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DETERMINATION OF INORGANIC ANTIMONY SPECIES BY HYPHENATED TECHNIQUE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY DETECTION

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Abstract: The article presents the hyphenated technique of high performance liquid chromatography and hydride generation atomic absorption spectrometry (HPLC-HG-AAS) in determination of antimony inorganic species: Sb(III) and Sb(V) in ground water samples. While carrying separation of these forms on an anion-exchange column in a chromatographic system and detection by means of hydride generation atomic absorption spectrometry method, the analytical signals of the determined forms were separated at the detection limits of 6.8 ng/cm^3 (peak high) or 2.7 ng/cm^3 (peak area) in the case of Sb(III) and 4.8 ng/cm^3 (peak high) or 3,2 ng/cm^3 (peak area) in the case of Sb(V) with RSD below 20% at the concentration of 25 ng/cm^3. The hyphenated technique was applied for antimony determinations in polluted ground water.

INTRODUCTION

Procedures of species separation and their selective determinations available in different analytical methods (spectrophotometric, spectrometric or electrochemical) are usually complicated [10, 11, 13, 14] and their results may be approximate (with high uncertainty). The most favorable methods for determination of different forms of elements which occur in the environment are hyphenated techniques with the element-specific detection followed by chromatographic separation. The research on determination of inorganic Sb(III) and Sb(V) forms with use of the selective detection methods [6] prevail in the field of antimony species determinations, however, the researchers are also interested in determination of antimony organic forms, e.g. methylo-derivatives [15]. Although the plasma methods dominate distinctly in the field of antimony species detection after their separation in the system of liquid chromatography [3, 19, 21], in several investigations, systems of atomic absorption spectrometry [20] or atomic fluorescence [15] hyphenated with hydride generation technique were used as the chromatographic detectors. The article presents optimization procedures and determinations of antimony species: Sb(III) and Sb(V) in ground water samples with use of the hyphenated technique: HPLC-HG-AAS.

EXPERIMENTAL

Instruments

The hyphenated system constructed is schematically shown in Figure 1. It comprises a liquid chromatograph Shimadzu LC-10A equipped with a pump (LC-10AT) with a degassing unit (GT-104) and 250 mm anion-exchange column Supelco LC-SAX1 (250 mm, 4.6 mm i.d., resin particle size 5 μm) thermostatted by column oven (CTO-10ASvp). The tubings used were made of PEEK. The detector was an atomic absorption spectrometer Varian SpectrAA 220FS equipped with a hydride generation unit VGA 77 (flow-through system) with an electrothermally heated quartz cell (ETC-60) as an atomizer and a lamp with hollow cathode (HCL Varian) for antimony determination. Argon was a carrier gas. The conditions of chromatographic separation experimentally established, concentrations of reagents used in the system of hydride generation and the conditions of AAS determinations are presented in Table 1.

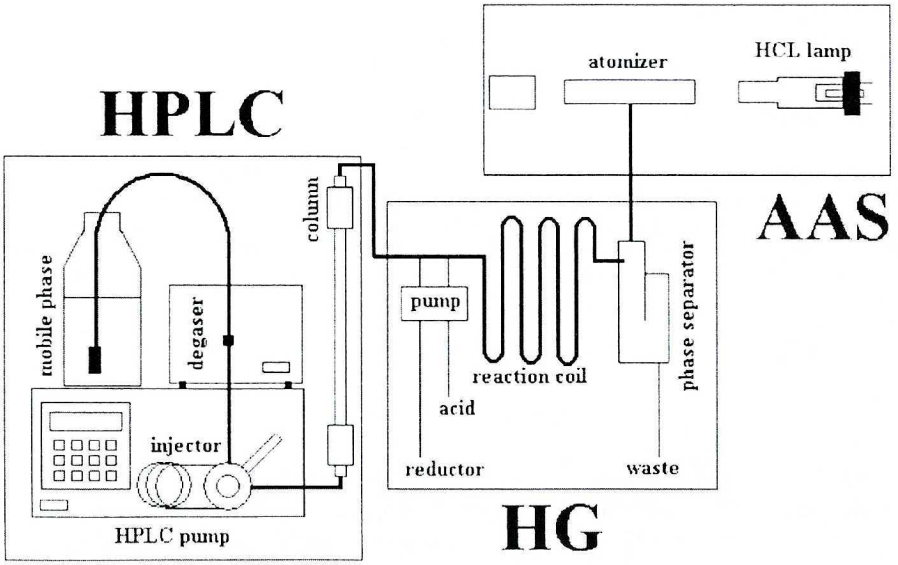

Fig. 1. Schematic diagram of the HPLC-HG-AAS instrumentation

Table 1. Experimental condition for antimony inorganic speciation analysis by HPLC-HGAAS

HPLC	
Column	Supelco LC-SAX1 250 mm, 4.6 mm i.d.
Mobile phase	ammonium tartrate
Flow rate	4 cm^3/min
Injection volume	$200 \cdot 10^{-6}$ dm^3
Hydride generation	
HCL concentration / flow	10 mol/dm^3 / 1 cm^3/min
$NaBH_4$ concentration / flow	2% / 1 cm^3/min
AAS	
Atomizer temperature	900°C
Lamp current	10 mA
Wavelength / slit	217.6 nm / 0.2 nm

Reagents

The chemical reagents used were analytically pure and the water was redistilled and subjected to the ion-exchange process in a Milli-Q unit (Milipore). The solutions of the standards at a different degree of oxidation (Sb(III) and Sb(V)) of the concentration of 1 mg/cm^3 were made of appropriate weighted portions of potassium hexahydroxyantimonate(V) ($KSb(OH)_6$) and potassium antimony tartrate ($C_4H_4KO_7Sb$) (Sigma-Aldrich). The solutions of lower concentration were obtained by their dilution. For determination in the hyphenated system the standard solutions containing both antimony species in one solution were prepared. The solution of sodium borohydride was made on the day of the analysis by dissolving $NaBH_4$ in a 1% (w/v) solution of sodium hydroxide. The solution of hydrochloric acid was made from a 10 mol/dm^3 Suprapur solution (Merck). The solutions of different pH and concentration were made from the solution of ammonium tartrate (Merck). The buffer was initially degassed at the ultrasonic bath.

Samples

Ground water samples were collected from the piezometers located in the waste disposal area of chemical industry using a pump for ground water sampling. After sampling the samples were transported and stored in polyethylene vessels for trace analyses (Nalgene). The samples were stored in a laboratory for no longer than a few days at the temperature close to minus 30°C.

Hyphenated analytical system HPLC-HG-AAS

The inorganic species of antimony: antimonates(III) and antimonates(V) were separated on an anion-exchange column Supelco LC-SAX1. The mobile phase was ammonium tartrate ensuring the presence of at least one Sb form in the dissociated form. The chromatographic separation was carried out in the isocratic conditions at a constant flow rate of a mobile phase (4 cm^3/min – to decrease the effect of peak dispersion). The eluent from the column was directed to the hydride generation system, which plays a role of an interface between the chromatographic system and the AA spectrometer. The coherence of the introduced phase (the eluent from the column) and of the flow rates, enabled a direct introduction of the eluent into the reaction system of hydride generation. Hydrides were directed in a stream of neutral gas (argon) to the atomizer of the AA spectrometer: electrothermally heated quartz cell.

RESULTS AND DISCUSSION

Optimization of chromatographic separation conditions

In the research on the chromatographic separation of antimony species, conducted by different authors different reagents were used as the mobile phase: ammonium tartrate [20], potassium hydroxide [1], EDTA with phthalic acid [21], ammonium hydrogen carbonate with tartaric acid [4, 5], phthalic acid and modifier: acetone, methanol, acetonitrile [17], organic acids [18], tetramethylammoniumhydroxide [7], potassium hydroxide with ammonium tartrate [9], nitric acid [18], ethylenediamine tetraacetic acid [3], phosphate buffer (KH_2PO_4/K_2HPO_4) [7], carbonate buffer or potassium hydroxide [5]. In most of the research on the antimony species determination potassium antimony tartrate or antimony trichloride [6, 9] as well as commercial standards for spectroscopy [21] are applied as the

standard for Sb(III) form [6, 12]. When potassium antimony tartrate ($C_4H_4KO_7Sb$) is used as the standard for Sb(III) form it seems correct to use the solution of ammonium tartrate as an eluent in the chromatographic system in order to ensure the coherence of standardization conditions of the analytical system and samples analyses.

In this work it was impossible to obtain a satisfactory separation of Sb(III) and Sb(V) forms on the Supelco LC-SAX1 column with use of EDTA and phosphate buffer (KH_2PO_4/K_2HPO_4) as eluents [6, 8]. The attempt to apply a short column (20 mm guard column for Supelco LC-SAX1) as a self-sufficient analytical column [20] was also unsuccessful. However, a single analytical signal representing unseparated Sb(III) + Sb(V) forms at the retention time of approx. 12 s and 24 s for the flow rates of 4 cm^3/min and 2 cm^3/min, respectively, was obtained. Hence, the system of 250 mm analytical column and ammonium tartrate as a mobile phase was used during the further research.

Studies of eluent pH, concentration and column temperature

Several investigations were carried on the optimization of the eluent concentration and pH, without a simple changing by means of e.g. ammonia solution [3, 21], potassium hydroxide [18], hydrochloric acid [15] or nitric acid [21], which would cause a considerable change of the eluent composition. The research presented in this paper was carried out keeping one of the parameters constant (pH or concentration) while analyzing the influence of the other one on the conditions of chromatographic separation.

The influence of pH changes on the retention time of Sb(III) and Sb(V) speciation forms was observed in the range of 5.0 to 6.7, at the concentration level of ammonium tartrate amounted to 400 ± 25 mmol/dm^3. With an increase of the eluent pH, Sb(III) retention time changed, like in the work of [20], in the range from approx. 93 s (superimposition of Sb(V) and Sb(III) peaks took place) to approx. 245 s at the constant retention time of Sb(V) form coming to approx. 71 s (Fig. 2). The optimum pH range was 6.5–6.7.

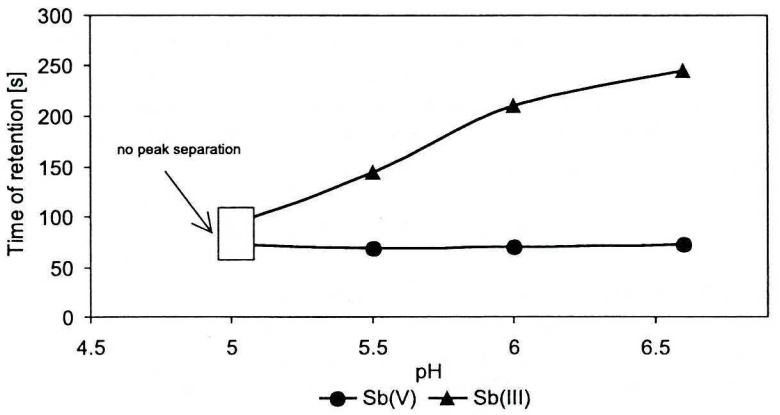

Fig. 2. Effect of the eluent (ammonium tartrate) pH on retention time of Sb(V) and Sb(III) species (eluent concentration 400 mmol/dm^3; for standard 250 ng/cm^3 of each form of antimony)

The influence of eluent concentration changes on the chromatographic separation conditions of Sb(III) and Sb(V) forms was observed for the eluent concentrations of 100 to 400 mmol/dm^3 at the constant pH values of the solutions at the level of 6.6 ± 0.1. With an increase of eluent concentration (ammonium tartrate) from 100 to 400 mmol/dm^3,

the retention time of Sb(III) form decreased from approx. 650 s to 255 s at the constant retention time of Sb(V) form of 70 s (Fig. 3). Similar effect was observed in studies of other researchers [21]. The value of 400 mmol/dm^3 was accepted as optimum concentration ensuring the shortest time of an analysis. Other authors, however, suggest applying a lower concentration and pH of the mobile phase [15].

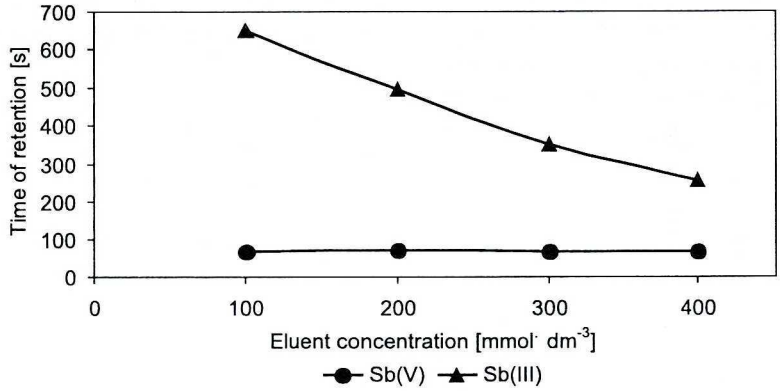

Fig. 3. Effect of the eluent concentration (ammonium tartrate) on retention time of Sb(V) and Sb(III) species (eluent pH 6.6–6.7; for standard 250 ng/cm^3 of each form of antimony)

It was also found that the temperature of an analytical column has a crucial influence on the retention time of Sb(III) form. In the temperature range from 7 to 30°C the retention time of Sb(III) from decreased from approx. 235 s to approx. 195 s and remained at this level to the temperature of 80°C. In the case of Sb(V) form the retention time did not change (approx. 71 s) in the temperature range from 7 to 80°C. Considerably wide scope of the Sb(III) retention time changes showed that it is necessary to apply thermostabilization of an analytical column with the accepted temperature of 20°C (Fig. 4).

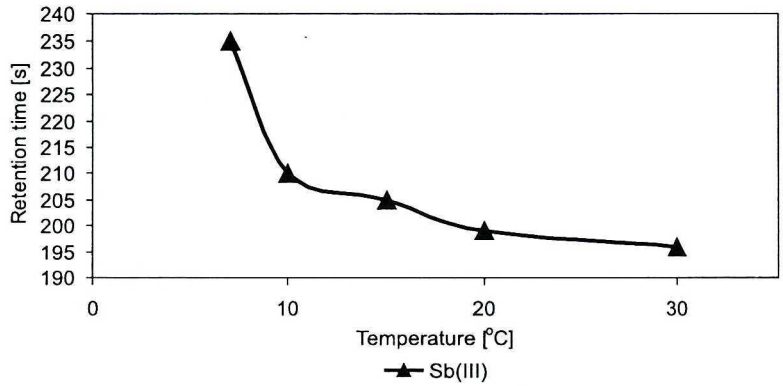

Fig. 4. Effect of the column temperature on retention time of Sb(III), retention time of Sb(V) is constant (eluent 400 mmol/dm^3, pH 6.6–6.7; for standard 250 ng/cm^3 of antimony)

Analytical figures of merit and sample analysis

The content of the antimony species was proportional both to the peak area and height, which were measured in each analysis (typical chromatogram in Fig. 5). Several valida-

tion parameters, which characterize an analytical method, were determined (Tab. 2). The obtained detection limits as well as the results of RSD are comparable with those obtained by other authors [20]. However, they are worse than those obtained by means of ICP-MS [2, 8] or AFS [9, 15] as a detector. Use of the high flow velocity of the mobile phase allowed to shorten the time of the analysis [9]. In view of the lack of the certified references material for determination of antimony inorganic speciation forms, it was impossible to investigate traceability. Therefore, recovery of each investigated form by addition of the standards to an environmental sample was determined.

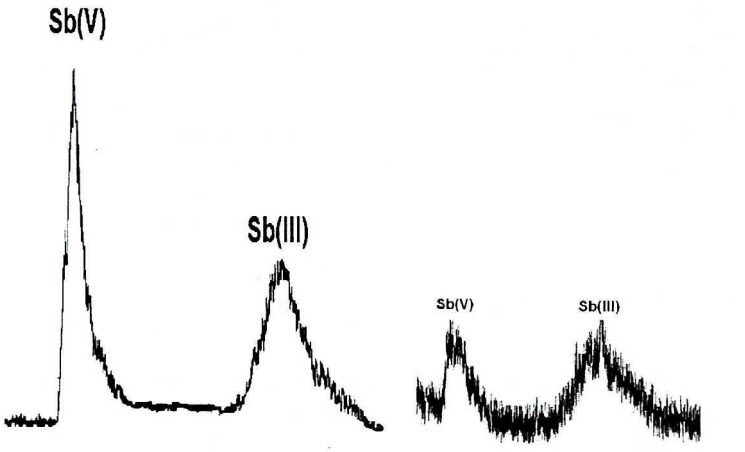

Fig. 5. Typical chromatograms for antimony speciation

Table 2. Analytical characteristic obtained for antimony species (n = 6)

Antimony species	Retention time [s]	Linear range of calibration curve [ng/cm^3]	Intercept	Slope	Correlation coefficient	Detection limit (3σ) [ng/cm^3]	RSD at 25 ng/cm^3 [%]
Sb(V) peak high	75.1 ± 1.8	DL* – 250	0.0135	0.0012	0.9916	4.8	17.0
peak area		DL* – 250	-0.2909	0.0279	0.9953	3.2	16.0
Sb(III) peak high	244.8 ± 0.5	DL* – 250	0,0117	0.0006	0.9915	6.8	13.5
peak area		DL* – 250	-0.8282	0.0488	0.9899	2.7	12.7

* – detection limit

Ground water analysis

The method of standards addition was applied to the analysis of the environmental samples as well (Tab. 3). Together 15 analyses of ground water samples from the region of the intensive anthropopressure were carried out. In the case of 13 samples, presented in the Table 3, no contents of antimony species exceeding the detection limits were found. In the case of 2 other samples (no. 1 and 2 in Table 1) a high concentration of antimony at the level of approx. 20 ng/cm^3 was determined. It was crucial to find the presence of

Sb(III) and Sb(V) forms in both samples, while in the case of arsenic [12], As(V) form prevailed in the same water.

Table 3. Results of speciation analysis of Sb(III) and Sb(V) by HPLC-HGAAS in contaminated ground water samples and recovery of antimony at the addition of standards (n = 6) [ng/cm³]

Peak area

Sample	Added [ng/cm³]	Sb(V) found [ng/cm³]	Recovery [%]	Added [ng/cm³]	Sb(III) found [ng/cm³]	Recovery [%]
1	0	13	–	0	8	–
	50	66	106	50	59	103
	100	125	112	100	109	101
	150	174	107	150	168	107
2	0	14	–	0	12	–
	100	109	95	100	102	90
3	0	< 3	–	0	< 3	–
	50	53	106	50	54	108
4	0	< 3	–	0	< 3	–
	50	54	108	50	52	104
5	0	< 3	–	0	< 3	–
	50	48	97	50	47	96
6–15	0	< 3	–	0	< 3	–

Peak high

Sample	Added [ng/cm³]	Sb(V) found [ng/cm³]	Recovery [%]	Added [ng/cm³]	Sb(III) found [ng/cm³]	Recovery [%]
1	0	12	–	0	9	–
	50	66	107	50	67	117
	100	123	111	100	120	111
	150	175	108	150	148	92
2	0	12	–	0	15	–
	100	106	94	100	107	92
3	0	< 5	–	0	< 7	–
	50	55	111	50	53	106
4	0	< 5	–	0	< 7	–
	50	59	118	50	52	104
5	0	< 5	–	0	< 7	–
	50	46	93	50	48	96
6–15	0	< 5	–	0	< 7	–

CONCLUSION

The hyphenated HPLC-HG-AAS method allows carrying the determination of antimony: Sb(III) and Sb(V) inorganic speciation forms. Considerably low detection limits and a wide dynamic scope for the determination of both antimony species with good accuracy make this method a useful analytical tool, competitive to other speciation analysis methods of this element [13, 16].

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OZNACZANIE NIEORGANICZNYCH FORM ANTYMONU Z ZASTOSOWANIEM TECHNIKI ŁĄCZONEJ WYSOKOSPRAWNEJ CHROMATOGRAFII CIECZOWEJ Z DETEKCJĄ ABSORPCYJNEJ SPEKTROMETRII ATOMOWEJ Z GENEROWANIEM WODORKÓW

W artykule przedstawiono technikę łączoną wysokosprawnej chromatografii cieczowej z detekcją absorpcyjnej spektrometrii atomowej z generowaniem wodorków (HPLC-HG-AAS) w oznaczeniach specjacyjnych nieorganicznych form antymonu: Sb(III) i Sb(V) w próbkach wód. Prowadząc rozdzielanie form specjacyjnych na kolumnie jonowymiennej w układzie chromatograficznym i detekcję z użyciem absorpcyjnej spektrometrii atomowej z generowaniem wodorków uzyskano rozdzielenie sygnałów analitycznych oznaczanych form antymonu przy granicach wykrywalności 6,8 ng/cm^3 (wysokość piku) lub 2,7 ng/cm^3 (powierzchnia piku) Sb(III) i 4,8 ng/cm^3 (wysokość piku) lub 3,2 ng/cm^3 (powierzchnia piku) Sb(V) z RSD odpowiednio poniżej 20% dla stężenia 25 ng/cm^3. Technikę łączoną zastosowano w oznaczeniach antymonu w zanieczyszczonych wodach podziemnych.