Journal of Chromatography, 598 (1992) 105–114
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 019

Determination of selenium by capillary gas chromatography after high-temperature derivatization with 1,2-diamino-3,5-dibromobenzene

K. Johansson* and Å. Olin

Department of Analytical Chemistry, University of Uppsala, P.O. Box 531, S-751 21 Uppsala (Sweden)

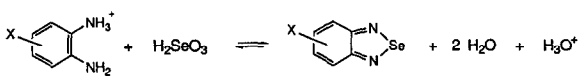
(First received October 2nd, 1991; revised manuscript received January 20th, 1992)

ABSTRACT

The determination of selenium as 4,6-dibromopiazselenol by gas chromatography with electron-capture detection (GC–ECD) was investigated. The rate of formation of the piazselenol from selenium(IV) and 1,2-diamino-3,5-dibromobenzene was studied as a function of temperature. At 100°C the time for quantitative piazselenol formation can be reduced to 5 min without adverse effects on the chromatograms. The use of capillary column results in the separation of the piazselenol from the background without clean-up steps. A capillary column and derivatization at a high temperature facilitate application of the GC–ECD method. The method was applied to the determination of total soluble selenium in water.

INTRODUCTION

The determination of selenium(IV) by gas chromatography with electron-capture detection (GC–ECD) is an established method. It is based on the formation of a volatile compound, generally called a piazselenol (PIAZ), in the reaction between selenious acid and a substituted *o*-phenylenediamine (PDA):



where X is one or several of Cl, Br, NO_2, F or CF_3. The piazselenol is extracted into an organic solvent before injection into the chromatograph.

The unsubstituted piazselenol was first synthesized in 1889 by Hinsberg [1], who showed that only Se(IV) reacts with PDA to form the selenium complex. In 1968, Nakashima and Tôei [2] published a GC method for the determination of selenium using 4-chloro-1,2-diaminobenzene as the ligand. Since then, several papers have been published on the determination of selenium in a range of samples by GC [3–12]. Various substituted *o*-phenylenediamines [2,3,9,10,12–14] have been employed in order to increase the ECD sensitivity. Shimoishi [3] synthesized a range of differently substituted piazselenols and investigated their chromatographic properties, distribution ratios and relative ECD sensitivities. Amongst the thirteen piazselenols investigated, he found that 1,2-diamino-3,5-dibromobenzene (Br_2-PDA) was the best as regards sensitivity and distribution ratio. Br_2-PDA is perhaps the most commonly used, commercially available ligand today, although it is not the most sensitive.

When fluorine is present in a compound it generally enhances volatility and the ECD sensitivity. Dilli and Sutikno [13] investigatad 1,2-diamino-4-fluorobenzene and 1,2-diamino-4-trifluoromethylbenzene. Al-Attar and Nickless [14] prepared and investigated 3-bromo-5-fluoro-1,2-diaminobenzene and 3-bromo-5-trifluoromethyl-1,2-diaminobenzene (Br-CF_3-PDA). Of these ligands, only Br-CF_3-PDA has a higher ECD sensitivity than Br_2-PDA.

In addition, the substituted PDAs 2,3-diaminonaphthalene [12,15] and 1,4-dibromo-2,3-diaminonaphthalene [16] have been used as ligands, but the

0021-9673/92/$05.00

sensitivities are inferior to those of the best PDA ligands.

The GC–ECD method is only one of several methods available for the determination of Se(IV). Atomic absorption spectrometry after hydride generation (HG-AAS) [17,18] and cathodic stripping voltammetry (CSV) [19,20] are two commonly used methods. The GC–ECD method is more time consuming than the other two for water samples. In earlier work it has been reported that the piazselenol reaction takes several hours to reach completion at room temperature [3,4] and generally several clean-up steps are needed before the extract can be injected into the gas chromatograph [4,5,10,13]. Reduction of the time necessary for the formation of the piazselenol and in the number of clean-up steps required would make the GC–ECD method more attractive. An advantage of the GC–ECD method is that it makes possible the direct determination of Se(IV) in natural water samples. This is not always possible with the CSV and HG-AAS methods, which are subject to interferences from organic material in such samples. Further, low limits of detection can be reached with the GC–ECD method owing to the sensitivity of the electron-capture detector and the preconcentration obtained in the extraction of the piazselenol.

This paper reports investigations with Br_2-PDA as the ligand. This ligand was selected for several reasons. It is commercially available in high purity, and the lower volatility of the piazselenol derived from Br_2-PDA is favourable when the derivatization is applied at higher temperatures. It also means a longer retention time, which was needed as natural water samples and the chemicals used generated peaks at short and medium retention times. Further, as will be shown, the values of the protonation constants of Br_2-PDA make the adjustment of the optimum pH for the piazselenol reaction easy. The rate of piazselenol formation was determined as a function of temperature and ligand concentration. Further, the chromatographic behaviour of a toluene extract of the reaction products obtained at various temperatures was studied on a capillary column. Derivatization at a high temperature in conjunction with a capillary column separation greatly facilitates the application of GC–ECD to the determination of selenium. The method was applied to the determination of total soluble selenium in water.

EXPERIMENTAL

Instrumentation

Kinetic studies were carried out in a 1-cm quartz cuvette in a Perkin-Elmer Lambda 17 UV–VIS spectrophotometer equipped with a thermostated (±0.1°C) cell holder and a magnetic stirrer.

For the GC measurements a Shimadzu GC-14 gas chromatograph equipped with a constant-current ^{63}Ni electron-capture detector was used. The column was DB-1701 (15 m × 0.25 mm I.D.) with a 0.25-μm film thickness (J&W Scientific) with the following conditions: carrier gas, helium at 40 cm/s; split vent, 60 ml/min; purge vent, 1 ml/min; make-up gas, nitrogen at 45 ml/min; injector temperature, 225°C; detector temperature, 325°C; and column temperature programme, 100°C held for 2 min, increased at 8°C/min to 175°C, held for 5 min, increased at 15°C/min to 265°C, held for 5 min.

The injections (0.5 μl of sample + 1 μl of toluene) were made in the splitless mode with a Shimadzu AOC-14 automatic injector, with a change to the split mode after 1 min. The peak areas were evaluated with a Shimadzu CR-5A integrator.

The UV destruction of organic material in water samples was performed in a locally built device [21]. The closed quartz tubes (60 ml, 22 mm I.D.) were placed around a 700-W UV lamp (Original Hanau, Hanau, Germany). A temperature-controlled aluminium block [22], made in the laboratory, heated the sample when Se(VI) was reduced to Se(IV) with hydrochloric acid at 100°C.

Reagents

All chemicals except lindane were of analytical-reagent grade. Water purified with a Milli-Q system (Millipore) ("Milli-Q water") was used for preparing standards and dilutions. All glassware was cleaned in 4 *M* nitric acid and rinsed with Milli-Q water.

1,2-Diamino-3,5-dibromobenzene. A 5.5 m*M* solution of Br_2-PDA (Merck) in 0.5 *M* perchloric acid was prepared. The solution was purified by extraction once with toluene and should preferably be kept in the dark. When 100 ml was extracted with 5 ml of toluene the Br_2-PDA concentration decreased to about 5.2 m*M*.

Selenium(IV). A stock standard solution containing 1 g/l of Se(IV) was prepared from an ampoule of

selenium dioxide in dilute nitric acid (Merck) and its concentration was checked by amperometric titration with thiosulphate after addition of a large excess of iodide [23]. Working standard solutions were obtained by serial dilution of the stock standard solution and contained 1 ml/l of perchloric acid (70–72%).

Lindane. A stock standard solution containing 2 mg/ml of lindane was prepared by dissolving lindane (99%, Applied Science Labs., State College, PA, USA) in toluene. A working standard solution containing 18 ng/ml of lindane was prepared by stepwise dilution of the stock standard solution with toluene.

Lindane is an insecticide and precautions should be taken to avoid inhalation and skin contact, especially when preparing solutions.

4,6-Dibromopiazselenol (PIAZ). This was synthesized according to the literature [3,12–14]. The synthesized piazselenol was analysed for selenium after wet digestion according to a variation of Gould's method [24] using a 10:1 (v/v) mixture of concentrated sulphuric acid and fuming nitric acid and the amperometric titration method mentioned above. The purity was 99.8 ± 0.4%. A stock standard solution containing 0.5 mg/ml of piazselenol was prepared by dissolving piazselenol in toluene. Working standard solutions were prepared by serial dilution with toluene.

Kinetic experiments

To a quartz cuvette 3 ml of 0.10 or 0.30 m*M* Br_2-PDA were added and the cuvette was placed in a thermostated cuvette holder. When the desired temperature had been reached, 100 μl of 10 mg/l Se(IV) solution were added. The automatic absorbance measurement was started immediately and the absorbance was measured every 15 s at the beginning and every 1 min at the end of the reaction. The reaction was followed at 20, 30, 40, 50 and 60°C. The wavelength used was 343 nm, where the piazselenol has an absorption maximum and the ligand has an absorbance of only 0.002 (Fig. 1).

Lindane as internal standard and the clean-up step

A stock standard solution containing 17 ng/ml of lindane and 1.9 ng/ml of piazselenol in toluene was prepared. A 1-ml volume of this solution was shaken with 1.5 ml of 4, 6, 7, 8 or 9 *M* perchloric acid. The aqueous phase was discarded and the toluene phase shaken twice with 1.5 ml of Milli-Q water and then injected into the chromatograph. The Milli-Q water and the perchloric acid solutions were extracted with toluene before use.

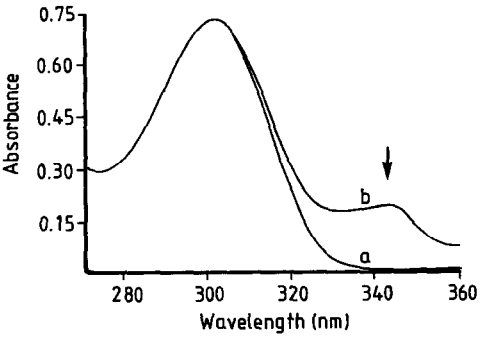


Fig. 1. UV absorption spectrum of 0.23 m*M* monoprotonated 1,2-diamino-3,5-dibromobenzene. (a) No selenium added; (b) 10 μ*M* selenium added; formation of 10 μ*M* 4,6-dibromopiazselenol. The analytical wavelength (343 nm) is indicated by an arrow.

Extraction efficiency

To five 25-ml volumetric flasks, 15 ml of Milli-Q water, 0.5 ml of 70–72% perchloric acid and 0.5 ml of 5.2 m*M* Br_2-PDA were added. To five 100-ml volumetric flasks, 75 ml of Milli-Q water, 2 ml of 70–72% perchloric acid and 2 ml of 5.2 m*M* Br_2-PDA were added. Finally, to five 250-ml volumetric flasks, 200 ml of Milli-Q water, 5 ml of 70–72% perchloric acid and 5 ml of 5.2 m*M* Br_2-PDA were added. This means that the concentrations of Br_2-PDA and perchloric acid in all flasks were 0.1 m*M* and 0.25 *M*, respectively. To each set of five volumetric flasks 0, 1, 2, 3 and 4 ml of a standard solution containing 1 ng/ml of Se(IV) were added, then, the flasks were diluted to the mark. After 3 h the solutions were extracted with 1 ml of toluene containing lindane as internal standard. The aqueous phases were discarded and the toluene phases were transferred into 5-ml test-tubes with PTFE-faced screw-caps. The toluene phases were shaken once with 1.5 ml of 6 *M* perchloric acid and twice with 1.5 ml of Milli-Q water. Before injection into the chromatograph the organic phase was dried with anhydrous sodium sulphate. Rapid phase separation was obtained by spinning the test-tubes.

Derivatization at elevated temperatures

A 100-ml volume of test solution containing 10 ng/l of Se(IV) and 0.25 *M* perchloric acid was heated in a conical flask to the appropriate reaction temperature. At temperatures between 20 and 80°C the flask was placed in a low-temperature oven and at 100°C the flask was heated to boiling on an ordinary hot-plate. When the temperature had stabilized, 1.9 ml of 5.5 m*M* Br_2-PDA were added and after $5t_{\frac{1}{2}}$ (where $t_{\frac{1}{2}}$ is the half-life of the piazselenol reaction) the solution was cooled to room temperature in a water-bath and then transferred quantitatively with 5 ml of Milli-Q water to a separating funnel for extraction. The extract was then treated as described above. Blanks were run with Milli-Q water.

Determination of total soluble selenium in water

The following method was applied to the determination of total soluble selenium in water.

Filter the sample through a 0.45-μm filter, acidify with 1 ml/l of perchloric acid and store the sample in a refrigerator at 4°C. Add 25 ml of pretreated water sample and 25 μl of 30% hydrogen peroxide to a 60-ml quartz tube. Irradiate the sample with a 700-W UV lamp for 3 h. Add 12.5 ml of concentrated hydrochloric acid (extracted with toluene) to the sample [to reduce Se(VI) to Se(IV)] and place the test-tubes in a temperature-controlled aluminium block or equivalent. Boil the sample for 30 min. Remove the tube from the heating source and add 1 ml of 5.5 m*M* Br_2-PDA extracted with toluene. Wait for 5 min, then cool the sample to room temperature and transfer it quantitatively to a separating funnel with 5 ml of Milli-Q water. Add 1 ml of toluene containing lindane (20 ng/ml) and extract the sample for 2 min by vigorous shaking. Discard the aqueous phase and transfer the toluene phase to a 5-ml test-tube with a PTFE-faced screw-cap. Wash the toluene twice with 1.5 ml of Milli-Q water (rapid phase separation can be obtained by spinning the test-tube). Dry the toluene phase by adding anhydrous sodium sulphate prior to injection into the chromatograph. Evaluate the result from a calibration graph obtained with PIAZ or selenium(IV) standards and lindane as internal standard.

The procedure is adapted to waters containing 10–200 ng/l of selenium. Outside this range, the sample volume should be adjusted.

RESULTS AND DISCUSSION

Rate of the piazselenol reaction

In the following, the previous abbreviation Br_2-PDA for 1,2-diamino-3,5-dibromobenzene will be retained in general discussions of the ligand; however, if its chemical form is of importance in the context, the ligand will be referred to in italics by *Br_2-PDA*, *Br_2-PDAH$^+$* or *Br_2-PDAH$_2^{2+}$* to indicate a particular species. A symbol in italics within square brackets denotes concentration.

The formation of the piazselenol has been shown repeatedly [3,25–28] to occur by the reaction between undissociated selenious acid and the diamine in the monoprotonated form. Hence the rate of reaction will be optimum in the pH range where these species predominate. As the dissociation constants of *Br_2-PDAH$_2^{2+}$* were unknown, they were determined by spectrophotometric measurements. The $pK_{a,2}$ value was found to be 2.6. The value of $pK_{a,1}$ could not be properly established but it must be small as it was observed that the spectrum of *Br_2-PDAH$^+$* did not change until the medium was 3 *M* in perchloric acid and it changed continuously in the range 3–7 *M* acid.

The $pK_{a,1}$ value of selenious acid is 2.6. Hence the reaction rate should be virtually independent of the acidity in the range 0.1–3 *M*. Outside this region the amount of monoprotonated ligand decreases and the reaction rate diminishes. Tôei and Shimoishi [3,8] found that *Br_2-PDAH$^+$* reacts quantitatively with selenious acid in 0.01–6 *M* hydrochloric acid if the ligand is present in more than a 12 000-fold molar excess over selenium(IV).

The kinetic experiments were carried out in 0.25 *M* perchloric acid. In this medium, Se(IV) will be present almost exclusively as H_2SeO_3 and the ligand as *Br_2-PDAH$^+$*. The reason for using perchloric acid instead of the commonly employed hydrochloric acid medium is the greater solubility of Br_2-PDA in perchloric acid. This acid also yielded cleaner blank chromatograms than hydrochloric acid.

From the previous discussion, the expected rate law for the formation of the piazselenol is

$$r = \frac{\mathrm{d}[PIAZ]}{\mathrm{d}t} = -\frac{\mathrm{d}[Se(IV)]}{\mathrm{d}t} = k'_2[H_2SeO_3][Br_2\text{-}PDAH^+] \quad (1)$$

Under the current experimental conditions, eqn. 1 can be written as, and a new second-order rate constant, k_2, defined by,

$$r = -\frac{d[Se(IV)]}{dt} = k_2[Se(IV)]_{tot}[Br_2\text{-}PDA]_{tot} \quad (2)$$

as the reactants are constant fractions, almost equal to 1, of the total selenium(IV) and ligand concentrations. In the following equations and discussions the subscript tot will be omitted.

Eqn. 2 was experimentally tested by Ostwald's isolation method using an excess of the ligand. Integration of eqn. 2 gives

$$\ln[Se(IV)] = \ln[Se(IV)]_0 - k't \quad (3)$$

where $k' = k_2[Br_2\text{-}PDA]$ and $[Se(IV)]_0$ represents the initial selenium concentration. First $[Se(IV)]_0$ was varied at constant $[Br_2\text{-}PDA]$. The results are presented in Fig. 2. The slopes of the three lines are equal, which is in agreement with eqn. 3. In a second series of experiments $[Br_2\text{-}PDA]$ was varied at constant $[Se(IV)]_0$. The slopes of the lines in Fig. 3, k', are different but the k_2 values are the same. The proposed rate equation is thus confirmed. These experiments were carried out at 20°C and eqn. 3 was assumed to be valid also at higher temperatures.

The values of k' at higher temperatures were evaluated in the following way in order to account for a possible time lag before the reaction mixture became homogeneous with respect to temperature

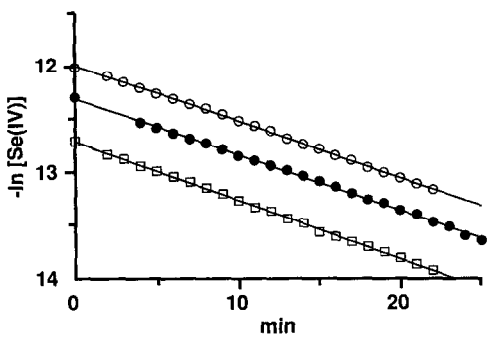


Fig. 2. Determination of the rate constant of piazselenol formation at 20°C by fitting eqn. 3 to the experimental data. The concentration of Br_2-PDA was kept constant at 0.3 mM and the total selenium(IV) concentration was varied: □ = 3.02; ● = 4.52; ○ = 6.03 μM.

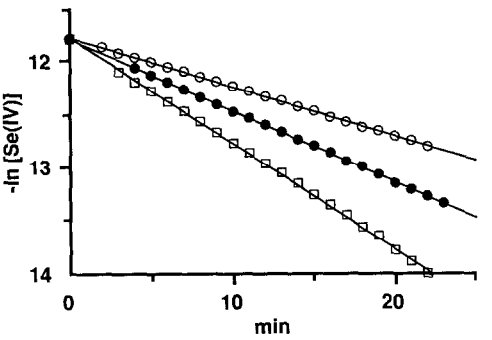


Fig. 3. Determination of the rate constant of piazselenol formation at 20°C by fitting eqn. 3 to the experimental data. The total concentration of selenium was kept constant at 7.5 μM and the concentration of Br_2-PDA was varied: ○ = 0.177; ● = 0.265; □ = 0.353 mM.

and concentration. The total concentration of selenium, C_0, is

$$C_0 = [PIAZ] + [Se(IV)] \quad (4)$$

With the ligand in excess the combination of eqns. 1, 2 and 4 yields

$$\frac{d[PIAZ]}{dt} = k'(C_0 - [PIAZ]) \quad (5)$$

The selenium(IV) solution is added to the Br_2-PDA solution in the cuvette at $t = 0$. A certain time will elapse before the solution is homogeneous and the temperature has stabilized at the preset value. At that time, $t = t_0$, the concentrations are $[Se(IV)] = C_0'$ and $[PIAZ] = C_0 - C_0'$. Integration of eqn. 5 between t and t_0 then results in

$$[PIAZ] = C_0 - B\,e^{-k't} \quad (6)$$

where $B = C_0'\,e^{k't_0}$. As the absorbance, A, of the piazselenol formed was measured, eqn. 6 is more conveniently expressed as

$$A_t = A_\infty - b\,e^{-k't} \quad (7)$$

Eqn. 7 was fitted to the experimental data shown in Fig. 4 by non-linear least-squares calculations.

The results from the kinetic studies of the piazselenol formation are presented in Table I. The temperature dependence of the second-order rate constant between 20 and 60°C can be expressed by

$$\ln k_2 = 22.9 - \frac{42900}{RT} \quad (8)$$

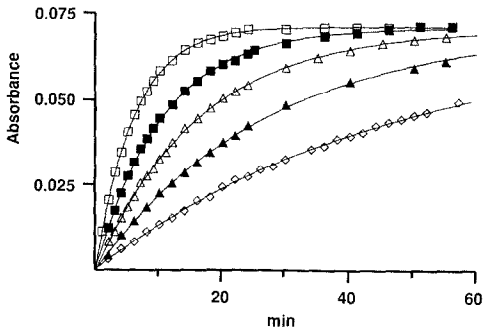


Fig. 4. Determination of the temperature dependence of piazselenol formation. The rate constant at each temperature was found by fitting eqn. 7 to the experimental data. Temperature: □ = 60; ■ = 50; △ = 40; ▲ = 30; ◇ = 20°C.

with k_2 in l mol^{-1} min^{-1} and the activation energy in J mol^{-1}. Half-lives, $t_{\frac{1}{2}}$, can be calculated from eqns. 8 and 9:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_2[Br_2\text{-}PDA]} \quad (9)$$

Table II contains estimates, expressed as $5t_{\frac{1}{2}}$, of the time for quantitative piazselenol formation with 0.1 m*M* Br_2-PDA at temperatures between 20 and 100°C in 0.25 *M* perchloric acid. The data indicate that the reaction time could be shortened from about 3 h to 4 min if the piazselenol can be formed at 100°C instead of room temperature (20°C). An increased temperature has been used for the piazselenol reaction in the analysis of biological samples [7,10,13,29], but it has also been reported that a high temperature in the derivatization step leads to a number of spurious peaks in the chromatogram [29].

TABLE I

SECOND-ORDER RATE CONSTANTS OF THE PIAZSELENOL FORMATION REACTION

The second-order rate constants (k_2) were determined once with 0.1 and twice with 0.3 m*M* Br_2-PDA. The estimated standard deviation is given in parentheses.

Temperature (°C)	k_2 (l mol^{-1} min^{-1})
20	200 (3)
30	362 (6)
40	616 (12)
50	1035 (39)
60	1658 (73)

TABLE II

TIME REQUIRED FOR QUANTITATIVE PIAZSELENOL FORMATION

The estimated times correspond to five half-lives ($5t_{\frac{1}{2}}$) calculated from Table I or, above 60°C, by extrapolation of eqn. 8. The ligand concentration was 0.1 m*M*.

Temperature (°C)	$5t_{\frac{1}{2}}$ (min)	Temperature (°C)	$5t_{\frac{1}{2}}$ (min)
20	175	70	13
30	98	80	9
40	57	90	6
50	34	100	4
60	21		

Chromatographic conditions and clean-up procedure

The piazselenol reaction yields by-products, which together with a co-extracted excess of the diamine result in chromatographic peaks that may overlap the piazselenol peak. Back-extractions of the organic extract with hydrochloric acid [3], perchloric acid [4,6,7,13,14], perchloric acid and sodium hydroxide [5,11] or by passing the organic phase through a Fluorosil column [10] have been used to eliminate the interferences. For Br_2-PDA it has been reported [29] that a peak partially overlapping the piazselenol peak could not be eliminated completely.

In earlier investigations on the GC determination of selenium, packed columns have been used. With the higher efficiency of a capillary column, interferences are absent in the present system even without a clean-up procedure, as the piazselenol is well separated from the by-products (Fig. 5a). The ligand, which is added in large excess, elutes after the piazselenol as two broad peaks. The effect of one clean-up step on the toluene extract is shown in Fig. 5b. The two broad peaks have almost disappeared and some of the peaks around the piazselenol peak have decreased. A clean-up step could be included in the method to prolong the lifetime of the column and detector and was therefore investigated.

In the clean-up step the toluene phase was extracted once with 1.5 ml of 6 *M* perchloric acid

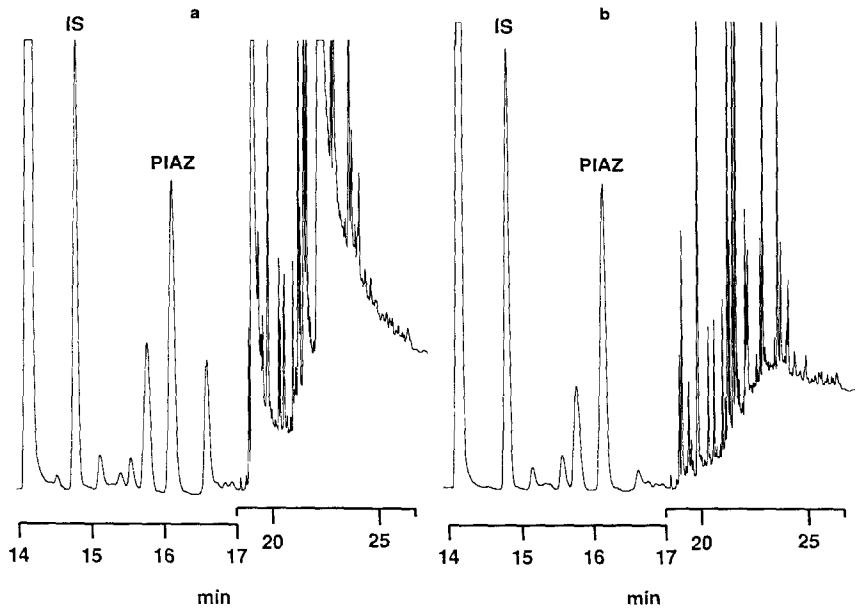


Fig. 5. Chromatograms of toluene extract of the reaction mixture after reduction of Se(VI) to Se(IV) in 4 *M* hydrochloric acid and piazselenol formation at 100°C. Only the analytically relevant part of the chromatogram is shown. A 25-ml volume of 130 ng/l Se solution was made to react with 0.1 m*M* Br_2-PDA for 5 min. The reaction mixture was extracted with 1 ml of toluene containing lindane as internal standard. The volume injected was 0.5 μl. (a) Without clean-up; (b) with clean-up; extraction with 6 *M* perchloric acid.

followed by two washings with 1.5 ml of Milli-Q water. This treatment removed most of the Br_2-PDA (Fig. 5b). The piazselenol was not back-extracted (Table III) unless the perchloric acid concentration exceeded 7 *M*. This finding is in fair agreement with a report [4] that two extractions can be made with 8 *M* perchloric acid without loss. For 4-bromo-6-fluoropiazselenol losses were observed after washings with 6 *M* perchloric acid [14]. Such differences between piazselenols might be due to differences in their first protonation constants [30].

TABLE III

BACK-EXTRACTION OF PIAZSELENOL AND LINDANE

A 1-ml volume of toluene containing 1.9 ng of piazselenol and 17 ng of lindane was extracted with perchloric acid solution. *R* is the ratio between the areas of the piazselenol and lindane peaks.

$[HClO_4]$ (*M*)	*V* (ml)	*R*
–	–	0.353
4	1.5	0.355
6	1.5	0.355
7	1.5	0.350
8	1.5	0.299
9	1.5	0.112
0.25	100	0.346

The temperature programme used gave a good separation of the piazselenol peak from some minor peaks close to it. The resolution between the piazselenol and the two closest peaks were $R_s(1) = 2.2$ and $R_s(2) = 3.3$. It would therefore be possible to use a shorter column. This was not investigated because in the future the column will be used to determine selenium(IV) in water. Decomposition of organic matter (UV destruction) then cannot be made and this will lead to additional peaks in the chromatogram from concomitants in the water.

If the Br_2-PDA reagent was not extracted with toluene before use, then a large peak appeared next to the piazselenol peak.

Lindane as internal standard

Lindane was used as internal standard and about 18 ng/ml of lindane were added to the toluene used

TABLE IV

EFFICIENCY OF THE PIAZSELENOL EXTRACTION

The stated amounts of selenium were derivatized in different volumes of 0.25 *M* perchloric acid and then extracted into 1 ml of toluene. *R* is the ratio between the areas of the piazselenol and lindane peaks corrected for the blank. The blank is the value of *R* when no selenium was added.

Se (ng)	*R*		
	V_{aq} = 25 ml	V_{aq} = 100 ml	V_{aq} = 250 ml
Blank	0.0263	0.0384	0.0890
1	0.320	0.324	0.317
2	0.630	0.638	0.579
3	0.870	0.885	0.812
4	–	1.14	1.09

for extraction of the piazselenol. It is well separated in the chromatogram (Fig. 5) and gives a good response in the electron-capture detector. No loss of lindane has been observed in the extraction or the clean-up step.

Extraction efficiency

The efficiency of the extraction of the piazselenol was investigated by extracting 0, 1, 2, 3 and 4 ng of selenium(IV) derivatized in different volumes of 0.25 *M* perchloric acid into 1 ml of toluene. The results presented in Table IV indicate that the distribution constant is so large that the extraction can be considered to be quantitative. There was, however, a small loss when extraction was made from 250 ml of the aqueous phase. Owing to the sensitivity of the method, the use of sample volumes larger than 100 ml is seldom required. For larger volumes the losses can be compensated for by establishing a calibration graph from standards of the appropriate volume.

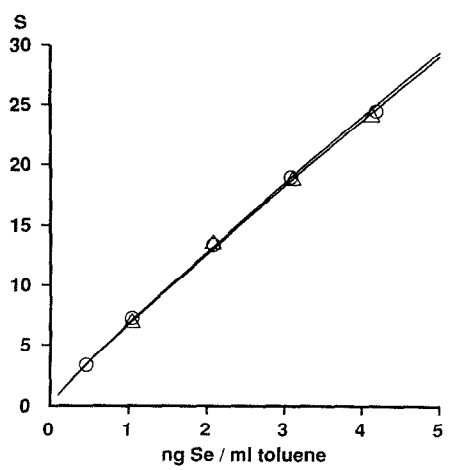


Fig. 6. Calibration graphs. *S* is the area ratio between the piazselenol peak and the internal standard (lindane) peak recalculated to an internal standard concentration of 1 ng/ml in toluene. Results from (□) synthesized piazselenol standards and (○) derivatized selenium(IV) standards.

Calibration

There was good agreement between calibration graphs obtained from solutions of synthesized piazselenol and derivatized standard solutions of selenium(IV) (Fig. 6). Standard solutions of synthesized piazselenol can therefore be used for calibration purposes. As a check of the calibration, a standard solution of selenium(IV), treated as the sample, should be included in the analysis of real samples. This procedure will correct for extraction losses with large sample volumes.

The calibration graph is non-linear. The function $Y = AX^B$ reproduces the calibration data well after subtraction of the blank. The value of *B* is typically about 0.9.

Derivatization at higher temperatures

The formation of the piazselenol at higher temperatures and hence shorter reaction times, $5t_{\frac{1}{2}}$, was investigated in two series of experiments. In the first series the toluene extracts were subjected to the previously described clean-up step with 6 *M* perchloric acid. This step was excluded in the second series. No special precautions were taken to minimize possible loss of piazselenol, other than covering the mouth of the conical reaction flask with a watch-glass. Losses of the volatile piazselenol would be greatest from boiling solution. In the experiments performed at 100°C the reaction vessel was therefore removed from the hot-plate before adding Br_2-PDA. The calculated reaction time was prolonged by 1 min instead.

At all temperatures the derivatizations resulted in very similar chromatograms. The analytical data are presented in Table V. No decrease in recovery is observed at higher temperatures. The blank value is

TABLE V

RECOVERY OF SELENIUM WHEN THE PIAZSELENOL IS FORMED AT HIGHER TEMPERATURES

The sample consisted of 1 ng of Se(IV) added to 100 ml of 0.25 *M* perchloric acid. The results are averages of triplicate determinations with relative standard deviations in parentheses.

Temperature (°C)	Reaction time (min)	Selenium found (ng)			
		Without clean-up		With clean-up	
		Sample	Blank	Sample	Blank
20	174	1.00[a] (4.8%)	0.050	1.01 (2.4%)	0.064
40	56	1.02 (0.2%)	0.051	1.02 (4.2%)	0.049
60	21	1.00 (1.0%)	0.048	1.00 (0.8%)	0.054
80	9	1.03 (7.8%)	0.051	0.99 (1.5%)	0.086
100	5	1.01 (5.0%)	0.060	1.01 (5.4%)	0.062

[a] Used as a reference.

TABLE VI

TOTAL SOLUBLE SELENIUM IN A LAKE WATER SAMPLE

The determination was made according to the procedure described in the text. The results obtained with an HG-AAS method was 131 ± 5 ng/l. This was an average of five different digestion procedures [31].

[HCl] (*M*)	$t_{react.}$ (min)	Selenium concentration (ng/l)
4	5	128
		124
4	10	132
		129
3.2	5	127
		129
1.5	180[a]	130

[a] The derivatization was made at room temperature.

not affected by temperature and the clean-up step does not influence the results.

The time for the derivatization step can be reduced by increasing the concentration of Br_2-PDA. Short reaction times can then be achieved at moderate temperatures. For instance, the reaction would be complete after 7 min when the concentration of Br_2-PDA is 0.3 m*M* and the temperature 60°C. However, a higher Br_2-PDA concentration adversely affects the chromatogram unless one or several clean-up steps are included. Therefore, it is recommended to use a lower Br_2-PDA concentration and a higher temperature.

Determination of total soluble selenium in water

In the determination of total soluble selenium in water it is necessary to decompose organic selenium compounds and reduce selenium(VI) to selenium(IV). The decomposition of the organic material was achieved by UV irradiation of the sample contained in closed quartz tubes after addition of hydrogen peroxide [21]. The lake water analysed contained 24 mg/l of carbon and was completely discoloured after the UV irradiation. The reduction was carried out in 4 *M* hydrochloric acid at 100°C for 30 min.

It would be convenient to carry out the derivatization directly in the quartz tube after the reduction step. The concentration of monoprotonated ligand is lower in 4 *M* hydrochloric acid than in 0.25 *M* perchloric acid, so that the previous kinetic data are not directly applicable. Table VI contains data from derivatizations at high hydrochloric acid concentrations. Obviously the high temperature leads to such a high reaction rate that neither the acid concentration need be decreased nor the reaction time increased.

The results from the GC–ECD method agree with those from a study with an HG-AAS method in which also different digestion procedures were evaluated [31].

CONCLUSIONS

Most analytical procedures for selenium determine only selenium(IV). Therefore, selenium(VI) must be reduced to selenium(IV). This is generally accomplished by boiling the sample in about 4 *M* hydrochloric acid. In the GC–ECD method the derivatization step follows the reduction step. For easy performance, the derivatization conditions should be compatible with the conditions prevailing after the reduction step. This is the case for Br_2-PDA owing to the small value of its second protonation constant. The piazselenol reaction is complete in about 5 min at boiling temperature with little or no increase in the background from decomposed ligand. The use of a capillary column facilitates the isolation of the piazselenol peak and a clean-up step may be

omitted. The sensitivity is good and quantitative determination of a few ng/l of selenium in water can be made.

REFERENCES

1 O. Hinsberg, *Ber. Dtsch. Chem. Ges.*, 22 (1889) 862.
2 S. Nakashima and K. Tôei, *Talanta*, 15 (1968) 1475.
3 Y. Shimoishi, *J. Chromatogr.*, 136 (1977) 85.
4 Y. Shimoishi and K. Tôei, *Anal. Chim. Acta*, 100 (1978) 65.
5 K. Kurahashi, S. Inoue, S. Yonekura, Y. Shimoishi and K. Tôei, *Analyst (London)*, 105 (1980) 690.
6 H. Uchida, Y. Shimoishi and K. Tôei, *Environ. Sci. Technol.*, 14 (1980) 541.
7 H. Uchida, Y. Shimoishi and K. Tôei, *Analyst (London)*, 106 (1981) 757.
8 K. Tôei and Y. Shimoishi, *Talanta*, 28 (1981) 967.
9 Y. Shimoishi and K. Tôei, *Talanta*, 17 (1970) 165.
10 T. Stijve and E. Cardinale, *J. Chromatogr.*, 109 (1975) 239.
11 Y. Shimoishi, *Analyst (London)*, 101 (1976) 298.
12 C. F. Poole, N. J. Evans and D. G. Wibberley, *J. Chromatogr.*, 136 (1977) 73.
13 S. Dilli and I. Sutikno, *J. Chromatogr.*, 298 (1984) 21.
14 A. F. Al-Attar and G. Nickless, *J. Chromatogr.*, 440 (1988) 333.
15 J. W. Young and G. D. Christian, *J. Chromatogr.*, 65 (1973) 127.
16 O. Zheng, P.-Y. Xu, G.-L. Xiong and Y. Liu, *Talanta*, 33 (1986) 443.
17 J. Piwonka, G. Kaiser and G. Tölg, *Frezenius' Z. Anal. Chem.*, 321 (1985) 225.
18 G. A. Cutter, *Anal. Chim. Acta*, 98 (1978) 59.
19 C. M. G. van den Berg and S. H. Khan, *Anal. Chim. Acta*, 231 (1990) 221.
20 M. Zelić and M. Branica, *Electroanalysis*, 2 (1990) 455.
21 I. Gustavsson and L. Hansson, *Int. J. Environ. Anal. Chem.*, 17 (1984) 57.
22 J. Pettersson, L. Hansson and Å. Olin, *Talanta*, 33 (1986) 249.
23 T. A. Bengtsson, unpublished results.
24 E. S. Gould, *Anal. Chem.*, 23 (1951) 1502.
25 L. Barcza, *Microchim. Acta*, 1 (1964) 136.
26 H. Ariyoshi, M. Kiniwa and K. Tôei, *Talanta*, 5 (1960) 112.
27 J. Neve, M. Hanocq and L. Molle, *Microchim. Acta*, 1 (1980) 41.
28 J. Neve, M. Hanocq and L. Molle, *Talanta*, 26 (1979) 1173.
29 T. Stijve and G. Philoppossian, *Trav. Chim. Aliment. Hyg.*, 69 (1978) 74.
30 J. Neve, M. Hanocq and L. Molle, *Talanta*, 26 (1979) 15.
31 U. Örnemark, J. Pettersson and Å. Olin, *Talanta*, in press.