Journal of Chromatography, 109 (1975) 239–245

CHROM. 8202

RAPID DETERMINATION OF SELENIUM IN VARIOUS SUBSTRATES BY ELECTRON CAPTURE GAS–LIQUID CHROMATOGRAPHY

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(Received January 20th, 1975)

SUMMARY

In the proposed procedure for the determination of selenium, 0.5–1 g of sample is wet ashed with concentrated nitric acid. After adding 1,2-diamino-4,5-dichlorobenzene to the digest at pH 1, the resulting dichloropiazselenol derivative is extracted with toluene. The extract is purified by column chromatography over Florisil and analyzed by gas–liquid chromatography with electron capture detection. Recoveries of selenium added to various substrates ranged from 72 to 102%. The limit of detection is approximately 0.01 ppm, but smaller amounts can be determined by increasing the sample size or by concentration of the final extract.

INTRODUCTION

Present methods for the determination of low levels of selenium in plant and animal tissues, foods and other materials are based on the reactions between selenious acid and aromatic *o*-diamines that yield piazselenol compounds which are determined by fluorimetry[1–3]. Other methods for the determination of trace amounts of selenium involve the use of neutron activation[4] and isotope dilution[5], which require equipment that is not available to many laboratories.

In this laboratory, there was a need for the analysis of many samples of the same nature with selenium contents that ranged from 0.01 to 20 ppm. The fluorimetric methods mentioned above were considered to be unsuitable for this purpose, because the average analyst needs considerable training before he is proficient in obtaining consistently high recoveries. For this reason, we considered the use of the gas chromatographic procedure described recently by Young and Christian[6], who treated selenium with 2,3-diaminonaphthalene at pH 2 and extracted the corresponding piazselenol compound with hexane. The latter compound could be readily determined by gas chromatography with electron capture detection, but the limit of detection was only 0.5 ng which was not sensitive enough for our purpose. It was decided, therefore, to develop an improved method, based on the determination of a derivative that has a much higher electron affinity.

Such a derivative was obtained by the reaction of tetravalent selenium with 1,2-diamino-4,5-dichlorobenzene:

$$NH_2C_6H_2(Cl_2)NH_2 + H_2SeO_3 \rightarrow SeN:C_6H_2(Cl_2):N + 3H_2O$$

This reaction was formerly used for the gravimetric determination of selenium[7]. The dichloropiazselenol compound was formed quantitatively in the pH range 1–1.5 and could be extracted completely from aqueous media with organic solvents such as toluene. Its response to the electron capture detector was found to be 50 times greater than that of the 4,5-benzopiazselenol employed by Young and Christian[6]. A detailed description of the improved method is given in this paper.

EXPERIMENTAL

Reagents

All chemicals used were of micro-analytical reagent grade. The solvents used were free from electron capturing impurities.

Florisil (synthetic magnesium silicate), 60–100 mesh, adsorbent was purified by heating overnight at 550°. After cooling, the adsorbent was standardized by adding 3% (w/w) of distilled water, vigorously mixing for at least 20 min and allowing the mixture to equilibrate for 10–12 h. The partly deactivated adsorbent thus obtained could be used only up to 5 days after its preparation, after which time it was heated and standardized again.

1,2-Diamino-4,5-dichlorobenzene was obtained from Ferak, Berlin, G.F.R. The reagent was recrystallized from hydrochloric acid solution by addition of sodium hydroxide. A 0.6% solution of the purified diamine in 1 *N* hydrochloric acid was used as a reagent for conversion of tetravalent selenium into the dichloropiazselenol derivative.

Dichloropiazselenol standard was synthesized as follows. A 240-ml volume of a 0.6% solution of the purified diamine in 1 *N* hydrochloric acid was added to 100 ml of distilled water containing 210 mg of selenium dioxide. Upon adjusting the pH to 1.5 by adding 4 *N* sodium hydroxide solution, a copious precipitate was obtained. After allowing the mixture to stand for 1 h so as to complete precipitation, the solid was separated from the liquid by centrifuging at 3000 rpm. The supernatant liquid was discarded and the precipitate washed successively with 0.1 *N* hydrochloric acid and distilled water until acid-free. After drying at 110°, the piazselenol was purified by recrystallization from a mixture of light petroleum–diethyl ether (7:3, v/v). The purified compound was found to contain 30.85% of selenium upon elemental analysis. The theoretical value is 31.34%. Reference solutions for gas chromatography containing 10, 20 and 40 pg/μl were prepared in toluene.

A standard solution of selenium was prepared by dissolving 50.0 mg of pure black selenium in 5 ml of nitric acid, sp. gr. 1.42. This solution was made up to 50 ml with water in a calibrated flask. For recovery experiments, dilutions were used containing 0.1, 1.0 and 10.0 μg/ml of selenium.

Field of application of the method

The method can be applied to vegetables, fruits, flours, mushrooms and soil samples. Among fatty substrates, only whole egg powder was tested, but the procedure will probably work equally well for other fatty foods.

Digestion of samples

Mineralise 0.50 g of sample with 3 ml of concentrated nitric acid in a PTFE and stainless-steel decomposition vessel according to Bernas[8] at 100° for 15–20 min. If these decomposition vessels are not available, perform the digestion in 170 × 20 mm tubes, made of 2-mm thick Pyrex glass, provided with a venting side-tube and a Quickfit Rotaflo TF 6/24 PTFE valve. When using these tubes, the sample size may be increased to 1 g, but in that case add 5 ml of concentrated nitric acid. In order to avoid too vigorous reaction upon heating, allow the mixture to digest overnight in open tubes at room temperature. Subsequently, evacuate air from the tubes with a water-jet pump, close it tightly with the valve and heat it in a boiling water-bath for 15 min. *Caution*: when handling tubes under pressure, wear safety goggles.

After cooling, slowly and cautiously vent the pressure and transfer the reaction mixture quantitatively with small portions of distilled water into a 100-ml conical flask. Add slowly about 2 g of pure urea and swirl the flask so as to expel nitrogen oxides. Adjust the pH of the mixture to approximately 1 by adding a few drops of 0.2% ethanolic thymol blue solution and sufficient concentrated ammonia solution until the red colour changes to orange-yellow. At this point, the pH is about 2.5. Make the final adjustment to pH 1 by adding dropwise 2 *N* hydrochloric acid and check after each addition with narrow-range pH paper.

Conversion of selenium into its dichloropiazselenol derivative

Extract any possibly present electron capturing impurities by shaking the reaction mixture with 10 ml of toluene in a 100-ml separating funnel for about 1 min. Allow the layers to separate. Drain the aqueous lower phase into a 100-ml conical flask and discard the toluene upper layer. Add 0.5 ml of 0.6% purified diamine solution in 1 *N* hydrochloric acid, close the flask tightly and heat it in a water-bath at 80° for 10 min. Allow the mixture to cool, then transfer it quantitatively into a 100-ml separating funnel, using a few millilitres of distilled water for rinsing. Extract the derivative by vigorous shaking with 20 ml of light petroleum–toluene (3:1, v/v) for 1 min. Allow the layers to separate completely and discard the aqueous phase.

Clean-up

Use a glass chromatography column, 8 × 200 mm, fitted with an outlet stopcock and having a 20-ml reservoir at the upper end. Tamp a small plug of glass-wool into this column and add 2.5 g of standardized Florisil. Ensure tight packing of the adsorbent by tapping the sides of the column with a glass rod.

Allow the light petroleum–toluene extract obtained above to pass through the column at a rate of 1–2 ml/min. Discard the liquid that has run through, then allow a further 15 ml of light petroleum–toluene (3:1, v/v) to pass through the column in order to wash out impurities. When the liquid has reached the top of the Florisil column, close the stopcock and discard the eluate. Elute the selenium derivative with

18 ml of toluene into a 20-ml calibrated flask, make the volume up to 20 ml and mix. The solution is now ready for gas–liquid chromatography.

Gas–liquid chromatography

The dichloropiazselenol can be chromatographed on all columns that are normally used in pesticide residue analysis, such as Dow 11, OV-17, QF-1 and DEGS. The conditions used for two of these columns are listed in Table I. It is not possible to give definite instructions concerning the parameters associated with optimal performance, because they are different for each instrument. The response for the selenium derivative is not only dependent on detector performance, but also on the state of the column.

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS USED FOR THE DETERMINATION OF THE DICHLOROPIAZSELENOL DERIVATIVE

Parameter	*Stationary phase*	
	1.5% OV-17 + 1.95% QF-1 coated on Chromosorb W H.P., 100–120 mesh	*2% DEGS + 0.5% H_3PO_4 coated on Chromosorb W H.P., 100–120 mesh*
Instrument	Perkin-Elmer 3920	Perkin-Elmer F-11
Detector	Electron capture, nickel-63 source	Electron capture nickel-63 source
Detector operation	Pulse-modulated with extended linear range	Conventional negative d.c.
Attenuation	1 × 64	1 × 8
Carrier gas	Argon–methane (95:5, v/v), 40 ml/min	Nitrogen, 35 ml/min
Injection port temperature	250°	250°
Oven temperature	200°	170°
Detector temperature	275°	245°
Column	1.5 m × 3 mm, Pyrex	1.5 m × 3 mm, Pyrex
Sample volume injected	5 µl	5 µl
Approximate retention time of the dichloropiazselenol compound	3 min	2 min

Select a sensitivity at which 100 pg of the standard produce at least 50% full-scale deflection. Inject 5 µl of sample extract into the gas chromatograph with a micro-syringe. Compare the size of the peak of the selenium derivative with the size of the peak from a known amount of the standard. Check if the amount of the derivative in the injected sample aliquot falls within the linear range of the detector. If this is not so, prepare a suitable dilution and inject again. Sufficient accuracy is achieved when simply using the peak height (expressed in millimetres) for quantitation.

Calculate the selenium content of the sample aliquot by multiplying the dichloropiazselenol concentration by 0.3134.

Thin-layer chromatography for confirmation of identity

Concentrate the toluene sample solution to 0.5 ml by evaporation. Spot suitable aliquots of this concentrate, *i.e.*, volumes to give spots within the range 0.05–0.25 μg, on a neutral aluminium oxide thin-layer plate (Merck pre-coated No. 5550 aluminium sheets are suitable). Apply standard solutions to give spots of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 μg. Develop the plate under conditions of saturation with light petroleum–acetone (98:2, v/v) as the mobile phase. After development, allow the adherent solvent to evaporate and spray abundantly with 0.5% ethanolic silver nitrate solution. Subsequently, irradiate the plate for 10 min with a high-power photochemical lamp. Remove the plate from the lamp and spray it lightly with distilled water until the plate is just moistened. Expose the plate again to the UV lamp. Spots should now become visible at R_F 0.50 in 1–2 min. The limit of detection is approximately 0.05 μg.

Alternatively, thin-layer chromatography can also be performed on silica gel layers using the same mobile phase. Reveal the spots of the selenium derivative by spraying the chromatogram with a 5% solution of tin(II) chloride in 6 *N* hydrochloric acid–ethanol (1:1, v/v) and subsequent UV irradiation as described above. The piazselenol is reduced to elemental selenium and red spots become visible at R_F 0.20. The limit of detection with this chromogenic reagent is only 0.5 μg. It is, therefore, only suitable for confirming parts per million levels of selenium.

RESULTS AND DISCUSSION

The digestion procedure in the PTFE and stainless-steel decomposition vessels or in the glass tubes equipped with a PTFE valve was chosen because it allowed the rapid mineralization of large series of samples*. It is possible, of course, to use the more conventional procedures in Kjeldahl flasks[2,3], provided that charring of the samples, which may result in loss of selenium, is avoided.

Initially, it was thought that it was necessary to base our calculations upon a standard curve obtained by taking different aliquots of selenium standard solution

TABLE II

RECOVERY OF SELENIUM FROM AQUEOUS SOLUTIONS

Amount of selenium added (μg)	*Recovery (%) (duplicate determinations)*
0.02	92, 94
0.10	92, 94
0.20	94, 95
0.50	89, 91
1.00	94, 96
10.0	89, 92
100.0	85, 87

*Caution: After submission of this paper for publication, the authors experienced two explosions, one occurring during the mineralization of fish tissue in glass tubes and the other during digestion of dehydrated mushrooms in the PTFE and stainless-steel vessel. Although they performed about 400 such digestions without accidents, the authors consider that it may be safer to use conventional wet-ashing procedures.

through the whole procedure with each series of samples. However, recoveries obtained by adding the dichlorodiamine reagent to 10-ml aqueous solutions of different concentrations of selenious acid and treating the reaction mixture as described for the sample digests were excellent, as shown in Table II. Consequently, we decided to use directly standard solutions of the derivative for quantitation. Recoveries were checked from time to time by running a sample to which a known amount of selenium had been added. Table III clearly indicates that the recoveries obtained were satisfactory at all levels.

The reaction of selenium with 1,2-diamino-4,5-dichlorobenzene seems to be highly selective for this element. Although we analyzed several different substrates, ranging from flours to egg powder, we never observed supplementary peaks on our chromatograms. Starace *et al.*[7], who employed the reagent for the gravimetric determination of selenium, reported no interference from 27 other elements, but they obtained precipitates with osmium(VIII) and cerium(IV). We tested the reaction of these two ions under conditions as specified for selenium, but did not obtain a derivative that could be chromatographed, even at the microgram level.

TABLE III

RECOVERY OF SELENIUM ADDED TO SAMPLES

Sample	*Selenium added (ppb)*	*Selenium determined (ppb)*	*Mean value (ppb)*	*Recovery (%)*
Tomato flakes	0	61, 64, 65	63	—
	10	73, 75, 69	72	90
	50	103, 98, 96	99	72
	100	170, 158, 155	161	98
	200	250, 240, 252	247	92
	500	560, 550, 540	550	97
	1000	990, 980, 1020	997	93
	2000	1820, 1870, 1900	1863	90
Dehydrated leeks	0	78, 63, 59	67	—
	50	110, 120, 107	112	90
	200	240, 228, 220	229	81
	500	580, 590, 560	577	102
	600	590, 610, 575	592	88
	800	810, 785, 776	790	90
	1000	1050, 1080, 1110	1080	102
Dehydrated mushrooms (*Agaricus arvensis*)	0	5640, 5800, 5700	5713	—
	10000	15170, 15300, 14800	15090	94
Barley flour	0	30, 35, 40	35	—
	100	107, 110, 106	108	73
	400	390, 398, 375	388	88
Whole egg powder	0	450, 500, 475	475	—
	1000	1420, 1380, 1370	1390	92

In order to obtain quantitative conversion of selenium into the dichloropiazselenol derivative, it is essential to use the pH range 0–2. Outside this range, the recovery of selenium decreases sharply.

Considering the high selectivity of the reagent, gas chromatographic determination is usually adequate for routine analysis. We developed the thin-layer chromatographic confirmatory procedure mainly for ascertaining the high selenium levels that we encountered in some species of mushrooms.

The clean-up step on Florisil is necessary in order to eliminate excess of reagent and impurities. The dichlorodiamine itself is strongly retained on the Florisil column, but small amounts of the impurities also pass into the toluene eluate together with the selenium derivative. However, these compounds do not interfere in the determination, because they are eluted before and after the dichloropiazselenol. As the method requires only small amounts of reagents, the blank value is generally very low. In most instances, we did not observe a measurable derivative peak when running a blank. With our gas chromatographic equipment, we could still obtain a measurable peak upon injecting 5 μl containing 10 pg of the dichloropiazselenol. This means that the limit of detection, based on a 1-g sample and a final volume of 20 ml, was approximately 0.01 ppm of selenium. Lower levels can undoubtedly be determined by increasing the sample size or by concentrating the final extract.

Other gas chromatographic detectors were also tried in order to determine their response to the piazselenol compound. The flame photometric detector[9] responded to nanogram amounts of the derivative when operated with a sulphur filter (394 nm). Slightly better sensitivity was obtained with the Perkin-Elmer P/N detector[10], using a very cool flame, but both devices were obviously unsuitable for the determination of selenium at the parts per billion level.

Experience with this method over a period of 1 year indicates that it is especially suitable for the routine determination of selenium. One technician can analyze without difficulty twelve samples per day, including gas chromatography and calculation of the results. It is not costly, because it requires only small amounts of reagents and uses equipment that is normally available in any laboratory dealing with trace analysis.

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