlet electrode dipping into this electrolyte reservoir. From there, the buffer flows coaxially around the CE capillary to the capillary's end at the point of nebulisation.

The nebuliser fitted tightly into a laboratory made spray chamber which was specially designed considering the nebulisation characteristics of the CE–ICP-MS nebuliser [10]: An additional Ar/H_2-gas stream was directed to the torch of the ICP-MS and 'coated' the inner surface of the spray chamber. This avoided an aerosol condensation at the walls of the chamber and the aerosol was transported completely into the plasma. The very small amount of aerosol coming from the CE capillary is transported more efficiently to the Ar plasma. Therefore detection limits and signal stability (especially at low concentrations) were improved.

2.7. Estimation of a possible suction driven flow by nebulisation gas stream

A liquid flow within the capillary, produced by a

Table 1
CE conditions for the separation methods A and B

Method	Injection	Stacking electrolyte 1	Electrolyte	Stacking electrolyte 2[a] (termination)	High voltage
Method A	15 s, 8 bar	Na_2CO_3 50 m*M*, pH 11.6 7 s, 8 bar	Na_2CO_3 20 m*M*, pH 11.6	Na_2CO_3 20 m*M* +acetonitrile $v+v=1+1$	−18 kV
Method B	15 s, 8 bar	NaOH 20 m*M*, pH 14 15 s, 8 bar	NaH_2PO_4/Na_2HPO_4, 20 m*M*, pH 5.6	Acetic acid, 1% pH 2	−18 kV

Before each run, the capillary was purged with Milli-Q H_2O (180 s, 8 bar) and running buffer (180 s, 8 bar).
[a] 'Stacking electrolyte 2' was positioned in the inlet vial, without injection into the capillary.

suction from the nebulisation gas stream might alter the separation. Therefore the occurrence of such an alteration was estimated according to [18].

2.8. Antimony determination

The ICP–MS (ELAN 5000, Perkin Elmer, Sciex) was used in the graphic mode with the laboratory made nebuliser fitting into a laboratory-made spray chamber (ID corresponding to OD of nebuliser). Antimony was determined at $m/z=121$. Instrumental parameters were derived from Ref. [19] with the following modifications:

RF power: 1200 W; nebuliser gas: Ar 0.85 l/min.; auxiliary gas for spray chamber: Ar/H_2 0.2 l/min. The total gas flow (nebuliser gas+auxiliary gas) was 1.085 l/min, being again in the optimal range already found in former investigations [8–11,18]. The dwell time was set to 50 ms.

For total Sb quantifications without CE separation conventional ICP–MS was used. Parameters were set in analogy to [19]. The detection limit of this method was 0.01 μg Sb/l.

3. Results

First investigations concerned a possibly occurring suction flow. The results showed that there was no flow produced by the nebulisation gas stream when our laboratory-made interface was used under the operational conditions described. This agrees with former findings [8–11,18].

The experiments for Sb speciation started with the application of 'method A' to standard solutions. A good peak shape was obtained especially for Sb (V). Detection time was highly reproducible at 18 s and the signal-to-noise ratio was excellent ($s/n=130$ at a Sb concentration of 100 μg/l, not shown). Unfortunately, the results for the other Sb standard solutions were less satisfactory with 'method A': Sb tartrate was not stable under these conditions. The baseline was noisy and the component produced three Sb selective peaks at 12 s, 18 s and 22 s (main peak), the latter just showing a $s/n=11$ at a Sb concentration of 100 μg/l. $Sb(OH)_3$ was unstable, too, and produced two peaks. The very small one appearing at 18 s, the main peak at 27 s, having a considerable tailing and a $s/n=80$ at a Sb concentration of 100 μg/l (not shown). When analysing trimethyl-antimony dichloride, again instability problems occurred. Two peaks were detected at ca. 15 s and ca. 20 s, showing only a $s/n=15$ (peak at 15 s) or 7 (peak at 20 s). The noise was significantly increased after the peaks appeared. Fig. 1 demonstrates the electropherogram of trimethyl antimony dichloride, using 'method A'.

Summarising these results, it turned out that 'method A' was resolved well but also destroyed the Sb species. Therefore, a 'method B', having conditions less destructive (e.g. pH in the middle range) was used. Here, the Sb species remained stable during analysis.

Fig. 2 shows the analysis of standard solutions at concentrations of 10 μg/l and 1 μg/l. Sb(V) appears at 3.3 s in both electropherograms. It is clearly resolved from $Sb(OH)_3$ but not totally separated from Sb(III) tartrate. At 1 μg/l the $s/n=7$, resulting in a $DL_{(Sb\ (V))}$ of 0.1 μg/l. This unsatisfactory resolution of Sb(V) from Sb(III) tartrate results from the broad peak shape of Sb(III) tartrate, which has its peak maximum at 10.4 s. However, the peak starts already at 6 s and lasts up to 22 s. At 1 μg/l, the $s/n=7$ and the $DL_{(Sb\ (III)\ tartrate)}$ is ca. 0.1 μg/l.

$Sb(OH)_3$ appears at 17 s, well resolved from Sb(V) and $(CH_3)_3SbCl_2$. Due to the broad peak of Sb(III) tartrate no complete separation from this compound is possible. At a concentration of 10 μg/l, $s/n=4$, resulting in a significantly worse detection

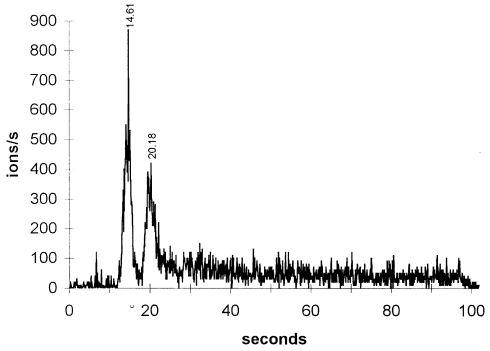

Fig. 1. Electropherogram of a trimethyl-antimony dichloride standard solution (100 μg/l) when using 'method A'. The integrity of the Sb species is obviously violated, shown by the two peaks for one species. Trimethyl-antimony dichloride is not stable under these alkaline conditions.

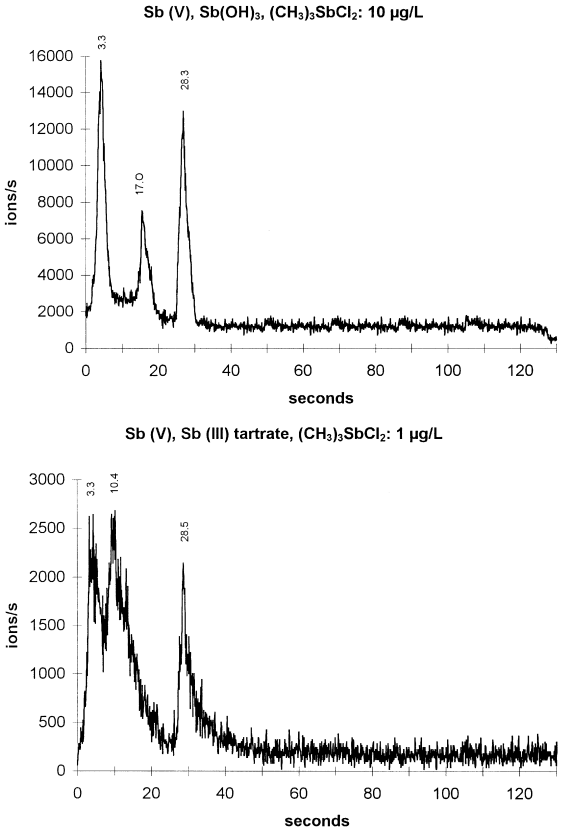

Fig. 2. Standard applications are shown for Sb(V), $Sb(OH)_3$ and $(CH_3)_3SbCl_2$, each 10 μg/l (upper trace) and Sb(V), Sb(III) tartrate and $(CH_3)_3SbCl_2$, each 1 μg/l (lower trace). Detection limits are calculated according to IUPAC recommendations (3σ criterion) between 0.1 μg/l [Sb(V)] and 0.7 μg/l [$Sb(OH)_3$].

limit of 0.7 μg/l. $(CH_3)_3SbCl_2$ showed a $s/n=6.5$ at 1 μg/l and a $DL_{[(CH_3)_3SbCl_2]}$ of 0.12 μg/l.

The concentration detection limits being below 1 μg/l for all Sb species and the successful separation (only Sb(III) tartrate caused some problems, but it was not considered very likely that this compound would be present in environmental samples) of most Sb species encouraged us to analyse environmental samples.

Fig. 3 shows the electropherogram of the liquid phase from a fouling sludge. Peak identifications and quantifications were performed by standard additions of the adequate species.

Peaks at different detection times compared to standards could not be identified. In this case, quantities were 'estimated' by relating peak areas to

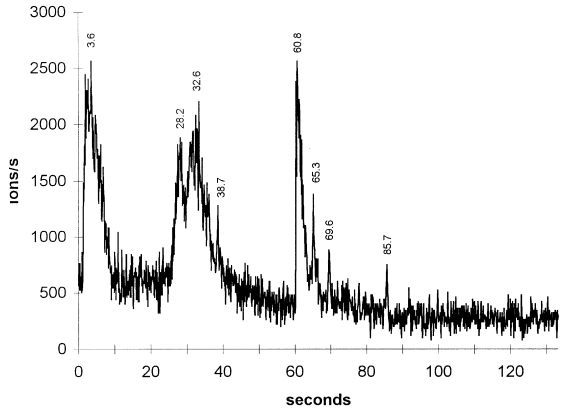

Fig. 3. Electropherogram of the liquid phase of a fouling sludge. The total Sb concentration in this sample is 5 μg Sb/l. Peaks at 3.6 s and 28.2 s were identified as Sb(V) and methylated Sb species by standard addition (c.f. Table 2).

a Sb(V) calibration curve. This estimation was done to get information about mass balances. Table 2 gives information about peak identity and quantity.

Only two peaks could be identified and quantified by standard additions: Sb(V) and $(CH_3)_3SbCl_2$. The latter was not totally resolved from an unknown peak at 32.6 s. This was one of the two main peaks remaining unidentified. Unidentified peaks were not quantified according an adequate Sb species (as it was still unknown). Therefore, their quantities were only estimated. Nevertheless the sum of all peak areas (quantified and estimated) was close to the total Sb amount in this sample (c.f. Table 2). Both peaks which were not identified (at 32.6 s and 60.8 s), contributed considerably to the total Sb amount in this sample.

Another example for analysing environmental samples is shown on an electropherogram in Fig. 4. There, a comparison of the electropherogram of a sewage sludge without (lower trace) or with a 5 μg/l standard addition of trimethyl-antimony dichloride is shown (upper trace). Several peaks are identified and quantified by standard additions as shown in Table 3. Peaks, which were not identified, were estimated in concentration by relating peak areas to a Sb(V) calibration curve. The sum of all peak areas (quantified and estimated) again was close to the total Sb amount in this sample (c.f. Table 3). In the native sample trimethyl-antimony dichloride is detected at 28.0 s at 0.25 μg/l and just resolved from an

Table 2
Information about detection times, species identity and species quantity in the liquid phase of a fouling sludge

Fouling sludge: total Sb amount in the liquid phase: 5.0±0.2 μg/l									
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Sum
Detection time (s)	3.6	28.2	32.6	38.7	60.8	65.3	69.6	85.7	
Identity std.-add.	Sb (V)	$(CH_3)_3SbCl_2$	[a]	[a]	[a]	[a]	[a]	[a]	
Quantity (μg/l)	1.8 ±0.19	0.4 ±0.11	1.5[b]	1[b]	0.1[b]	<DL[b]	<DL[b]	<DL[b]	4.8[b]

[a] Not identified.
[b] Quantities estimated according to a Sb(V) calibration curve. As no identification was possible, also no species-related quantification was possible. For elucidating mass balances the estimation of species quantity was performed. The mass balance (sum of species quantities/total Sb amount) was determined at 96%. <DL = Below detection limit. std.add. = Identification by standard addition procedure.
No standard deviations are given for 'estimated' values.

unknown Sb species having a Sb concentration of ca. 4.8 μg/l. The standard addition of 5 μg/l clearly increases the peak of trimethyl-antimony dichloride, giving an example for the identification of this compound. Again the Sb species at 32.0 s (not identified, cf. Fig. 3) is present in considerable amounts. This species is clearly differentiated from trimethyl-antimony dichloride by the standard addition procedure.

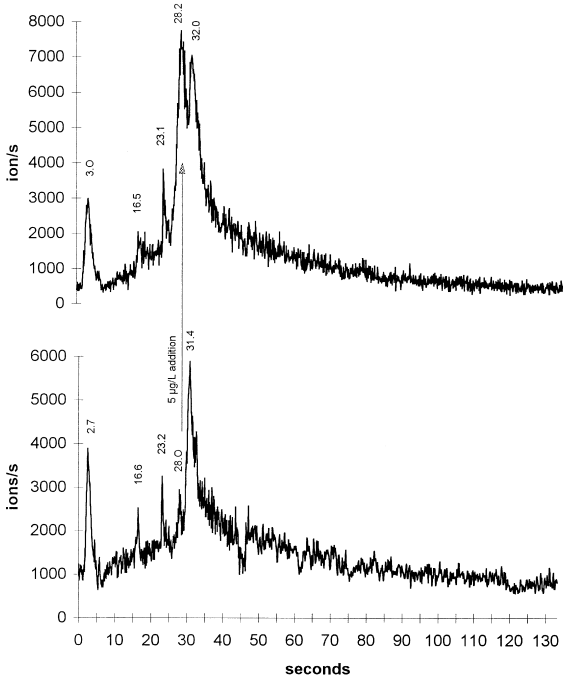

Fig. 4. A comparison of the electropherogram of a sewage sludge without (lower trace) or with a 5 μg/l standard addition of trimethyl antimony dichloride is shown (upper trace). Peaks at 2.7 s and 28.0 s were identified as Sb(V) and methylated Sb-species by standard addition (c.f. Table 3). The total Sb concentration in this sample is 8.9 μg Sb/l.

4. Discussion

The initial experiments with 'method A' showed that the separation of Sb species was sufficient but stability problems for Sb species occurred. This was possibly due to the alkaline pH of the background electrolyte [20] . Therefore, 'method A' was considered not to be suitable for Sb speciation.

The second 'method B' however, proved to fulfil the requirements for Sb speciation. Sb species obviously were not altered, when using a background electrolyte with pH 5.8. Separation efficiency was high and detection limits were low (0.1–0.7 μg/l).

The analysis of environmental samples resulted in electropherograms showing partly Sb species which could be identified, but also several Sb species remained unknown. This is due to the fact that CE–ICP–MS principally provides elemental information (within the species) but no structural information like e.g. CE–ESI–MS [21–23]. Identifications can only be obtained, when adequate standard solutions are available (for comparing detection times or performing standard additions). However, identifications and quantifications were clear when standard addition procedures were possible (c.f. Fig.

Table 3
Information about detection times, species identity and species quantity in the liquid phase of a sewage sludge

Sewage sludge: total Sb amount: 8.9±0.25 μg/l						
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Sum
Detection time (s)	2.7	16.5	23.2	28.0	31.4	
Identity std.-add.	Sb(V)	$Sb(OH)_3$	[a]	$(CH_3)_3$ $SbCl_2$	[a]	
Quantity (μg/l)	1.6 ±0.2	1.1 ±0.2	0.3	0.25 ±0.06	4.8	8.05

[a] = not identified.
[b] = Quantities estimated according to a Sb(V) calibration curve. As no identification was possible, also no species-related quantification was possible. For elucidating mass balances the estimation of species quantity was performed. The mass balance (sum of species quantities/total Sb amount) was determined at 90%. <DL = Below detection limit. std.add. = Identification by standard addition procedure.
No standard deviations are given for 'estimated' values.

4). Although, the concentration of not identified species were only 'estimated', mass balances were satisfactory. The sum of peak concentrations always met the total Sb amount in the samples. When considering inorganic Sb species, it is remarkable, that only Sb(V) was found in the liquid phase of the fouling sludge sample. This agrees with findings from reference [24]. There the authors found Sb(III) generally sticking to the solid-phase in a soil (not extractable). Sewage sludge, however, showed Sb(V) and ($Sb(OH)_3$) within the same concentration range. The small peak of ($Sb(OH)_3$) resulted in a similar concentration to the bigger Sb(V) peak, as ($Sb(OH)_3$) was detected less sensitively (c.f. Fig. 2).

The existence of trimethyl-antimony species in these fouling and sewage sludge samples was clearly proven by standard addition methods. This result principally agrees with the detection of volatile methyl-Sb species in the gas phase above the sludges [25].

Future investigations will focus on the identifications of the unknown Sb species.

5. Abbreviations

CE	Capillary electrophoresis
DL	Detection limit
ICP–MS	Inductively coupled plasma mass spectrometry
s/n	Signal-to-noise ratio.

References

[1] M.O. Andreae, J.F. Asmode, P. Foster, L. Van't Dack, Anal. Chem. 53 (1981) 1766.
[2] A.A. Benson, in: P.J. Craig, F. Glocking (Eds.), The Biological Alcylation Of Heavy Elements, Royal Society of Chemistry, London, p. 135.
[3] J. Feldmann, A.V. Hirner, Intern. J. Environ. Anal. Chem. 60 (1995) 339.
[4] E.M. Krupp, R. Gruemping, U.R.P. Furchtbar, A.V. Hirner, Fresenius' J. Anal. Chem 354 (1996) 546.
[5] M. Dodd, S.A. Pergantis, W.R. Cullen, H. Li, G.K. Eigendorf, K.J. Reimer, Analyst 121 (1996) 223.
[6] H. Gürleyük, V. Van Fleet-Stadler, T.G. Chasteen, Appl. Organomet. Chem. 11 (1997) 471.
[7] W.R. Cullen, K.J. Reimer, Chem. Rev. 89 (1989) 713.
[8] B. Michalke, P. Schramel, Fresenius' J. Anal. Chem. 357 (1997) 594.
[9] B. Michalke, S. Lustig, P. Schramel, Electrophoresis 18 (1997) 196.
[10] B. Michalke, P. Schramel, Electrophoresis 19 (1998) 270.
[11] B. Michalke, P. Schramel, Electrophoresis (1998) in press.
[12] M. Dood, S.L. Grundy, K.J. Reimer, W.R. Cullen, Appl. Organomet. Chem. 6 (1992) 207.
[13] G. Koelb, K. Kalcher, K.J. Irgolic, J. Autom. Chem. 15 (1993) 37.
[14] Z.K. Shihabi, 4th Göttinger Capillary Electrophoresis Symposium, Göttingen, 22-23.10.1997.
[15] M.S. Chen, J.E. Wiktorowicz, Anal. Biochem. 206 (1992) 84.
[16] F. Foret, O. Müller, J. Thorne, W. Götzinger, B.L. Karger, J. Chromatogr. A 716 (1995) 157–166.
[17] F. Kilar, 2nd Göttinger Capillary Elektrophoresis Symposium, Göttingen, 12-13.10.1994.
[18] B. Michalke, P. Schramel, J. Chromatogr. A 750 (1996) 51.
[19] P. Schramel, I. Wendler, S. Lustig, Fresenius' J. Anal. Chem. 353 (1995) 115.
[20] J. Lintschinger, personal communication.

[21] H. Chasseigne, R. Lobinsky, First International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences, München, 04.-07.05.1998.
[22] O. Schramel, B. Michalke, A. Kettrup, First International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences, München, 04.-07.05.1998.
[23] B. Michalke, O. Schramel, A. Kettrup, First International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences, München, 04.-07.05.1998.
[24] J. Lintschinger, B. Michalke, S. Schulte-Hostede, P. Schramel, Int. J. Environ. Anal. Chem. 67 (1998) 1.
[25] Dr. Eva Krupp, personal communication.