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Speciation of arsenic and selenium compounds by ion chromatography with inductively coupled plasma atomic emission spectrometry detection using the hydride technique

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Abstract

Different forms (species) of arsenic and selenium play an important role in biological and environmental samples. For example, arsenic is widely dispersed in the environment by emission of about $32 \cdot 10^6$ kg/year as a result of anthropological inputs. The feasibility of species and multi-element determinations after ion chromatographic separation was investigated using inductively coupled plasma atomic emission spectrometry (ICP-AES) as an atomic spectrometric detection technique. After the separation of arsenic(III), arsenic(V), dimethylarsinic acid and selenium(IV) on an ion-exchange column PRP X-100 with a mobile phase of 1 m*M* *p*-hydroxybenzoate–0.4 m*M* benzoate at pH 8.5 the analyte ions were transferred to the hydride forms. The analyte gas was separated from the aquatic phase by a laboratory-made low-volume gas–liquid separation chamber. With optimized reagent concentrations of sodium borohydride and hydrochloric acid the efficiency of the hydride generation of the investigated compounds is in the range of 85 to 96%. Compared with the pneumatic nebulization, the sensitivity was increased by a factor of 50 using on-line post-column hydride formation. In addition, the parameters of the plasma, *e.g.* plasma power, argon gas flows and emission lines were optimized to achieve a high sensitivity of the ICP detection. The comparison of the sensitivities and reproducibilities shows the improvement by ICP-AES detection.

1. Introduction

Various arsenic species found in the environment have different toxicological properties in living organisms, ranging from relatively nontoxic to extremely toxic [1–3]. Therefore, evaluations of arsenic exposure to humans or plants should include chemical speciation [4–6], not only the total amounts of the elements. Due to the increasing demand for quantitative information of the form of trace metals in environmental and biological samples, chemical and instrumental techniques have been developed that allow selective detection of different species at very low levels [7–10], usually in the ng/ml range. Most of these techniques consist of couplings of two independent analytical systems ("hyphenated techniques") [11–13], which combine the ability for separation of similar compounds with a selective and sensitive detection mode. Couplings of liquid [HPLC, ion chromatography (IC)] or gas chromatography and atomic spectrometric methods [atomic absorption spectrometry (AAS), inductively coupled plasma (ICP) atomic emission spectrometry (AES), ICP-MS] are often used to achieve the efficient

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separation and sensitive quantification of the species investigated [14–17]. The discontinuous monitoring normally used in AAS techniques in many cases does not correspond with the detection scheme of chromatographic methods. The two main reasons are: (1) the transfer of the sample from the column to the source of excitation leads to a drastical loss of sensitivity by low-efficient, high-volume nebulizers and (2) the data acquisition of the spectrometer does not allow the collection of data during the whole chromatographic run. To transfer elements that form volatile hydrides —As, Se, Bi, Pb, Sn, Te— into the plasma in a simple way, a on-line hydride generation (HG) by chemical reduction of the elements with higher oxidation states may be performed [18]. Rauret *et al.* [19] demonstrated the potential of coupling a HG system to ICP-AES for arsenic determination. A problem is the quick conversion of As(V) to the hydride because of the lower HG efficiency of this oxidation state. Nothing is reported about the improvement and how to reach nearly the same sensitivity as for As(III). The aim of this study was an evaluation and improvement of the hydride formation and of the gas–liquid separation to obtain high efficiencies for the determination of arsenic and selenium species.

2. Experimental

2.1. Instrumentation

For the IC separation a Perkin-Elmer HPLC system (LC 250 pump and ISS 200 autosampler) (Norwalk, CT, USA) equipped with a self-thermostating conductivity detector GAT 320 in series was applied. For separation of the ionic species two ion exchange columns Nucleosil SB 10, 125 × 3 mm and Hamilton PRP X-100, 125 × 4 mm (Bischoff, Leonberg, Germany) were used. The columns were thermostated to 25°C. The element-specific detection was performed by an ICP-atomic emission spectrometer Plasmaquant 100 (Carl Zeiss, Jena, Germany). The high optical resolution of this instrument results from the echelle polychromator with crossed lines. Twelve element lines are detectable simultaneously. Special software was developed to collect data with integration times of 0.1 to 2.0 s depending on the whole time of the chromatogram ranging from 20 to 400 min, respectively. The measured emission intensities of transient signals were saved as ASCII files and then converted to the graphic program Origin (Microcal Software, USA), so that the results could be presented graphically. The coupling unit was a miniaturized laboratory-made gas–liquid separator. The mixing of the hydride generation reagents and the mobile phase was realized with two-way splitters, polyether ether ketone (PEEK) tubes and a multichannel peristaltic pump with three different speeds (MLW, Germany). The diameters of the silicon tubes for pumping were varied to achieve different flow-rates. The scheme of the complete apparatus is shown in Fig. 1.

2.2. Chemicals

All reagents were of analytical-reagent grade. Double de-ionized water was used for the mobile phases and the standard solutions. Stock solutions (1000 μg/ml) of the species were prepared by dissolution: arsenite from As_2O_3 (Merck),

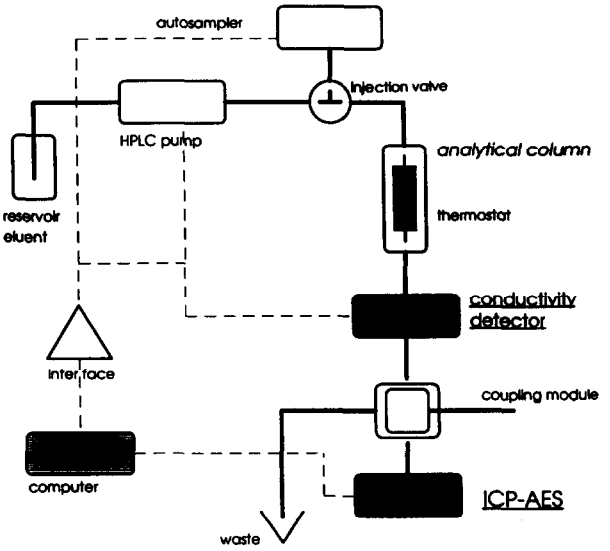

Fig. 1. Scheme of coupling IC with ICP-AES.

Table 1
Protonation constants of arsenic and selenium species [25]

Compound	pK_{a1}	pK_{a2}	pK_{a3}
Arsenic acid, As(V)	2.3	6.9	11.5
Arsenous acid, As(III)	9.2	13.5	–
Dimethylarsinic acid, DMA	1.3	6.2	–
Selenic acid, Se(VI)	−3.0	2.0	–
Selenous acid, Se(IV)	2.5	8.0	–

dimethylarsinate from cacodylic acid (Merck), selenite, selenate from Na_2SeO_3, Na_2SeO_4 (Alfa), respectively, and arsenate from a Titrisol solution (Merck). The working solutions were prepared daily by dilution of the stock solution to the required concentration in eluent. Sodium tetrahydroborate solutions were prepared by dissolution of $NaBH_4$ (Merck) and stabilized by 0.1 *M* NaOH. The mobile phases for the chromatographic separation consisted of phthalate or *p*-hydroxybenzoate, benzoate, methanol and NaOH to adjust the pH. The mobile phase was deaerated by helium and filtered (0.45 μm).

3. Results and discussion

3.1. Chromatographic separation

To determine the optimal conditions for an efficient IC separation of the species described above their protonation constants (pK_a) must be considered (Table 1). The difficulties getting uniform conditions for all components are caused by the quite different pK_a values and different electrochemical charges (Fig. 2).

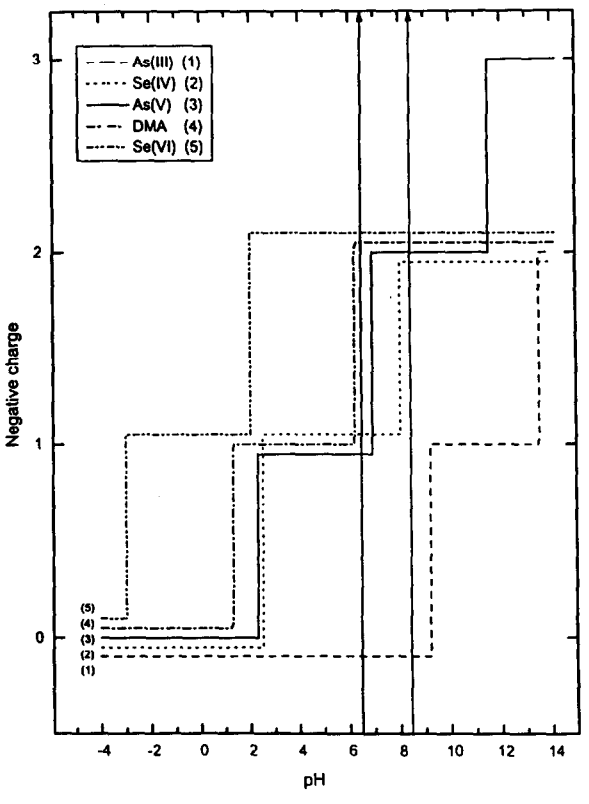

Fig. 2. Schematic dependence of the charge of selenium and arsenic compounds on pH value.

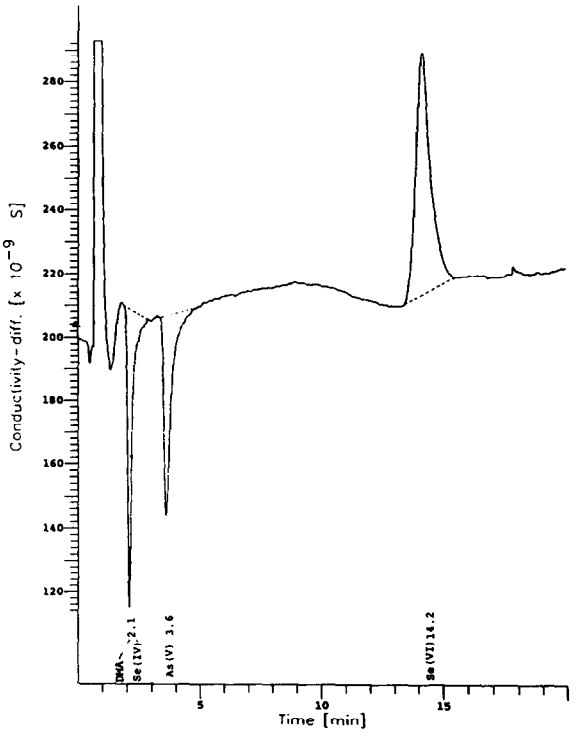

Fig. 3. Ion chromatogram of Se(IV), Se(VI), As(V), DMA with conductivity detection. Nucleosil SB 10; eluent: phthalate 2 m*M*, pH 6.5, 1 ml/min. Se(IV), Se(VI), As(V), DMA 100 mg/l.

Therefore, two different columns, Nucleosil SB 10 and Hamilton PRP X-100, were used in combination with eluents of different pH. In a weak acidic medium (pH 6.5) As(III) should not retain on the ion-exchange column Nucleosil, because of its non-ionic form. Otherwise, DMA and Se(VI) with a charge of −2 should interact very intensively with the stationary phase resulting in long retention times. As(V) and Se(IV) with a charge of −1 should show short retention times like chloride or nitrate. Fig. 3 and Table 2 show the separation and separation parameters, respectively, for a mixture of As(V), Se(IV) and Se(VI) in an identical sequence and a comparison with the traditional anions chloride, nitrate and sulphate. DMA has shown a diverging behaviour because of additional hydrophobic interaction with residual reversed-phase capacity and a pK_{a2} nearby the pH of the eluent. The existence of positive and negative peaks results from differences in conductivity compared with the conductivity of the eluent. In contrast, in a weak alkaline solution of pH 8.5 all species have a negative charge of −2 except As(III). Fig. 4 shows the separation of the five arsenic and selenium species during 30 min. The As(V) and Se(VI) signals were interfered by system peaks. The calibration plots show linear behaviour over two orders of magnitude and detection limits comparable to those of the other anions (Table 2).

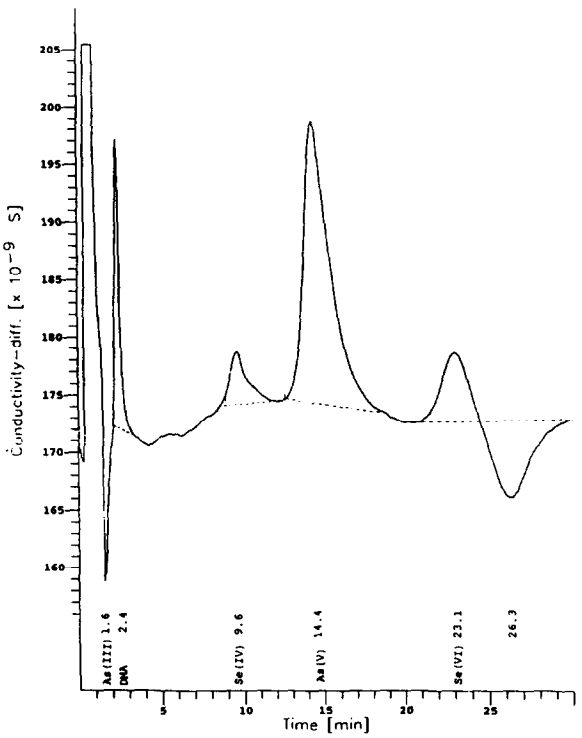

Fig. 4. Ion chromatogram of DMA, As(III), As(V), Se(IV), Se(VI) with conductivity detection. PRP X-100; eluent: 0.4 m*M* benzoic acid–1.0 m*M* *p*-hydroxybenzoic acid, methanol 2.5%, pH 8.5, 2 ml/min. DMA, As(III), As(V), Se(IV), Se(VI) 40 mg/l.

Table 2
Retention times and capacity factors of various anionic species, conductivity detection

Species	t'_R (min)	k'	Detection limit (mg/l)
$HSeO_3^-$	1.3	1.62	0.34
DMA^{2-}	1.3	1.62	0.90
$H_2AsO_4^-$	2.8	3.5	0.56
SeO_4^{2-}	13.4	16.7	0.40
Cl^-	1.9	2.4	0.08
HPO_4^{2-}	2.5	3.1	n.d.
NO_3^-	4.2	5.2	0.30
SO_4^{2-}	11.5	14.4	0.45

Conditions: Nucleosil SB 10, eluent phthalate 2.0 m*M*, pH 6.5. n.d. = Not determined.

3.2. ICP Detection

To overcome limitations of unspecific conductivity detection like unresolved or overlapping signals as well as system peaks, ICP-AES is a good choice. To enable this element-specific detection, the column eluate should be transferred to the plasma continuously. Many studies have been undertaken to enhance the poorer sensitivity if a Meinhardt or cross-flow nebulizer was used for sample introduction into the plasma [20]. With the formation of gaseous arsenic and selenium hydrides a more efficient technique was

applied. In comparison to the cross-flow nebulizer the introduction efficiency was nearly 100% with the hydride formation of As(III) and Se(IV). The optimization of the ICP-AES operating parameters such as gas flow-rates, radio frequency (rf) power and integration time was performed by continuously introducing a test solution. The best signal-to-background ratio (SBR) was chosen as the criterion for optimization. The three most sensitive analytical emission lines for arsenic —193.6, 197.1 and 228.8 nm— were tested. The observed SBR values determined the choice of the 228.8-nm line for further investigations. For selenium only the emission line of 196.0 nm was available. The argon gas streams were optimized with respect to additional hydrogen evaporation by HG. The carrier gas flow (Fig. 5) has shown a significant influence on the SBR with a maximum at 0.6 to 0.8 l/min similar to the selenium and arsenic species investigated. The optimized ICP-AES conditions are: rf power, 1.95 kW; view height, 7.5 mm; plasma gas flow, 11.5 l/min; auxilliary gas flow, 0.4 l/min; carrier gas flow, 0.75 l/min.

The transformation process to the hydrides strongly depends on the sodium borohydride concentration added to the eluent stream like a post-column derivatization. A continuous increase of the borohydride concentration yielded improved sensitivities for As(V) and DMA (Fig. 6). Concentrations higher than 4% were not suitable because the hydrogen production disturbed the plasma stability. The reduction efficiency, near 96% for the lower oxidation states, decreased from As(III) to As(V) or Se(IV) to Se(VI), so an additional reduction step by iodide [21,22] or cysteine [23,24] was recommended to accelerate the reaction, which is important considering the short period between mixing and gas–liquid separation. In this case the reduction of As(V) by iodide did not improve the sensitivity but decreased the signal intensity of Se(IV) by a factor of approximately 2 depending on the iodide concentration. Se(IV) is reduced to the

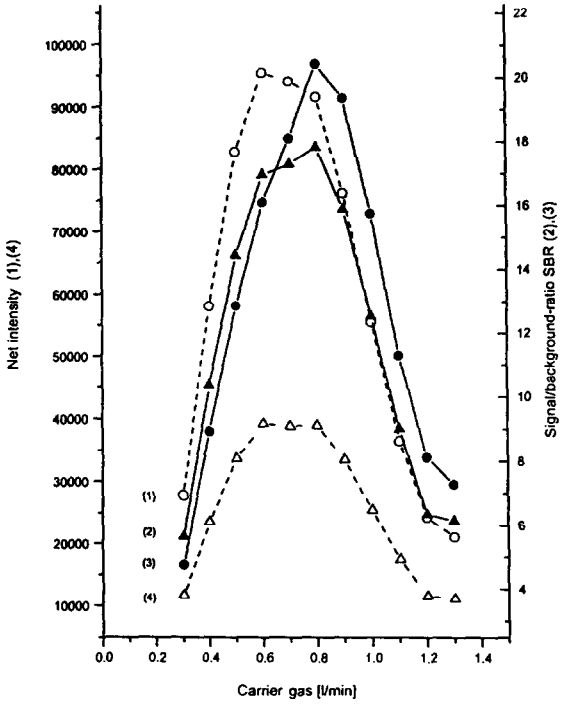

Fig. 5. Dependence of net intensity (○, △) and SBR (●, ▲) on carrier gas flow-rate. As(III) (○, ●) 5 mg/l, Se(IV) (△, ▲) 20 mg/l, power 1.95 kW, view height 7.5 mm.

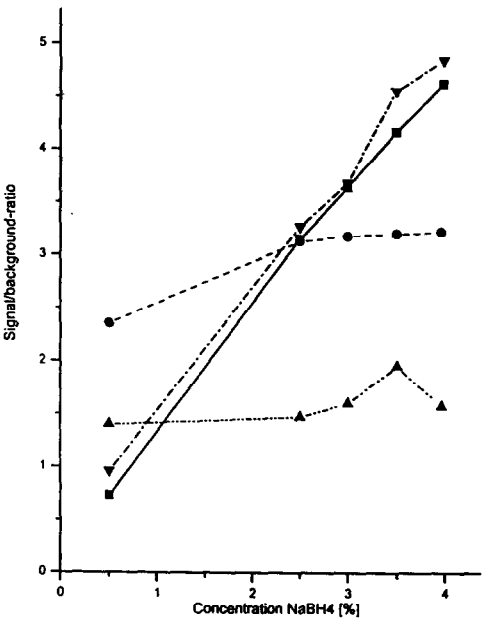

Fig. 6. Influence of $NaBH_4$ concentration on net intensity signals. DMA (▼) 10 mg/l, As(III) (●) 2 mg/l, As(V) (■) 10 mg/l, Se(IV) (▲) 8 mg/l, HCl 10%.

red elementary form, which is not converted to the hydride. The reduction of Se(VI) to the hydride form was not achieved. Here a combination of introduction techniques, *e.g.* hydride generation with an ultrasonic nebulizer, may increase the transfer for As(V) and Se(VI) by keeping the advantage of a complete introduction of the other species. Also, in this way the number of simultaneously detectable elements may be increased. An additional piece of software was used for the time-resolved ICP detection with different integration times and baseline correction. An example for a dual-channel monitoring of As and Se with overlapping signals is shown in Fig. 7, emphasizing the advantage of the element-specific detection mode. The calibration was performed under optimized conditions for the chromatographic system coupled to the ICP-AES detector: column, PRP X-100; *p*-hydroxybenzoate–benzoate, pH 8.5; HCl (added concentration), 10%; $NaBH_4$, 3.5%.

The calibration plots of arsenic and selenium species are illustrated in Fig. 8. The slopes of the

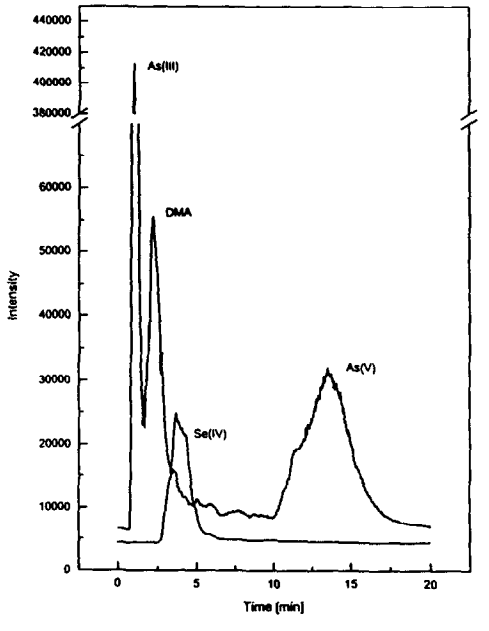

Fig. 7. Ion chromatogram of DMA, As(III), As(V), Se(IV); IC, HG, ICP-AES detection (rf power 1.95 kW, plasma gas 11.5 l/min, carrier gas 0.75 ml/min, HCl 10%, $NaBH_4$ = 3.5%, for chromatographic conditions see Fig. 4).

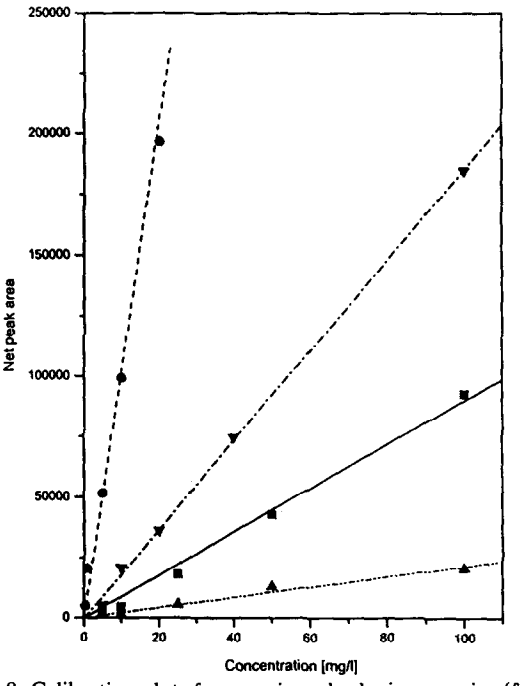

Fig. 8. Calibration plots for arsenic and selenium species (for conditions see Fig. 7). ● = As(III); ■ = As(V); ▼ = DMA; ▲ = Se(IV).

curves depend on the integration time, which varied from 0.2 to 2.0 s. Table 3 shows the analytical parameters of the calibration. The linearity of the method was determined for concentrations ranging over two orders of magnitude. Reproducibility was measured by five replicate injections of a test mixture of 1 mg/l as a relative standard deviation less than 5%. As(III) has the best limit of detection (17 μg/l), As(V) is the compound detected with the lowest sensitivity. This is due to the different kinetics of the reduction process.

The column eluate was always monitored with a conductivity cell in series (Fig. 1). In this way extra information about the anion content of the sample can be obtained concerning non-metallic ions like chloride, nitrate and sulphate, often present in natural materials and not normally detected by AES.

In the future this principle will be expanded to a variety of environmental samples and also include the ICP-MS technique.

Table 3
Calibration parameters IC–HG–ICP-AES

Species	Sensitivity (l/mg)		Detection limit	
	$t_{integr.} = 0.2$ s	$t_{integr.} = 2.0$ s	mg/l, $t_{integr.} = 2.0$ s	g, $t_{integr.} = 2.0$ s
As(III)	10620	10335	0.017	$1.7 \cdot 10^{-9}$
As(V)	811.5	890.2	0.64	$0.64 \cdot 10^{-7}$
Se(IV)	217.5	128.7	0.23	$2.3 \cdot 10^{-8}$
DMA	1217	187.9	0.10	$1.0 \cdot 10^{-8}$

Conditions: PRP X-100, eluent 1.0 m*M* *p*-hydroxybenzoate–0.4 m*M* benzoate, methanol 2.5%, pH 8.5, reagents HCl 10%, $NaBH_4$ 3.5%.

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