The Gas Chromatographic Determination of Selenium in Plant Material with 4-Nitro-*o*-phenylenediamine

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Selenium(IV) reacts with 4-nitro-*o*-phenylenediamine to form 5-nitropiaselenol, which is detected by means of a gas chromatograph equipped with an electron-capture detector. By this highly sensitive method, the determination of very small amounts of selenium in plant materials has been investigated. Fuming nitric acid digests the plant sample completely, and the selenium is converted to the quadrivalent state. No loss of selenium can be found by this treatment. A method is proposed for the determination of selenium in plant materials at levels as low as 0.005 μg/g in dried material.

In earlier studies of the determination of selenium contained in plant material, the interest of research workers has been directed toward the investigation of selenium toxicity for farm animals. In these studies, it was enough that the method could determine only the ppm levels of selenium in dried samples.

Recently, many investigators have demonstrated that selenium is an important micronutrient for farm animals. Scott[1] and Kubota *et al.*[2] have pointed out that the critical level for the deficiency of selenium for farm animal diets is 0.01—0.04 ppm. Therefore, it is necessary to have a method of analysis to determine amounts of selenium as low as 0.01 ppm with reasonable accuracy and reproducibility. To fulfill these requirements, a fluorometric method involving the use of 3,3'-diaminobenzidine[3,4] or 2,3-diaminonaphthalene[5,6] has been widely used in studies of animal nutrition. The neutron-activation[7] and isotope-dilution methods[8] have also been used to check the former method.

On the other hand, it is important to digest the plant material without any loss of selenium, because selenium is volatile. By the low-temperature ashing method, selenium could not be recovered completely.[9] In the oxygen-flask combustion method, selenium is obtained as an elemental state with a small amount of charred tissues in the platinum vessel, and the residue could not be dissolved completely by a bromine-bromide solution. Thus, the wet-digestion method was employed in the following experiments.

The author has recently proposed a far more sensitive method for determining selenium in pure sulfuric acid,[10] pure tellurium,[11] and sea water[12] by means of a gas chromatograph equipped with an electron-capture detector using 4-nitro-*o*-phenylenediamine. The present paper will describe an investigation of the determination of very small amounts of selenium in plant material.

Materials and Method

Reagents. 4-nitro-*o*-phenylenediamine hydrochloride solution: 1 g of the reagent was dissolved in 100 ml of 1 M hydrochloric acid. This solution is so stable that the solution can be used after standing for more than 1 week at room temperature.

Selenium(IV) stock solution: 351.3 mg of selenium dioxide was dissolved in 250 ml of distilled water (1 mg Se/ml). Working solutions were prepared by dilution. Selenium(0) stock solution: About 5 mg of elemental selenium was dissolved in 50 ml of carbon disulfide. The concentration is determined by gas chromatography[10] as 90 μg/ml. Working solutions were prepared by dilution. 7.5 M Hydrochloric acid solution: The acid solution was prepared each time before washing the toluene extract. The other reagents were of an analytical reagent grade.

Apparatus. A Shimadzu Model GC-3AE gas chromatograph equipped with an electron-capture detector was used. A glass column (1 m long, 4 mm bore) was packed with 15% SE-30 on 60—80 mesh Chromosorb W. The column and the detector temperature were maintained at 200 °C. The nitrogen flow-rate was 53 ml/min. A Shimadzu Model 250A recorder was used at a chart speed of 5 mm/min.

Standard Procedure. The whole digestion procedure must be carried out in a fume hood with an efficient extractor fan.

A tenth to 1 g portions of dried samples are put into a 100-ml conical flask. 5 ml of fuming nitric acid is added, and the mixture is heated on a sand bath (130—140 °C) in a fume hood. Vigorous oxidation reactions take place soon thereafter, and a copious brown vapor is evolved. After a few minutes, the main reaction comes to an end and the solution soon becomes clear (a few suspended particle of silicate are often observed). The solution is then heated further with frequent shaking until the white solid separates off and the volume is reduced to about 0.1—0.2 ml. During this procedure the temperature of the sand bath must be raised to 170—180 °C and maintained until the digestion is finished. At this time, if any charred material appears, erroneous results may be obtained. Therefore, special care has to be exercised to prevent the formation of a charred material by frequent shaking. If more than 0.5 g of a plant sample is needed for the determination, the digestion is not complete with the use of 5 ml of fuming nitric acid. Consequently, another 5 ml of fuming nitric acid is added and the digestion procedure has to be repeated. Digestion is usually completed by heating for about 1.5 hr. The flask is then removed and allowed to stand for 3 min. After redissolving the precipitate with 10 ml of distilled water, 2 ml of 1 M urea is added and the mixture is heated on the sand bath for 10 min to decompose the oxides of nitrogen. After cooling, the content is transferred into a 100-ml separatory funnel and the flask is rinsed with 10 ml of distilled water into the funnel. When the selenium content was too much, an aliquot containing 0.01—0.1 μg of selenium was used. Five ml of concentrated hydrochloric acid and 5 ml of toluene are added, and the toluene-soluble material is extracted with vigorous shaking for 5 min.

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After standing for 2 hr, the aqueous phase is transferred into another 100-ml separatory funnel, and the initial separatory funnel is rinsed with 10 ml of distilled water into the latter. The color of the solution is pale yellow or colorless. Two milliliters of a 1% 4-nitro-*o*-phenylenediamine solution are added, and the solution is allowed to stand for 2 hr. Then, the 5-nitropiaselenol formed is extracted into 1 ml of toluene by shaking for 5 min; the toluene extract is washed, first with 5 ml of 0.2 M sodium hydroxide and subsequently with 2 ml of 7.5 M hydrochloric acid, by shaking for a minute. Five microliters of the toluene extract are injected into the gas chromatograph and the peak height is measured.

Results and Discussion

Digestion of the Plant Material. A mixture of nitric and sulfuric acids or nitric and perchloric acids is usually used in the digestion method. Gorsuch[13] suggested in his detailed study that a considerable loss of selenium at the 1 ppm level was found when using the former mixture, whereas he obtained a 100% recovery with the latter one. In recent publications, when the digestion mixture containing a catalyst such as dichromate,[14] molybdate[15] or vanadate[16] was used, vigorous bumping was observed in the reactions.

Hall and Gupta[6] used nitric acid, hydrogen peroxide, and perchloric acid in the digestion of the plant samples. They stated that the selenium loss often occurred when the temperature rose too far as a result of the use of perchloric acid. Moreover, when the plant sample was digested with only fuming nitric acid or a mixed solution of fuming nitric and perchloric acids, the recovery of the above two methods showed the same value. Thus, fuming nitric acid was used for the digestion of the plant material.

0.2 g filter paper (Toyo Roshi No. 5B) was impregnated with carbon disulfide containing 0.045 μg elemental selenium and then dried. The filter paper was digested by various amounts of fuming nitric acid. On the other hand, the same filter paper and a 0.2 g stem of wheat containing less than 0.005 μg/g selenium were digested together. Figure 1 shows that more than 4 ml of fuming nitric acid can digest the filter paper and stem of wheat completely, and a good recovery is observed.

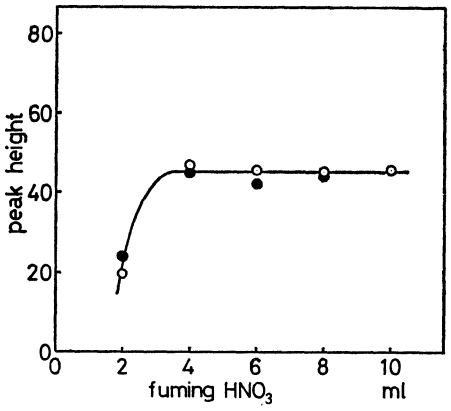

Fig. 1. Effect of the volume of fuming nitric acid.
-O- 0·045 μg selenium(0) permeated in filter paper
-●- 0.045 μg selenium(0)+0.2 g stem of wheat (selenium content below 0,005 μg/g)

Five ml of fuming nitric acid digest 0.2 g filter paper containing 0.027 μg selenium and a wheat stem which contains less than 0.005 μg Se/g. When a larger amount of wheat is to be digested, the digestion is repeated by the use of another five ml of fuming nitric acid. If charred materials do not appear during the digestion, the determination may be undertaken. The results are shown in Fig. 2. When more than 0.5 g of a sample is digested by 5 ml of fuming nitric acid, the background of gas chromatogram becomes larger. However, this trouble is overcome by further digestion with 5 ml of fuming nitric acid without any loss of selenium.

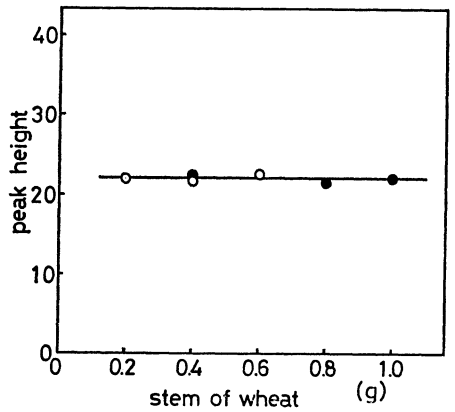

Fig. 2. The amount of the plant sample digested by 5 ml of fuming nitric acid.
-O- 0.027 μg selenium(0)+stem of wheat (selenium content below 0.005 μg/g) digested by 5 ml of fuming nitric acid.
-●- the same sample digested twice another 5 ml of fuming nitric acid

The digestion temperature is examined by the use of 0.2 g filter paper containing 0.045 μg of elemental selenium at 100—110, 160—180, and 210—220 °C (sand bath temperature). At 100—110 °C, the digestion is not enough, and at 210—220 °C the sample is sometimes charred, at which time selenium is lost. When a sample is heated at 160—180 °C for 80 min, digestion is complete. The heating is continued at this temperature till the volume of the digestion liquid reaches 0.1—0.2 ml; the smaller the volume of the liquid is, the better the result becomes. The whole digestion requires about one and half an hour.

In the digestion procedure, a small amount of undecomposed materials such as hydrocarbon constituents remains unchanged; it should be removed before gas chromatography. Five milliliters of toluene are used to eliminate any interfering substances. Selenium is not transferred into toluene by this procedure. Though a major part of the toluene-soluble substances can be eliminated by this treatment, the very small amount of interfering substances remaining disturbs the determination, because the sensitivity of the electron-capture detector is extremely high for the unknown impurities as well as for 5-nitropiaselenol. Thus, 1 ml of the toluene extract after the formation of 5-nitro-

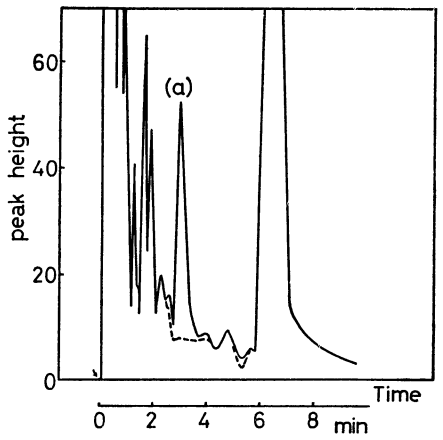

Fig. 3. Gas chromatogram of 5-nitropiaselenol.
---- 0.2 g stem of wheat.
—— 0.2 g stem of wheat + 0.045 μg selenium(0)
(a) 5-nitropiaselenol
Column: 15% SE-30/Chromosorb W, Carrier gas: N_2 (53 ml/min), Temperature: 200 °C, Range: 0.1 V, Sample: 5 μl.

piaselenol should be washed again with 5 ml of 0.2 M sodium hydroxide and then 2 ml of 7.5 M hydrochloric acid. Figure 3 shows the ECD gas chromatogram obtained by the use of the above-mentioned procedure.

Recovery Test. The recovery test by the standard procedure is carried out by the use of two samples: one is a mixed sample of 0.2 g filter paper containing 0.045 μg elemental selenium and 0.2 g of wheat stem which contains less than 0.005 μg Se/g, while the other is foliage leaves of wheat cultivated in a pot. The results, shown in Tables 1 and 2, indicate that fairly good results are obtained by this procedure.

Table 1. Recovery of the selenium added to stem of wheat

Stem of wheat[a] (g)	Added selenium(0) (μg)	Peak height	Selenium determined (μg)	Recovery (%)
0.200	none	2.0	0	
0.200	0.045	41.0	0.043	96
0.200	0.045	45.0	0.047	104
0.200	0.045	42.0	0.044	98
0.200	0.045	44.0	0.046	102

a) Containing less than 0.005 μg Se/g.

Table 2. Reproducibility of selenium in foliage of wheat

Foliage of wheat[a] (g)	Added selenium (μg)	Peak height	Selenium determined (μg/g)
0.200		3.5	0.020
0.200		4.5	0.025
0.500		12.0	0.026
0.500		12.0	0.026
0.500	0.027	35.0	0.024

a) Cultivated in the pot which contains the soil added no selenium.

Selenium(VI) in Plant. The oxidation number of the selenium contained in the plant sample should be −2, 0, or +4. The selenium in these oxidation states can be determined by the above-mentioned procedure. If selenium(VI) is present in the plant, it cannot be determined by the standard procedure as it stands. If selenium(VI) was contained in the plant, it would be reduced to the IV state by heating with 4 M hydrochloric acid on a sand bath for 10 min at about 100 °C, after the addition of 1 M urea to decompose oxides of nitrogen. By this procedure, selenium of the VI state could not be detected in the samples used in the experiment.

Effect of Chloride Ion. No loss of selenium was found during the reduction of selenium(VI) with 4 M hydrochloric acid for 10 min at about 100 °C. When the chloride ion is present together with selenium for a long digestion at 160—180 °C, selenium(VI) volatilizes to form its chloride. Even if the digestion procedure is carried out in the presence of 10 mg of sodium chloride, no loss of selenium is found.

Calibration Curves. The calibration curves are made by the use of four standard samples. The first is made from an acidic solution containing a known amount of selenium(IV). The second is prepared by treating filter paper containing selenium(IV) by the standard procedure. The third is made from filter paper containing elemental selenium. The fourth is made from a mixed sample of filter paper containing a known amount of elemental selenium and a wheat stem containing no selenium. The curves obtained are shown in Fig. 4. These are quite consistent with each other. This fact also shows that no loss of selenium is found in the standard procedure adopted.

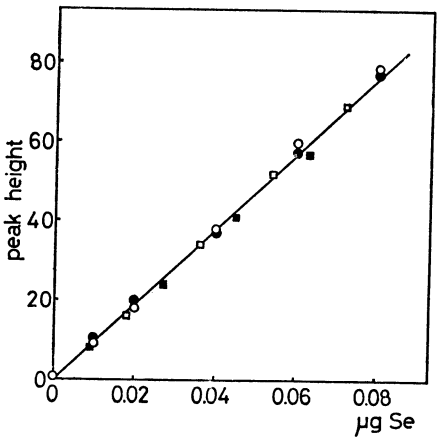

Fig. 4. Calibration curve.
-○- selenium(IV) in hydrochloric acid (no digestion)
-●- selenium(IV) permeated in filter paper
-⊟- selenium(0) permeated in filter paper
-■- selenium(0) + 0.2 g of stem of wheat (selenium content below 0.005 μg/g)

Determination of Selenium in Plant Material. The determination of the selenium involved in the plant material was carried out for cabbage, Chinese cabbage, wheat, and rice plants. The cabbages and Chinese cabbages were harvested from an ordinary field. The wheats and rice plants were obtained as follows: a seed-

TABLE 3. SELENIUM CONTENT OF PLANT MATERIALS

		Selenium content in soil[a] (ppm)	Plant sample (g)	Selenium added (μg)	Dilution	Selenium determined (μg)	Selenium content (μg/g)
Cabbage	Foliage	none	0.500			0	<0.005
		none	0.500			0	<0.005
		none	1.000			0	<0.005
		none	1.000	0.027		0.026	<0.005
	Average						<0.005
Chinese	Foliage	none	0.500			0.005	0.010
Cabbage		none	0.500			0.009	0.018
		none	0.500	0.027		0.026	<0.005
		none	1.000			0.004	0.004
		none	1.000			0.004	0.004
		none	1.000	0.027		0.024	<0.005
	Average						0.006
Wheat	Stem	none	0.400			0	<0.005
		none	0.400			0	<0.005
		none	0.800	0.027		0.025	<0.005
		none	1.000	0.027		0.026	<0.005
	Average						<0.005
	Foliage	none	0.200			0.004	0.020
		none	0.200			0.005	0.025
		none	0.500			0.013	0.026
		none	0.500			0.013	0.026
		none	0.500	0.027		0.039	0.024
	Average						0.024
	Ear	none	0.781			0	<0.005
		none	1.252			0	<0.005
		none	0.416	0.027		0.025	<0.005
		none	0.944	0.027		0.026	<0.005
	Average						<0.005
	Stem	5	0.100			0.018	0.18
		5	0.100	0.027		0.032	0.05
		5	0.200			0.038	0.19
		5	0.200			0.042	0.21
	Average						0.17
	Foliage	5	0.100			0.032	0.32
		5	0.100	0.027		0.052	0.25
		5	0.200			0.068	0.34
		5	0.200			0.066	0.33
	Average						0.31
	Ear	5	0.327			0.056	0.17
		5	0.547			0.196	0.36
		5	0.237	0.027		0.081	0.23
		5	0.320			0.066	0.21
	Average						0.24
	Stem	10	0.100		20/50[b]	0.022	0.55
		10	0.100	0.045	20/50	0.034	0.40
		10	0.200		20/50	0.048	0.60
		10	0.200		20/50	0.048	0.60
	Average						0.54
	Foliage	10	0.100		20/50	0.038	0.95
		10	0.100	0.045	20/50	0.053	0.88
		10	0.200		20/50	0.077	0.94
		10	0.200		20/50	0.086	1.08
	Average						0.97
	Ear	10	0.281		20/50	0.063	0.56
		10	0.548		20/50	0.165	0.75

TABLE 3. Continued

		Selenium content in soil[a] (ppm)	Plant sample (g)	Selenium added (μg)	Dilution	Selenium determined (μg)	Selenium content (μg/g)
		10	0.218	0.045	20/50	0.086	0.78
		10	0.403		20/50	0.084	0.52
	Average						0.65
	Stem	20	0.100		10/50	0.044	2.20
		20	0.100		10/50	0.052	2.60
		20	0.100		10/50	0.059	2.95
		20	0.100	0.18	5/50	0.047	2.90
	Average						2.7
	Foliage	20	0.100		10/50	0.077	3.85
		20	0.100		10/50	0.074	3.70
		20	0.100		10/50	0.075	3.75
		20	0.100	0.18	5/50	0.057	3.90
	Average						3.8
	Ear	20	0.174		5/50	0.037	2.13
		20	0.150	0.18	5/50	0.065	3.13
		20	0.163		5/50	0.032	1.96
		20	0.286		5/50	0.059	2.06
	Average						2.3
	Stem	50	0.100		5/50	0.034	3.40
		50	0.100		5/50	0.030	3.00
		50	0.100		5/50	0.036	3.60
		50	0.100	0.18	5/50	0.053	3.50
	Average						3.4
	Foliage	50	0.100		5/50	0.050	5.00
		50	0.100		5/50	0.057	5.70
		50	0.100		5/50	0.054	5.40
		50	0.100	0.18	5/50	0.068	5.00
	Average						5.3
	Ear	50	0.113		5/50	0.069	6.11
		50	0.112	0.18	5/50	0.057	3.48
		50	0.152		5/50	0.050	3.29
		50	0.164		5/50	0.052	3.17
	Average						4.0
	Stem	100	0.100		5/50	0.040	4.00
		100	0.100		5/50	0.042	4.20
		100	0.100		5/50	0.051	5.10
		100	0.100	0.18	5/50	0.062	4.40
	Average						4.4
	Foliage	100	0.100		5/50	0.078	7.80
		100	0.100		5/50	0.070	7.00
		100	0.100		5/50	0.074	7.40
		100	0.100	0.18	5/50	0.082	6.40
	Average						7.2
	Ear	100	0.126		5/50	0.104	8.25
		100	0.165	0.18	5/50	0.132	6.91
		100	0.139		5/50	0.069	4.96
		100	0.137		5/50	0.056	4.09
	Average						6.1
Rice plant	Mixture[c]	none	0.500			0.027	0.054
		none	0.500			0.032	0.064
		none	0.500	0.027		0.058	0.062
		none	0.500	0.027		0.070	0.086
	Average						0.067
	Stem	20	0.100		3/50	0.060	10.0
		20	0.100		3/50	0.064	10.7

TABLE 3. Continued

	Selenium content in soil[a] (ppm)	Plant sample (g)	Selenium added (μg)	Dilution	Selenium determined (μg)	Selenium content (μg/g)
	20	0.100		3/50	0.076	12.7
	20	0.100	0.27	3/50	0.077	10.2
Average						11
Foliage	20	0.100		3/50	0.109	18.2
	20	0.100		3/50	0.098	16.3
	20	0.100		3/50	0.106	17.7
	20	0.100	0.27	3/50	0.115	16.5
Average						17
Stem	50	0.100		1/50	0.039	19.5
	50	0.100		1/50	0.046	23.0
	50	0.100		1/50	0.039	19.5
	50	0.100		1/50	0.039	19.5
Average						21
Foliage	50	0.100		1/100	0.062	62.0
	50	0.100		1/100	0.057	57.0
	50	0.100		1/100	0.059	59.0
	50	0.100		1/100	0.065	65.0
	50	0.100		1/100	0.056	56.0
Average						60
Stem	100	0.100		1/50	0.043	21.5
	100	0.100		1/50	0.040	20.0
	100	0.100		1/50	0.042	21.0
	100	0.100		1/50	0.048	24.0
	100	0.100		1/50	0.046	23.0
Average						22
Foliage	100	0.100		1/100	0.067	67.0
	100	0.100		1/100	0.061	61.0
	100	0.100		1/100	0.079	79.0
	100	0.100		1/100	0.070	70.0
	100	0.100		1/100	0.066	66.0
Average						69

a) Planted soil (added as SeO_2). b) The example, indicates that the solution digested is diluted to 50 ml and 20 ml of it is used. c) A mixture of foliage and stem.

ling grown to 10 cm was transplanted into pots which had 3 kg of soil containing 0, 5, 10, 50, and 100 ppm selenium respectively (added as SeO_2). These plants were harvested after maturity. They were dried in a ventilated oven at 80 °C for 2 day. Subsequently, the dried sample was cut with scissors into portions about 1 mm long and mixed well. The selenium in 0.1—1 g of plant materials was determined by the procedure described above; also, the same sample containing 0.018—0.027 μg of selenium as the elemental form was used for the determination. As the added amount of selenium is always obtained by deducing the former values from the latter values, this method is proved to be usable for the determination of selenium in plant material. The results are shown in Table 3.

Since the sensitivity of an electron-capture detector varies during operation, a series of experiments should be carried out successively and an unknown content of selenium should be determined along with standard samples which contain similar amounts of selenium. The outstanding features are that it is simple, highly sensitive, and rapid, and no special skill is necessary to perform this method.

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