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Determination of Germanium in Medicinal Plants by Atomic Absorption Spectrometry with Electrothermal Atomization

Yoshiki Mino, Nagayo Ota, Satoshi Sakao,[1a] and Shigeru Shimomura,[1b]

Osaka College of Pharmacy[1a] and Faculty of Pharmaceutical Sciences, Tokushima University[1b]

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Flameless atomic absorption spectrometry combined with solvent extraction was developed for the determination of germanium in medicinal plants. A wet oxidation procedure using only nitric acid was suitable for ashing plant samples. Carbon tetrachloride and water were used as solvents for extraction and back-extraction. Measurements were carried out after preparing sample solutions in 0.5 M sodium hydroxide in order to enhance the sensitivity.

This high sensitive method (detection limit, 1 ppb) was applied to the determination of germanium in some medicinal plants. The analytical results obtained indicate that medicinal plants contain small amounts of germanium; it appeared that there was no connection between the pharmacological effects of medicinal plants and their germanium contents.

Keywords——germanium; flameless atomic absorption spectrometry; solvent extraction; back-extraction; medicinal plant; Ginseng radix; ashing method; wet oxidation; loss of germanium

Although several workers[2-8] have investigated the biological activity of germanium or its occurrence in biomaterials, it is generally accepted that germanium has little biological significance; its concentration in biomaterials is believed to be usually in the range of 0.1 to 1.0 ppm. On the other hand, it has been reported that some medicinal plants contain large amounts of germanium, *e.g.*, about 300 ppm for Ginseng radix.[9] Therefore, the relationship between their pharmacological effects and the presence of germanium has become of interest in recent years,[10,11] especially in Japan.

In view of its relative abundance in the upper lithosphere, its chemical nature, and the fact that many elements of similar atomic number are biologically essential trace elements, it seemed desirable to carry out a reinvestigation of the germanium concentrations in medicinal plants in order to assess the possibility of a relationship between the effects of these plants and the amount of germanium contained in them.

The present paper deals with the determination of germanium in medicinal plants by flameless atomic absorption spectrometry (AAS) combined with solvent extraction. The method was applied to the determination of germanium in various medicinal plants, such as Ginseng radix.

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1) Location: *a) 2-10-65 Kawai Matsubara City, Osaka, 580, Japan; b) 1-78 Shomachi, Tokushima, 770, Japan.*
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Experimental

Apparatus——A two-channel, dual-phase atomic absorption spectrometer (Nippon Jarrell-Ash model AA-8500) was used in combination with a graphite tube atomizer (model FLA-100), a two-pen recorder (Hitachi model 056), a germanium hollow-cathode lamp (Hamamatsu TV: L-233), and a deuterium lamp background corrector. Argon was used to provide an inert atmosphere in the graphite tube. Samples (20 μl × 2) were introduced by means of an Eppendorf micropipette with a disposable plastic tip. The optimal instrument parameters were as follows: analytical line, 265.12 and 265.16 nm (doublet); background correction, 265.3 nm; germanium lamp current, 15 mA; deuterium lamp current, 200 mA; argon flow, 2.0 l/min; ramp mode, 0. The atomization program was: injection of a 20 μl aliquot of sample solution into the graphite furnace; drying at 25 A for 20 sec; reinjection in the same manner; drying at 25 A for 30 sec; ashing at 60 A for 30 sec; and atomization at 300 A for 8 sec.

A fluorescence X-ray spectrometer (Rigakudenki Geigerflex SX) equipped with a scintillation counter was employed. The operating conditions were as follows: X-ray source, Mo Kα; analyzing crystal, LiF; voltage, 60 kV; current, 50 mA.

Materials——Stock solutions of germanium (500 ppm) were prepared as reported previously.[12] All chemicals used were of reagent grade or of the highest quality available.

Crude drug samples were purchased in Osaka market. Plant samples were collected from the authors' herb garden.

Preparation of Calibration Curve——Transfer 0, 0.25, 0.5, 1.0, 1.5, and 2.0 ml aliquots of standard germanium solution (0.2 ppm) into 60 ml Squibb-type separatory funnels. Add 2.5 ml of nitric acid to each, dilute to 5 ml with water, and add 14 ml of hydrochloric acid. Add 5 ml of carbon tetrachloride, stopper the funnel, and shake vigorously for 2 minutes. Allow the layers to separate and run the carbon tetrachloride layer into a second 60 ml separatory funnel. Add 2 ml of water to it, stopper the funnel, and shake vigorously for 2 minutes. Allow the layers to separate. Discard the lower layer.

Pipet 1.0 ml of the aqueous solution into a 10 ml test tube. Add 0.1 ml of 5 M sodium hydroxide. Measure this solution (20 μl × 2) using the above-mentioned atomization program.

Procedure for Dry Ashing——Transfer an air-dried sample (5.0 g) into a crucible. Place it in a muffle furnace at room temperature. Increase the furnace temperature to 400°, and hold for 2 hours. Further increase this temperature to 750° and hold for 5 hours. When the sample has cooled, wash it in a 60 ml separatory funnel with 5 ml of nitric acid (1→2), add 14 ml of hydrochloric acid, and continue as in the preparation of the calibration curve.

Recommended Procedure for Sample Analysis——Transfer an air-dried sample (5.0 g) to a 100 ml beaker, add 30 ml of nitric acid, cover with watch glass, and allow to stand overnight. Heat in a sand bath, add a suitable amount of nitric acid to destroy organic matter completely, and concentrate to about 2.5 ml by evaporation. Transfer the solution to a 60 ml separatory funnel, dilute to 5 ml with water, and continue as in the preparation of the calibration curve.

Results and Discussion

Flameless AAS combined with Solvent Extraction

As preliminary experiments, X-ray fluorescence measurements were carried out to examine the approximate germanium contents in medicinal plants and to identify the elements coexisting with the germanium. Such trace elements as iron, zinc, copper and manganese could be detected in all the samples tested, but germanium could not be detected in any sample by this method because the concentration was extremely low in each sample. Flameless AAS is suitable for the analysis of these samples, because the use of an atomizer such as a graphite tube furnace generally leads to an increase in the sensitivity of the atomic absorption technique by about 2 to 3 orders of magnitude.

Johnson *et al.*[13] first studied the determination of germanium by flameless AAS and suggested that difficulties in the determination of germanium might be due to the formation of volatile germanium monoxide. They recommended a rapid increase in temperature to prevent loss of sample as germanium monoxide. A ca. tenfold enhancement of sensitivity was obtained by using a specially designed tube, as compared with the most sensitive analy-

12) S. Shimomura, H. Sakurai, H. Morita, Y. Mino, and M. Inoue, *Anal. Chim. Acta*, **91**, 421 (1977).
13) D.J. Johnson, T.S. West, and R.M. Dagnall, *Anal. Chim. Acta*, **67**, 79 (1973).

sis[14] of germanium by flame AAS. In recent years, the authors[15] reported that the addition of sodium hydroxide to the sample solution provided a very sensitive analysis (0.004 ppm for 1% absorption) for germanium without matrix interference. The proposed method, in which the measurement was carried out after preparing sample solutions in 0.5 M sodium hydroxide, gave a significantly higher sensitivity than the method of Johnson *et al.* (0.017 ppm for 1% absorption).

For the determination of germanium in plant samples, flameless AAS after preparing sample solutions in 0.5 M sodium hydroxide should be combined with solvent extraction followed by back-extraction with water in order to prevent matrix interference and to concentrate the germanium sample solution. Although germanium can be extracted from appreciably concentrated hydrochloric acid solutions by several solvents, *e.g.*, carbon tetrachloride, chloroform, methyl isobutyl ketone, and *n*-butyl ether, carbon tetrachloride was selected. This is because the only metal that accompanies germanium into the carbon tetrachloride layer is trivalent arsenic. Furthermore, the addition of sodium hydroxide to the aqueous layer obtained when germanium is back-extracted from the carbon tetrachloride layer forms only a small amount of sodium chloride. The molecular absorption occurs just before the atomization of germanium in flameless AAS. Since even in the case of carbon tetrachloride a rather high signal of sodium chloride appears, an atomic absorption spectrometer having background correction is essential (Fig. 1).

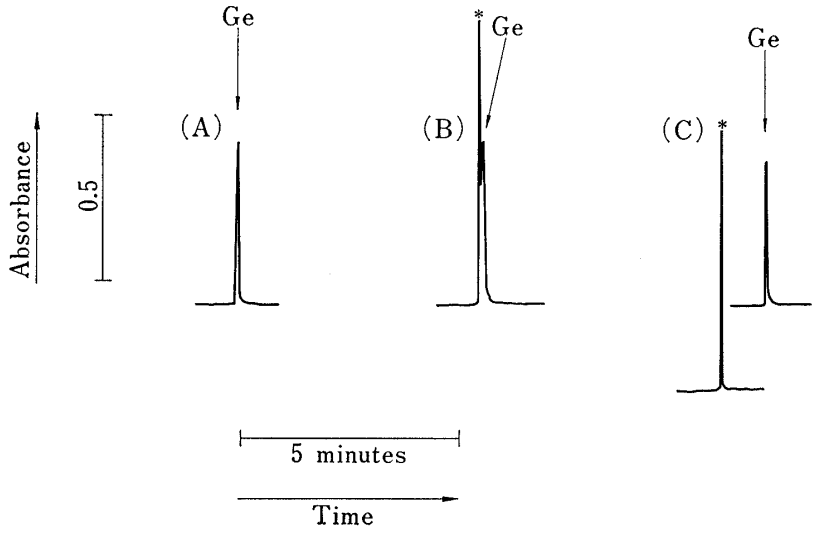

Fig. 1. Absorption Profiles of Germanium (0.2 ppm) in Flameless AAS

(A): Standard solution (1 channel), (B): 0.5 M NaOH solution obtained after back-extraction (1 channel), (C): Same solution as in (B) (2 channel).
*: Based on the molecular absorption of NaCl.

Loss of Germanium during Ashing

In flameless AAS, as well as all analytical tools for elemental analysis, except fluorescence X-ray analysis and radioactivation analysis, the sample has to be ashed by heat, with a low temperature asher, or by means of oxidizing agents, such as nitric acid, nitric acid plus sulfuric acid, nitric acid plus perchloric acid, and sulfuric acid plus hydrofluoric acid.

Heating is a convenient ashing method, but occasionally results in losses of material. No work regarding loss of germanium from plant samples has yet been reported. Morgan

14) S. Shimomura, H. Sakurai, H. Morita, and Y. Mino, *Anal. Chim. Acta*, **96**, 69 (1978).
15) Y. Mino, S. Shimomura, and N. Ota, *Anal. Chim. Acta*, **107**, 253 (1979).

and Davies[16] mentioned that on ashing coal a large part of the contained germanium was volatilized as germanium monoxide and its monosulfide. In contrast, Waring and Tucker[17] observed no detectable loss of germanium from low-rank coal under various conditions. This disagreement may be related to the chemical form of germanium contained in the samples. Consequently, loss of germanium from medicinal plants during ashing should be examined directly. In this experiment, *Oryza sativa* L. cultivated in the presence of germanium was used as a sample, because no plant naturally containing a suitable amount of germanium could be found.

Wet oxidation with a suitable agent is generally considered to be a most reliable ashing method. In the determination of germanium, halogen acids must not be used due to the formation of volatile halides, such as germanium tetrachloride (bp. 83.1°). Nitric acid was chosen as an oxidizing agent in order to avoid the coprecipitation of germanium with other metal as sulfates.

Two ashing methods were compared with respect to loss of germanium from samples during ashing under the above-mentioned conditions. The results (Table I) indicate that more than half the germanium contained in plants was volatilized during ashing by heat.

Table I. Loss of Germanium during Ashing

Material	Ashing method		Ratio (dry/wet)
	Wet oxidation[a] found (μg)	Dry ashing[b] found (μg)	
Oryza sativa L.[c]	19.30	8.50	0.440

a) Only HNO_3 was used.
b) 400° for 2 hours → 750° for 5 hours.
c) Cultivated in the presence of germanium (1 ppm). The above-ground part (1.0 g) was used for the measurements.

To test for loss of germanium during analytical procedures involving wet oxidation with nitric acid, four samples, *i.e.*, *Coicis* semen with addition of 0, 0.05, 0.5, and 40 μg of germanium, were analyzed. The results are given in Table II. The recovery of germanium was more than 85% even when 0.05 μg of germanium was added. Thus, even a very small amount of germanium in plant samples could be successfully determined.

Table II. Loss of Germanium during Analytical Procedures Involving Wet Oxidation with HNO_3

Material	Amount of Ge added (μg)	Found (μg)[a]	Recovery (%)
Coicis semen[b]	+0	0.030	—
	+40	38.6	96.4
	+0.5	0.485	91.0
	+0.05	0.073	86.0

a) Values are the means of four determinations (sample number, $n=4$).
b) A 5.0 g sample was used for the measurements.

Procedures and Calibration Curve

The above results led to a recommended procedure (described in "Experimental") for the determination of germanium in medicinal plant by flameless AAS.

16) G. Morgan and G.R. Davies, *J. Soc. Chem. Ind.*, **56**, 717 (1937).
17) C.L. Waring and W.P. Tucker, *Anal. Chem.*, **26**, 1198 (1954).

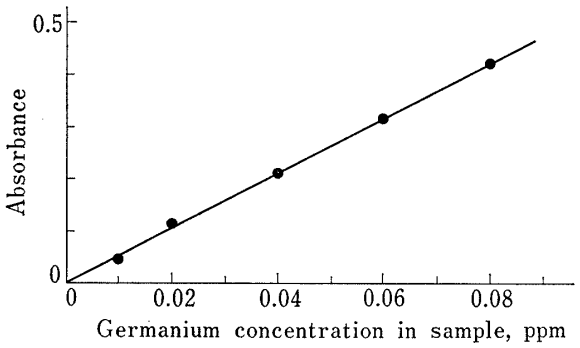

Fig. 2. Calibration Curve for Germanium

Table III. Analytical Results for Germanium in various Medicinal Plants

Material[a]	Germanium content (ppb)
Allii bulbus	<1
Coicis semen	6
Ginseng radix No. 1 (1976, 5 g piece)	6
2 (1976, 3 g piece)	<1
3 (1976, 3 g piece)	5
4 (1959, 10 g piece)	<1
5 (1974, 8 g piece)	<1
6 (1979, 4 g piece)	<1
Ginseng radix rubra	<1
Notoginseng radix	<1
Panacis japonici rhizoma	<1
Lithospermi radix	<1
Sophorae subprostratae radix	4
Theae folium	9
Fomitopsis rosea (Fr.) Karst.	<1
Lycium chinense Mill. (fruit)	<1
Symphytum officinale L. (leaf)	<1
Symphytum officinale L. (root)	2

a: Crude drug samples were purchased in Osaka market. Plant samples were collected from the authors' herb garden.

The calibration curve for analysis of germanium in medicinal plants is shown in Fig. 2. The detection limit for germanium was 1 ppb (concentration in sample), the sensitivity for 1% absorption was 0.8 ppb, and the standard deviation for a 0.08 ppm sample (*Coicis* semen with addition of 0.5 μg of germanium) was 5.0% for 8 consecutive measurements.

Analytical Results

Some medicinal plants were analyzed by the above procedures. The results obtained are listed in Table III. The largest value in all the plants tested was 9 ppb in the case of green tea. Germanium contents in medicinal plants, *e.g.* Ginseng radix, selected on the basis of Asai's report,[9] were less than 6 ppb in every case. The results obtained indicate that medicinal plants in general contain small amounts of germanium, and suggest that there may be no connection between the pharmacological effects of the medicinal plants and their germanium contents. The effects of differences in the soils where the samples were collected remain to be investigated.

The analytical values in this experiment were much lower than those of Asai's report[9] and even than those given in Schroeder's report.[7] The method used by Asai was not clear. The measurements of Schroeder were made by the method of Luke and Campbell[18] with phenylfluorone as a color-forming agent. This colorimetry (detection limit, 0.5 ppm) has such low sensitivity that the values obtained. 0.1—1.0 ppm, must be considered as unreliable. Therefore, the germanium contents in other biomaterials should also be reinvestigated by the present procedure using flameless AAS. This procedure should be useful as an analytical method for studies on the biological transport of germanium and on germanium uptake by plants.

18) C.L. Luke and M.E. Campbell, *Anal. Chem.*, **28**, 1273 (1956).