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Short communication

Simultaneous speciation of aqueous selenium(IV) and selenium(VI) by high-performance liquid chromatography with ultraviolet detection

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Abstract

An ion-pair liquid chromatography with UV detection was used to determine selenium(IV) and selenium(VI) ions simultaneously. The chromatographic behavior of the selenium species was examined in detail. Factors affecting chromatographic separation and quantitative determination, as well as potential interferences were systematically optimized. Calibration graphs were linear with very good correlation coefficients ($r>0.9999$). The applicability of the method to the analysis of a spiked (1.0 μg/ml) lake-water was demonstrated. © 1997 Elsevier Science B.V.

Keywords: Selenium; Metal cations

1. Introduction

Selenium has been recognized as an essential nutrient for humans at lower concentration, and becomes toxic at higher concentration [1]. The toxic dose of selenium is very much dependent on its chemical form [2,3]. In natural waters, selenium exists predominantly in the oxidation states of Se(IV) as selenite (SeO_3^{2-}) and Se(VI) as selenate (SeO_4^{2-}). For better understanding of the selenium effect, it is essential to develop a reliable method to determine the accurate concentration of Se(IV) and Se(VI) [4,5].

In previous studies, the detection of selenium species was generally based on a two-step procedure including measuring one species and obtaining the other by difference after total selenium measurement [6–9]. The simultaneous detection of selenium species has been performed using a number of chromatographic techniques hyphenated with atomic spectroscopy, inductively coupled plasma-atomic emission spectroscopy or mass spectroscopy, neutron activation analysis, or isotope dilution mass spectroscopy, after appropriate derivatization [6,10–16]. Fung et al. [17] used a single-column ion chromatography to determine selenite and selenate with spectrophotometric detection. However, a higher pH of eluent was used in this SCIC system, which might have the risk of species interchange due to the pH being far from that of surface water. This article describes the simultaneous determination of SeO_3^{2-} and SeO_4^{2-} achieved using RP-HPLC with a conventional UV detector and a neutral pH elution.

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2. Experimental

2.1. Apparatus

The HPLC used was a LC-9A system (Shimadzu, Kyoto, Japan) equipped with a Rheodyne 7125 injection valve (20 μl sample loop) and a reversed-phase Supelcosil LC-8 column (25 cm×4.6 mm I.D., 5 μm) (Supelco Inc., Bellefonte, PA, USA). A Soma Model S-3702 UV detector (Soma, Tokyo, Japan) and a Shimadzu C-R6A Chromatopac integrator were used to detect and obtain chromatograms. An atomic absorption spectrophotometer (Model Z-8100, polarized Zeeman AA, Hitachi, Japan) was also used to analyze the selenium species.

2.2. Reagents

Distilled-deionized water was used to prepare all solutions. Stock solutions of 1000 μg/ml SeO_3^{2-} and SeO_4^{2-} were prepared by dissolving 2.190 g of reagent grade Na_2SeO_3 (Aldrich, USA) and 2.393 g of Na_2SeO_4 (Aldrich) in 1000 ml water, respectively. Fresh working standards of SeO_3^{2-} and SeO_4^{2-} (single or mixed) were prepared daily by appropriate dilution of the stock solutions. Tetrabutylammonium hydroxide (TBAOH) and sodium dihydrogen phosphate were purchased from Riedel-dehaën (Hannover, Germany). The HPLC eluent was prepared from HPLC-grade acetonitrile (Mallinckrodt Inc., Kentucky, USA), water, TBAOH and NaH_2PO_4. Sulfuric acid (0.01 *M*) or sodium hydroxide (0.01 *M*) was used to adjust the pH. All eluents were filtered through a 0.45-μm PVDF membrane filter and degassed ultrasonically.

2.3. Procedure

A 2.0-ml sample solution containing SeO_3^{2-} and/or SeO_4^{2-} was pipetted into a 100-ml volummetric flask followed by dilution to the mark with water. The sample was injected into the HPLC system through a 20-μl sample loop after filtration through a membrane filter. The eluent was 15% (v/v) acetonitrile prepared in the 0.002 *M* TBA aqueous solution with 0.001 *M* NaH_2PO_4 solution at pH 7.0. The flow-rate was 1.0 ml/min. The eluent composition and the elution conditions were adjusted to obtain an optimal separation based on the purpose of the experiments.

3. Results and discussion

3.1. Selection of detection wavelength

In order to obtain the highest sensitivity, the detection is better set at or near the λ_{max}. However, both selenium species do not have specific absorption above 240 nm even being ion-paired with TBA. Thus, the wavelengths in the range of 200–240 nm were tested to obtain a better sensitivity. Because a worse baseline was obtained at lower wavelength detection and the sensitivity decreased as the wavelength increased. Compromising both, 210 nm was selected for monitoring selenium species.

3.2. Effect of pH and organic modifier

Because the stability and the ion-pairing of selenium species with TBA are all affected by pH, the pH of the eluent should be adjusted carefully. From our study, the retention time increased as the pH changed from 3 to 4, and decreased significantly from 4 to 7. The decrease of retention time for SeO_4^{2-} was more rapid than for SeO_3^{2-}. As to the influence of pH on quantitative detections, there was no remarkable change of peak area for both species, except that SeO_3^{2-} increased from pH 3 to 4. Overall, elution at pH 7 was selected because of its similarity to the neutral pH of surface water.

Acetonitrile was selected as the organic modifier to shorten the elution time. Good resolution was kept until 25% addition and no significant change on the detection sensitivity was observed. Compromising the resolution with the analytical time, 15% of acetonitrile was added into the eluent to perform the separation.

3.3. Calibration graphs and detection limits

In order to test the applicability of the method to the simultaneous determination of SeO_4^{2-} and SeO_3^{2-}, calibration graphs were built-up for SeO_3^{2-} over a concentration range of 0.1–100 μg/ml, and for SeO_4^{2-} over 0.5–100 μg/ml. The linear relationship between the peak area and the injected quantity was excellent for both selenium species. The correlation coefficients were all above 0.9999. The reproducibility was examined with five replicate injections of 20 μl of each species in the concentrations for

build-up of calibration plots. Peak area was measured and the relative standard deviation (R.S.D.) was calculated. The R.S.D. values for SeO_3^{2-} and SeO_4^{2-} were below 1.98 and 2.78%, respectively. The detection limits [18] were calculated from the concentration of analyte that gives a signal equal to 3-times the peak-to-peak noise level of the baseline (blank injections) which are 0.16 ng (8 μg/l) for SeO_3^{2-} and 1.8 ng (90 μg/l) for SeO_4^{2-}.

3.4. Interferences

Some anions might also form ion pairs in the elution. Thus, interferences by potential species were studied. Table 1 lists the retention time of these species. Fortunately, no serious interference occurred in the elution except for over 20 μg/ml of acetate ion which might interfere the SeO_3^{2-}. Although the concentration of acetate is not always so high in an aqueous sample, elimination of this interference should be further studied in order to broaden the application of this method. Fig. 1a is the chromatogram of SeO_3^{2-}, SeO_4^{2-} and some potential species in aqueous sample. Peaks 1–7 all agreed well with those of system peak, SeO_3^{2-} (5 μg/ml), Br^- (3 μg/ml), NO_3^- (1 μg/ml), I^- (5 μg/ml), $Cr_2O_7^{2-}$ (5 μg/ml), and SeO_4^{2-} (20 μg/ml), respectively. As can be seen, the SeO_3^{2-} and SeO_4^{2-} were free from interferences except the acetate ion. This indicates that the proposed method can be used to analyze aqueous samples with very lower acetate content. That is, the elution condition should be modified if it is applied to the simultaneous determination of selenium species in a sample with a high acetate content.

Table 1
Retention time of some potential anions in water

Anions/50 μg/ml	Average retention time (min) (n=3)
F^-	—[a]
Cl^-	—[a]
CO_3^{2-}	—[a]
SO_4^{2-}	—[a]
SeO_3^{2-}	5.19±0.04
CH_3COO^-	5.32±0.06
Br^-	6.37±0.07
NO_3^-	7.08±0.07
I^-	10.60±0.09
Cr(VI)	11.46±0.11
SeO_4^{2-}	14.40±0.12
$C_2O_4^{2-}$	16.70±0.15

[a]Non-detectable.

3.5. Analysis of selenium species in a lake-water matrix

The applicability of this method to simultaneous determination of SeO_3^{2-} and SeO_4^{2-} in real samples was examined by analyzing a lake-water sample under the optimum conditions. Unfortunately, both selenium species in lake-water samples were non-detectable. Spiking 1.0 μg/ml of both selenium species into the lake-water and then analyzing by the proposed HPLC method and a graphite atomic absorption spectrometric method (GFAA) was carried out. The same experiment was repeated five times. The proposed HPLC method offers recoveries of 98.3% (2.74% R.S.D.) and 97.4% (2.94% R.S.D.) for 1.0 μg/ml of SeO_3^{2-} and SeO_4^{2-}, respectively; whereas the GFAA method obtained 98.6% recovery with 2.31% R.S.D. In general, they are in good agreement. Fig. 1b is the spiked lake water chromatogram. Obviously, both selenium species can be analyzed simultaneously without interference by the proposed HPLC–UV method.

4. Conclusion

This study shows the potentiality of the simultaneous determination of aqueous SeO_3^{2-} and SeO_4^{2-} by using a simple HPLC–UV instrument with ion-pair elution. A convenient procedure for sample analysis is thus built-up. Although the method did not detect at the concentration levels present in natural samples, it can be a reference method for the simultaneous determination of selenium species (SeO_3^{2-} and SeO_4^{2-}) at the levels of above 0.5 μg/ml in samples with a low acetate content.

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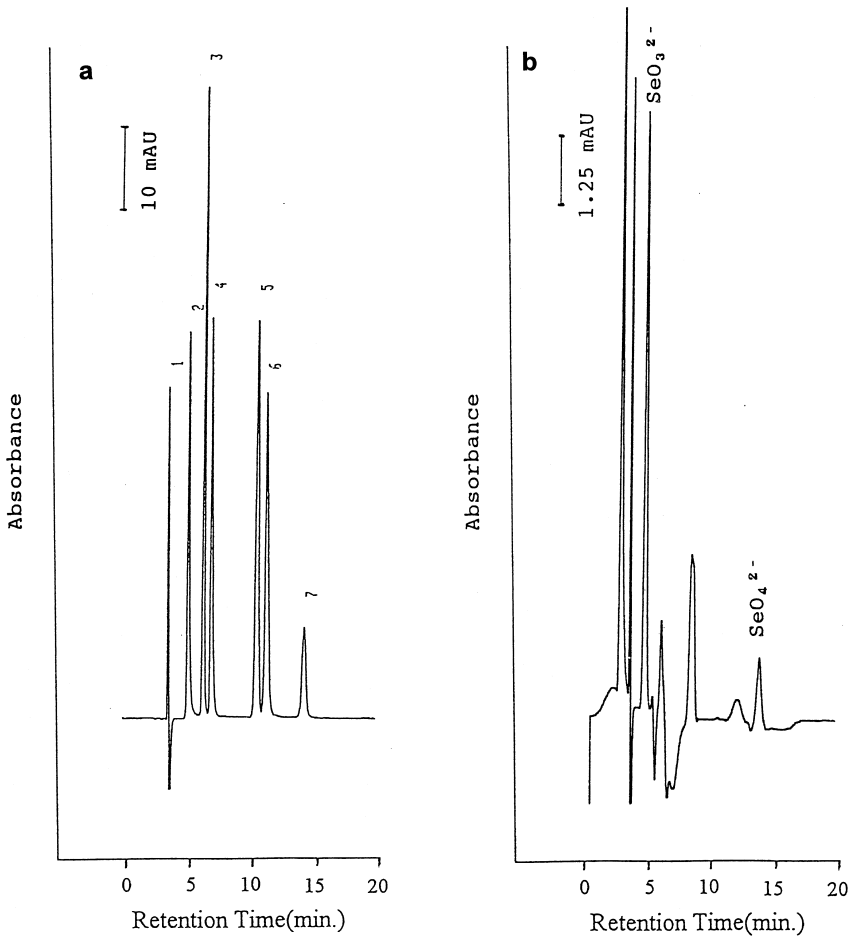

Fig. 1. (a) Chromatogram of SeO_3^{2-}, SeO_4^{2-} and some potential species in aqueous sample. Chromatographic conditions: eluent, 2 m*M* TBAOH+1 m*M* Na_2HPO_4+15% ACN, at pH 7.0; flow-rate, 1.0 ml/min; detection, UV at 210 nm. Peak 1, system peak; peak 2, Se(IV), 5 μg/ml; peak 3, Br^-, 5 μg/ml; peak 4, NO_3^-, 1 μg/ml; peak 5, I^-, 5 μg/ml; peak 6, Cr(VI), 5 μg/ml; peak 7, Se(VI), 20 μg/ml. (b) Chromatogram of the spiked lake water.

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