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Determination of selenium in organic compounds on a silica gel sintered thin-layer chromatographic plate with 2,3-diaminonaphthalene after direct digestion

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ABSTRACT

Biologically interesting selenium compounds such as selenomethionine, selenocystine and trimethylselenonium were digested directly on a silica gel sintered thin-layer chromatographic (TLC) plate. Digestion was performed with nitric acid–perchloric acid (2:1, v/v) by heating at 210°C for 15 min. Selenite formed on the plate was revealed with 2,3-diaminonaphthalene reagent and determined using a TLC scanner. The recoveries of selenomethionine, selenocystine and trimethylselenonium were 94 ± 7, 99 ± 9 and 97 ± 10%, respectively, with a detection limit of 0.4 ng of selenium. Linearity between fluorescence intensity and selenium content was found in the range 3–250 ng of selenium.

INTRODUCTION

Selenium is an essential trace element for animals [1], and the metabolism of selenium compounds has become of interest in connection with its role in human health and diseases. It is important to know what chemical species are present and how they are distributed in tissues.

Concentrations of selenium compounds in biological materials are usually very low. For example, the concentration of selenium in tissues and urine from healthy humans is less than 1 μg/g [2] and 100 μg/l [3], respectively. The mean daily intake of selenium estimated for the Japanese population is about 100 μg [4].

Selenoamino acids are readily separated by paper chromatography [5] or thin-layer chromatography (TLC) [6] using conventional solvent systems for the separation of amino acids. Fluorimetry [7] is a suitable method for the determination of trace amounts of selenium. However, when fluorimetry is applied directly to the TLC plate, the usual precoated layer is destroyed by the hot acid treatment. Extraction of

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samples from cut sections scraped off the plate is another approach for fluorimetry, but is very tedious.

To overcome this drawback, we used a silica gel sintered TLC plate [8]. The purpose of this study was to establish standard procedures for the direct digestion and determination of separated selenium compounds on the silica gel sintered TLC plate.

It has been reported that the complete digestion of trimethylselenonium (TMSe) ion requires drastic conditions (210°C, 30 min) [9]. We found, however, that the time was shortened to 10 min when the reaction occurred on a silica gel thin layer.

A linear relationship between the fluorescence intensity and the selenium content in the separated compounds was found in the range 3–250 ng of selenium.

EXPERIMENTAL

TLC plates

Silica gel sintered TLC plates (10 × 10 cm), prepared with uniform-sized silica gel (15 μm) by Iatron Labs. (Tokyo, Japan) were used. Similar plates, now available commercially from Gasukuro Kogyo (Tokyo, Japan), were also used.

Before use, the plates were dipped in fuming nitric acid overnight at room temperature, washed and rinsed with distilled water and heated at 400°C for 2 h in an electric furnace.

Precoated silica gel TLC plates (Kieselgel 60; Merck, Darmstadt, F.R.G.), were used for examination of the purity of authentic samples. The plates were developed in normal glass chambers (26 × 26 × 13 cm) saturated with the solvent system used.

Reagents

A mixture of 60% nitric acid (for determination of harmful metals; Wako, Osaka, Japan) and 60% perchloric acid (for determination of harmful metals; Wako) (2:1, v/v) was used for digestion.

2,3-Diaminonaphthalene (DAN) reagent was prepared by dissolving 1 g of DAN (Wako) in 100 ml of 0.3 *M* hydrochloric acid at 50°C for 20 min. The solution was washed with 10 ml of cyclohexane (for HPLC; Wako) four times to remove impurities [10] and filtered off. The filtrate was mixed with 20 ml of 1 *M* glycine–HCl buffer (pH 1.5) and adjusted to 100 ml with distilled water. The solution was stored in a freezer and thawed before use.

Other chemicals were of analytical-reagent grade.

Authentic samples

Seleno-DL-methionine (SeMet) (>99.9%) and seleno-DL-cystine [$(SeCys)_2$] (>90%) were purchased from Sigma (St. Louis, MO, U.S.A.). TMSe iodide (>99.999%) was obtained from TRI Chemical Lab. (Kanagawa, Japan). Selenium standard solution (1000 ppm, as selenious acid, for atomic absorption spectrometry) and other selenium-containing compounds were purchased from Wako. Their purities were checked on a precoated silica gel plate and on a silica gel sintered TLC plate. Detection was performed with ninhydrin and by the present method, respectively. All the compounds were found to be chromatographically pure.

Authentic sample solutions

Authentic samples were dissolved in distilled water. Their concentrations were evaluated by the method reported previously [7] and adjusted to 3000 μg/ml of selenium for the stock solutions of SeMet and TMSe iodide and to 300 μg/ml of selenium for that of $(SeCys)_2$ owing to the poor solubility in the latter instance [11]. Authentic sample solutions were used after dilution to a concentration of 100 μg/ml of selenium.

An "authentic mixture" of SeMet, $(SeCys)_2$ and TMSe was prepared so as to contain 200 μg/ml of selenium for each constituent.

Solvent systems

The following solvent systems were used all proportions by volume: (A) *n*-propanol–28% ammonia–water (8:1:1); (B) chloroform–methanol–28% ammonia–water (60:35:4:4); (C) *n*-butanol–acitic acid–water (4:1:1); (D) *n*-propanol–1 *M* acetic acid (7:3); (E) isopropanol–formic acid–water (20:1:5); and (F) phenol–water (5:1, ammonia vapour saturated).

Procedure

To establish the standard procedures for digestion, aliquots (0.5 μl) of authentic sample solutions were spotted separately on a TLC plate and digested at 150, 180, 210 or 250°C for various times. Recoveries of selenium were calculated from the ratios of the fluorescence intensities of the spots to that of the selenium standard solution on the same plate. Fluorescence intensities were measured according to the following procedures.

"Authentic mixture" was applied to a silica gel sintered TLC plate together with the selenium standard solution in a volume of 0.5 μl with a Microcaps (Drummond Scientific, Broomall, PA, U.S.A.). After development, the solvents were removed in an air oven. The TLC plate was sprayed with the digestion reagent, covered with a clean glass plate to keep it wet with the reagent, placed upside down on a hot-plate and heated at 210°C. After 15 min, the glass cover-plate was removed to evaporate the digestion reagent. After about 30 s, the TLC plate was cooled and sprayed with 6 *M* hydrochloric acid and heated again at 100°C for 5 min to convert selenate to selenite. The plate was cooled, spotted in one corner with 0.1% cresol red as an indicator and neutralized in a vessel saturated with ammonia vapour until the colour became yellow. The plate was sprayed with DAN reagent and heated at 60°C for 10 min to form a fluorophore, 4,5-benzopiazselenol [7], and sprayed a solution of Triton X-100–chloroform (1:4, v/v) to enhance the fluorescence intensity [12,13]. The fluorescence intensity of spots on the TLC plate was scanned with a Shimadzu CS-910 dual-wavelength TLC scanner (fluorescence mode, 540-nm cut filter, excitation at 388 nm) and recorded with a Hewlett-Packard 3390A integrator.

RESULTS AND DISCUSSION

To evaluate the purities of the authentic samples, the selenium contents were determined by the method reported previously [7]. Table I gives the analytical results for authentic samples. The purities from selenium contents of SeMet, $(SeCys)_2$ and TMSe iodide were 98 ± 2, 90 ± 2 and 101 ± 2% (mean ± standard deviation) from five runs, respectively, and agreed with the manufacturers' data.

TABLE I

ANALYTICAL RESULTS FOR AUTHENTIC SAMPLES

An aliquot of each authentic sample was weighed and dissolved in distilled water. The concentrations were determined by the method reported previously [7]. The values are means ± standard deviations from five runs.

Authentic sample	Selenium content (μg/ml)		Purity (%) (mean ± S.D.)
	Calculated	Found (mean ± S.D.)	
SeMet (>99.9%)[a]	246	240 ± 6	98 ± 2
$(SeCys)_2$ (>90%)[a]	219	198 ± 5	90 ± 2
TMSe (>99.999%)[a]	174	176 ± 3	101 ± 2

[a] Manufacturers' data.

Table II gives the R_F values of the selenium compounds with each solvent system. In general, good separations were obtained. Fig. 1 shows a thin-layer chromatogram of the authentic mixture which contained 200 ng of selenium in one spot (1 μl).

Selenoamino acids could be easily digested on the TLC plate at 150°C. TMSe is a metabolite of selenium that has been identified in the urine of mammals [14,15], and it has been reported that the compound is resistant against digestion [16]. To complete digestion of the compound, the reaction temperature had to be raised above 150°C. The relationship between digestion temperature and recoveries of the authentic sample [SeMet, $(SeCys)_2$ and TMSe] is shown in Fig. 2. $(SeCys)_2$ was most easily digested in 10 min at 150°C. The digestion of TMSe was difficult at 150 or 180°C.

Table III shows the recoveries of the authentic sample [SeMet, $(SeCys)_2$ and TMSe] at 210°C. The digestion for the selenium compounds almost complete in 10 min at 210°C.

TABLE II

R_F VALUES OF SELENIUM COMPOUNDS ON SILICA GEL SINTERED PLATES DEVELOPED WITH VARIOUS SOLVENT SYSTEMS

Systems A–F as specified under *Solvent systems* were used. Detection was performed both with ninhydrin and by the present method.

Selenium compound	Solvent system					
	A	B	C	D	E	F
Selenomethionine	0.68	0.67	0.62	0.73	0.78	0.80
Selenocystine	0.41	0.19	0.34	0.58	0.44	0.34
TMSe iodide	0	0	0.14	0.33	0.21	0.94
Selenotaurine	0.70	0.68	0.66	0.76	0.71	0.62
Selenious acid	0.26	0.26	0.48	0.70	0.71	0.35
Selenic acid	0.21	0.10	0.51	0.64	0.47	0.21

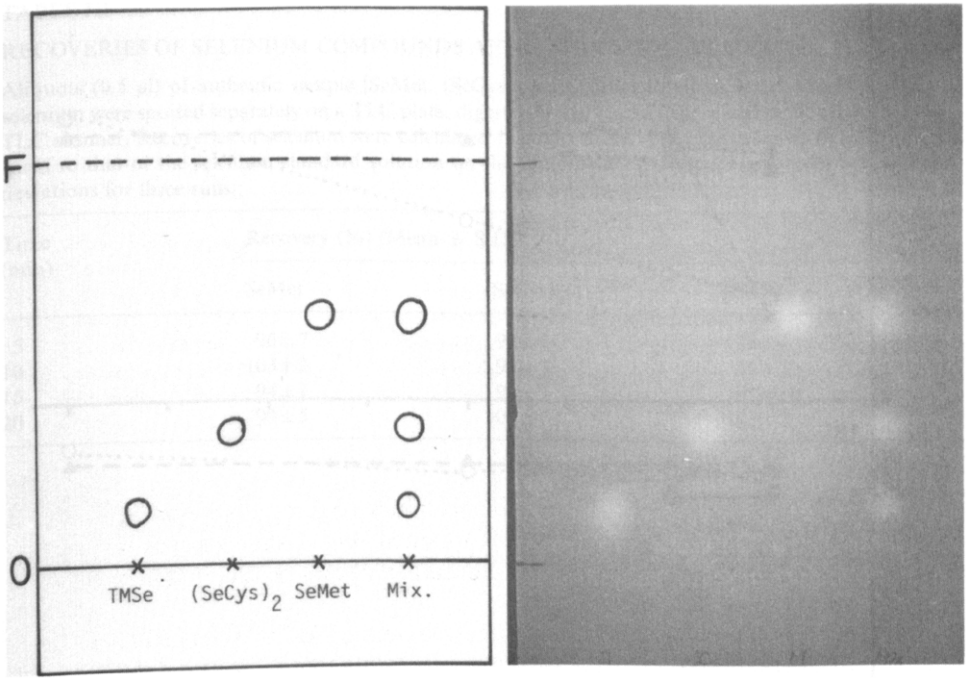

Fig. 1. Separation of selenium compounds on a silica gel sintered TLC plate. The authentic samples [SeMet, $(SeCys)_2$ and TMSe] and the authentic mixture were spotted (1 μl) and developed with *n*-butanol–acetic acid–water (4:1:1, v/v/v) followed by digestion and UV detection (365 nm). Each spot contained about 200 ng of selenium.

After the colour development with DAN reagent, the TLC plate was sprayed with a solution of Triton X-100–chloroform (1:4, v/v) [12] and the fluorescence intensity was enhanced [13] by a factor of 100. If a solution of either paraffin oil–*n*-hexane (2:1, v/v) [17], glycerol–ethanol (1:1, v/v) [17] or 1.8% cycloheptaamylose [18] was sprayed, the fluorescence intensity was enhanced by a factor of only 30, 3 or 3, respectively. The standard procedures for the digestion were adopted as described under *Procedure*.

Fig. 3 shows the calibration graph for selenium standard solution in the range 12.5–300 ng of selenium. A linear relationship exists up to 100 ng selenium. Lower responses at selenium levels > 100 ng may be attributed to self-quenching due to the high conentration. If the plate was untreated with the solution for fluorescence enhancement, the linearity was extended up to 250 ng of selenium.

Fig. 4 shows the calibration graphs for SeMet, $(SeCys)_2$ and TMSe ion in the range 3–100 ng of selenium. It is noteworthy that each selenium compound gave the same calibration graph. This result strongly suggests that these organic selenium compounds were digested completely and reacted stoichiometrically with DAN without any interfering effect. The detection limit was 0.4 ng of selenium with a signal-to-noise ratio of 3.

Using the proposed method, it became possible to identify selenium-containing

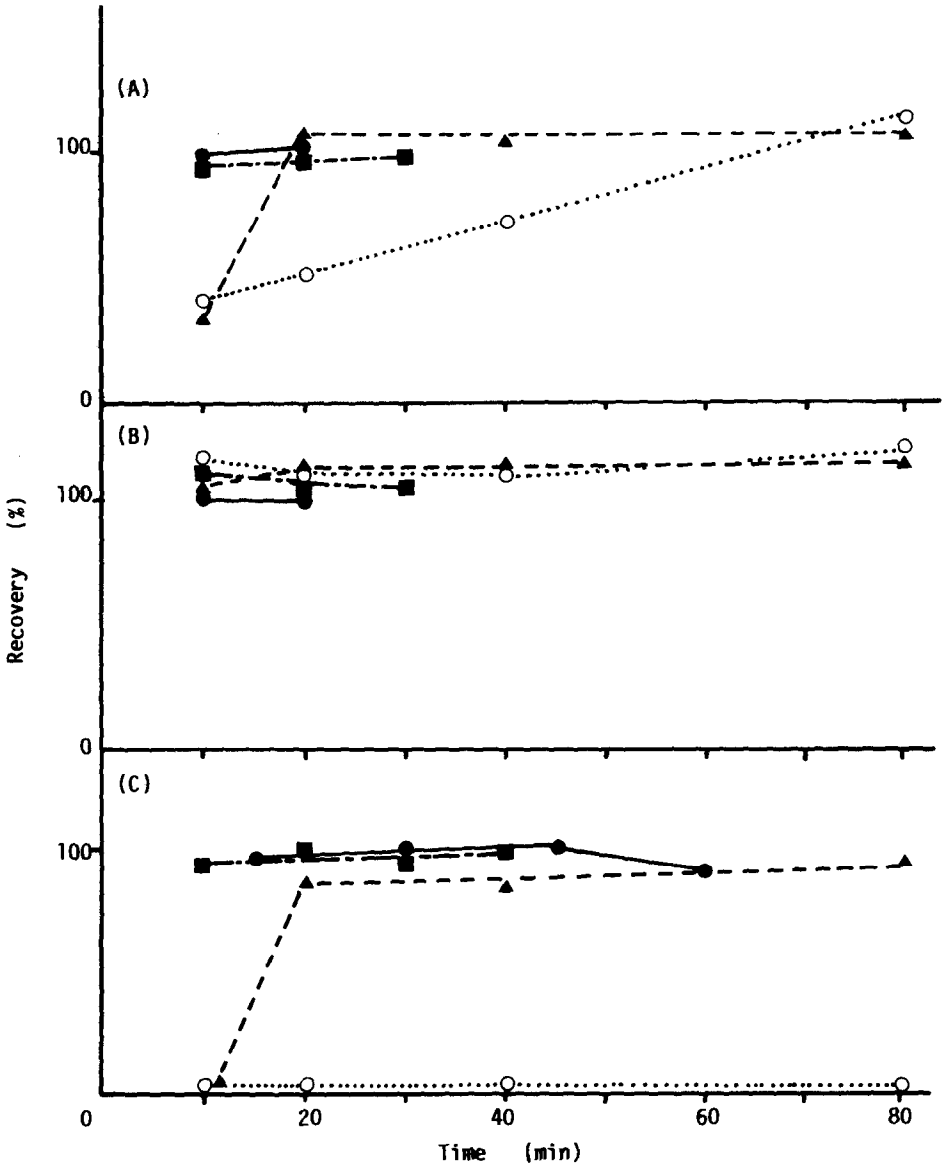

Fig. 2. Temperature and recoveries of selenium compounds. Aliquots (0.5 μl) of authentic sample [(A) SeMet; (B) $(SeCys)_2$; (C) TMSe] solutions which contained 100 μg/ml of selenium were spotted separately on a TLC plate and digested at (○) 150, (▲) 180, (●) 210 or (■) 250°C for various times. Recoveries of selenium were calculated from the ratios of the fluorescence intensities of the spots to that of the selenium standard solution on the same plate. The values are means of three runs.

compounds directly and to determine selenium in them on a TLC plate on which biological materials have been separated. Although organic selenium compounds so far known as constituents of biological materials are confined broadly to those used

TABLE III

RECOVERIES OF SELENIUM COMPOUNDS AFTER DIGESTION AT 210°C

Aliquots (0.5 μl) of authentic sample [SeMet, $(SeCys)_2$ and TMSe] solutions containing 100 μg/ml of selenium were spotted separately on a TLC plate, digested at 210°C and determined by fluorimetry using a TLC scanner. Recoveries of selenium were calculated from the ratios of the fluorescence intensities of the spots to that of the selenium standard solution on the same plate. The values were means ± standard deviations for three runs.

Time (min)	Recovery (%) (Mean ± S.D.)		
	SeMet	$(SeCys)_2$	TMSe
5	96 ± 7	92 ± 6	88 ± 4
10	103 ± 2	99 ± 3	96 ± 5
15	94 ± 7	99 ± 9	97 ± 10
20	99 ± 5	101 ± 7	103 ± 3

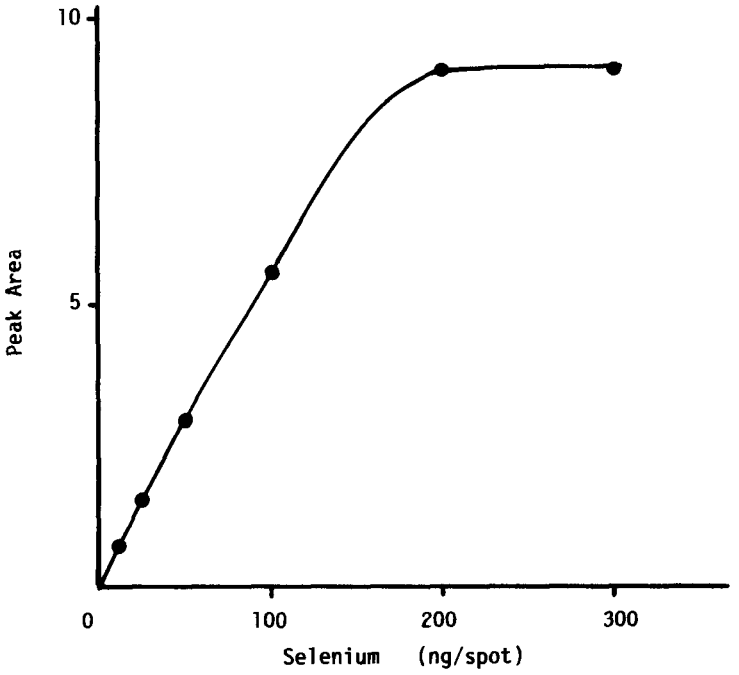

Fig. 3. Linear relationship between fluorescence intensities and selenium concentration. The selenium compound in the range 12.5–300 ng of selenium was developed with *n*-butanol–acetic acid–water (4:1:1, v/v/v) and determined by fluorimetry using a TLC scanner.

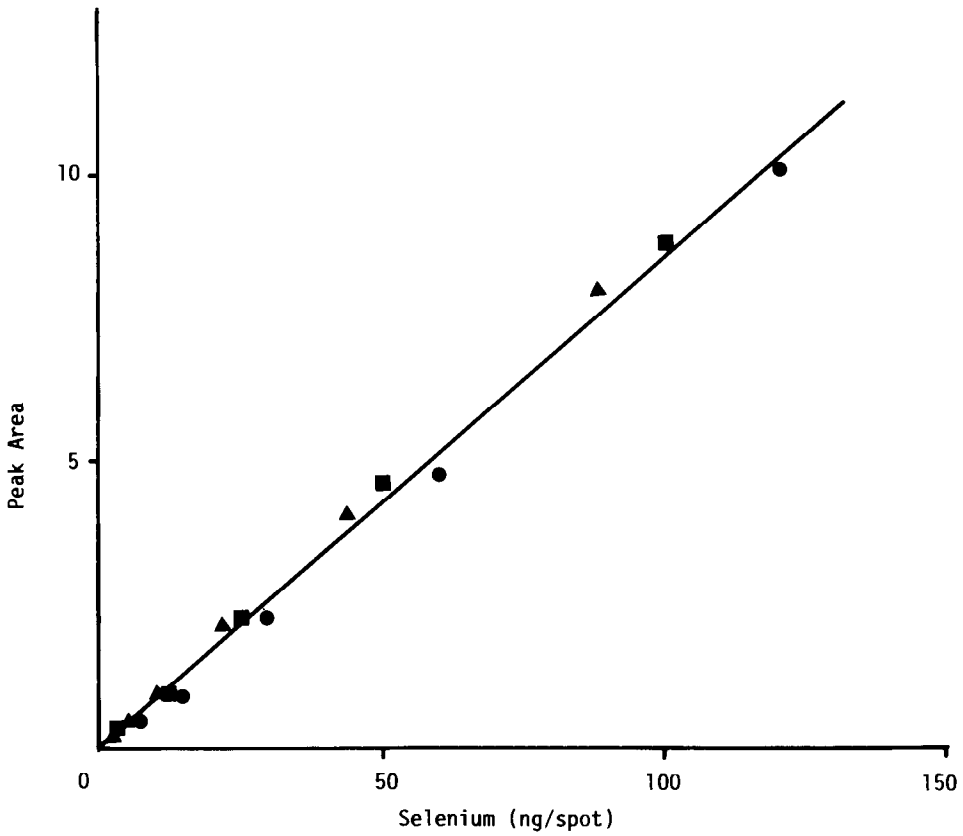

Fig. 4. Calibration graphs for (●) SeMet, (■) $(SeCys)_2$ and (▲) TMSe in the range from 3–100 ng of selenium. The authentic mixture [SeMet, $(SeCys)_2$ and TMSe] was developed with *n*-butanol–acetic acid–water (4:1:1, v/v/v) followed by digestion and determination by fluorimetry using a TLC scanner.

in this study, the proposed method showed open up a new approach to acquiring a better understanding of selenium metabolism.

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