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Determination of Seven Organosulfur Compounds in Garlic by High-Performance Liquid Chromatography

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The properties of garlic (*Allium sativum* L.) are attributed to organosulfur compounds. Although these compounds change during cultivation and storage, there is no report of their simultaneous analysis. Here, a newly developed analytical method with a rapid and simple sample preparation to determine four sulfoxides and three γ-glutamyl peptides in garlic is reported. All garlic samples were simply extracted with 90% methanol solution containing 0.01 N hydrochloric acid and prepared for analysis. Alliin, isoalliin, methiin, cycloalliin, and γ-L-glutamyl-*S*-methyl-L-cysteine were determined by normal-phase HPLC using an aminopropyl-bonded column. γ-L-Glutamyl-*S*-(2-propenyl)-L-cysteine and γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine were separated on an octadecylsilane column. The overall recoveries were 97.1−102.3%, and the relative standard deviation values of intra- and interday precision were lower than 2.6 and 4.6%, respectively. This newly developed method offers some advantages over the currently accepted techniques including specificity, speed, and ease of use and would be useful for chemical and biological studies of garlic and its preparations.

KEYWORDS: Organosulfur compounds; HPLC; garlic; validation; *Allium sativum*

INTRODUCTION

Garlic (*Allium sativum* L.) has been used universally as a food, spice, and traditional medicine. Numerous studies have previously demonstrated that garlic may be useful for the prevention of carcinogenesis, cardiovascular, and age-related diseases (*1*). Especially, it has been strongly suggested that its medicinal and beneficial properties are attributed to specific organosulfur compounds (*2*−*6*). It is known that garlic contains three γ-glutamyl peptides, that is, γ-L-glutamyl-*S*-(2-propenyl)-L-cysteine (GSAC), γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC), and γ-L-glutamyl-*S*-methyl-L-cysteine (GSMC); their corresponding sulfoxide derivatives, that is, (+)-*S*-(2-propenyl)-L-cysteine sulfoxide (alliin), (+)-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (isoalliin), and (+)-*S*-methyl-L-cysteine sulfoxide (methiin), respectively; and (1*S*,3*R*,5*S*)-5-methyl-1,4-thiazane-3-carboxylic acid 1-oxide (cycloalliin), as shown in **Figure 1**. Sulfoxides have been reported to have some medicinal properties; for example, alliin shows anticancer effects (*2*, *3*), and alliin and cycloalliin present lipid-lowering effects (*3*, *4*). On the other hand, γ-glutamyl peptides have been reported to lower blood pressure (*5*) and to have cholesterol-lowering effect (*6*). However, once garlic is crushed, these compounds are transformed into other compounds such as allicin, ajoene, dithiins, and diallylpolysulfides (*2*, *7*). Therefore, it becomes important to control sample preparation to minimize artificial errors caused by their chemical characters and the different stabilities of organosulfur compounds.

The biosynthetic pathway of organosulfur compounds in garlic has been proposed to involve transformation of γ-glutamyl peptides into their corresponding sulfoxides by γ-glutamyl transpeptidase and oxidase (*8*, *9*). Moreover, it has been reported that contents of organosulfur compounds in garlic bulb change during cultivation (*10*, *11*) and storage (*12*). Therefore, an analytical method for the simultaneous determination of all sulfoxides and their precursors, γ-glutamyl peptides, in a garlic sample is required for evaluating the quality of garlic.

It has been previously reported that these compounds in garlic were analyzed using high-performance liquid chromatography (HPLC) (*13*−*23*), gas chromatography (*24*), thin-layer chromatography (*25*), and biosensors (*26*). Sulfoxides except cycloalliin were analyzed by derivatization with *o*-phthalaldehyde (*13*−*17*), dansyl chloride (*18*), or 9-fluorenylmethyl chloroformate (*19*), whereas cycloalliin and γ-glutamyl peptides were determined by normal-phase (NP) (*10*, *20*) and reversed-phase (RP) HPLC (*15*, *21*−*23*), respectively, with UV detection. All of the above methods needed different sample preparation procedures and did not allow simultaneous determination. More recently, the simultaneous analysis has been reported to determine the constituents in garlic preparations (*21*−*23*).

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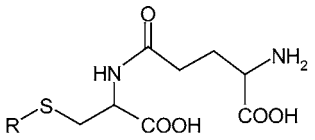
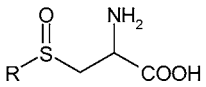
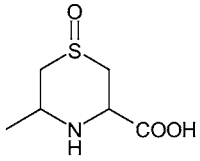


	R
γ-L-Glutamyl-*S*-methyl-L-cysteine (GSMC)	$-CH_3$
γ-L-Glutamyl-*S*-(2-propenyl)-L-cysteine (GSAC)	$-CH_2-CH=CH_2$
γ-L-Glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC)	$-CH=CH-CH_3$



	R
(+)-*S*-Methyl-L-cysteine sulfoxide (Methiin)	$-CH_3$
(+)-*S*-(2-Propenyl)-L-cysteine sulfoxide (Alliin)	$-CH_2-CH=CH_2$
(+)-*S*-(*trans*-1-Propenyl)-L-cysteine sulfoxide (Isoalliin)	$-CH=CH-CH_3$



Cycloalliin

Figure 1. Chemical structures of organosulfur compounds in garlic.

However, these methods were not sufficiently validated with respect to the analytical performance characteristics (e.g., specificity, linearity, limit of detection, accuracy, and precision) despite quantitative analysis.

In the present study, we report a simple, rapid, and precise analytical method using a one-step sample preparation procedure, followed by NP and RP HPLC techniques to determine four sulfoxides (alliin, isoalliin, methiin, and cycloalliin) and three γ-glutamyl peptides (GSMC, GSAC, and GSPC) in garlic clove and its powder. In addition, this method was validated with respect to specificity, linearity, limit of detection, accuracy, and precision. Thus, this newly established method will be useful for chemical and biological studies of garlic and its preparations.

MATERIALS AND METHODS

Chemicals. Alliin, methiin, cycloalliin, GSAC, and GSMC were synthesized as previously described (*12*, *26*−*28*). Isoalliin and GSPC were isolated from onion and garlic bulbs, respectively, according to the methods of Shen et al. (*29*) and Lawson et al. (*12*) with slight modifications. Acetonitrile and methanol for HPLC were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals of reagent grade were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) or Yoneyama Yakuhin Kogyo Co., Ltd. (Osaka, Japan). Water purified through a Milli-Q Labo (Millipore, Bedford, MA) water system was used for all sample preparation and mobile phase.

Garlic Samples. Garlic grown in China was purchased from local markets in Japan. Other garlic used was harvested in July 2004 at Hokkaido and Hiroshima (Japan) or in California (United States). Garlic powder was prepared as follows: 500 g of freshly peeled garlic cloves was collected in 2003 (Hokkaido, Japan), then frozen in liquid nitrogen, and freeze-dried using an FRD-50P freeze-dryer (Iwaki, Tokyo, Japan). The resulting lyophilisate was ground into powder with a mortar and pestle and stored at −35 °C until analysis.

HPLC Analysis of Alliin, Isoalliin, Methiin, Cycloalliin, and GSMC. A Shimadzu LC-10AVP system coupled with an SPD-M10AVP photodiode array detector (scan rage, 190−300 nm; Shimadzu, Kyoto, Japan) was used. HPLC conditions were as follows: column, Shodex Asahipak NH2P-50 2D (5 μm, 150 mm × 2 mm, Showa Denko, Tokyo, Japan); column temperature, 25 °C; flow rate, 0.2 mL/min; mobile phase, acetonitrile/water (84:16, v/v) containing 0.2% phosphoric acid; wavelength, UV 210 nm; injection volume, 1 μL.

HPLC Analysis of GSAC and GSPC. GSAC and GSPC were analyzed by RP HPLC, which was adopted for GSAC assay in garlic from that listed in the United States Pharmacopoeia (USP) (*13*). An LC-10AVP system, that is, the same construction system as described above, was used. HPLC conditions were as follows: column, Symmetry C18 (5 μm, 150 mm × 3.9 mm, Waters, Milford, MA); column temperature, 25 °C; flow rate, 0.8 mL/min; mobile phase, 50 mM phosphate buffer (pH 2.6)/methanol (85:15, v/v); wavelength, UV 205 nm; injection volume, 10 μL.

Moisture Content. Moisture content of garlic clove was determined by measuring weight loss after the clove had been sliced and dried using an FD-230 infrared moisture determination balance (80 °C, Kett Electric Laboratory, Tokyo, Japan).

Sample Preparation of Garlic Powder. In a 50-mL flask, garlic powder (1 g) was added to 30 mL of 90% methanol solution containing 0.01 N HCl, and the mixture was shaken for 30 min using an SR-II recipro shaker (Taitec, Tokyo, Japan). Additional 90% methanol solution containing 0.01 N HCl was added to the mixture to make exactly 50 mL. The resulting mixture was centrifuged at 11000*g* for 5 min using a benchtop KM-15200 microcentrifuge (Kubota, Tokyo, Japan). The obtained supernatant was analyzed by HPLC.

Sample Preparation of Garlic Clove. A peeled garlic clove (4−10 g) in a 250-mL homogenizing cup was added to 150 mL of 90% methanol solution containing 0.01 N HCl and then homogenized at the highest speed for 1 min using a 7012 laboratory blender (Waring Products, Inc., Torrington, CT). The homogenate was decanted into a 250-mL flask. The homogenizing cup was washed with a 90% methanol solution containing 0.01 N HCl, and the washing solution was combined with the homogenate to make exactly 250 mL. The mixture was centrifuged at 11000*g* for 5 min, and the supernatant was analyzed by HPLC.

RESULTS AND DISCUSSION

Selection of Extraction Solvent for Sample Preparation. When garlic cloves are cut or crushed, the enzyme alliinase (EC 4.4.1.4) cleaves sulfoxides except cycloalliin to give thiosulfinates, pyruvate, and ammonia (*7*, *26*). Several studies have demonstrated that sulfoxides were extracted with acidic solutions to inhibit enzymatic activity of alliinase, which is irreversibly inhibited at acidic pH values below 3.6 (*30*). In addition, higher methanol concentration as extraction solvent protects the analytical column due to decrease in the solubility of carbohydrates and proteins in garlic. To select an extraction solvent, effects of HCl and methanol concentrations on extraction efficiency of organosulfur compounds in garlic powder were evaluated (**Table 1**). Increasing methanol concentrations resulted in decreases in extraction efficiency of γ-glutamyl peptides (GSMC, GSAC, and GSPC) in the presence of 0.001 N HCl. This might be due to solidification of prepared samples, which decreased the solubility of polar compounds. On the other hand, at high HCl concentrations (0.01 and 0.1 N HCl), samples extracted with 80 or 90% methanol showed higher contents of γ-glutamyl peptides than those with 95% methanol. This might result from enhancement of solubility in all concentrations of methanol by suppression of ionization of γ-glutamyl peptides at high HCl concentrations. The sample extracted with a solution containing 90% methanol and 0.01 N HCl resulted in the highest contents of sulfoxides (alliin, isoalliin, methiin, and cycloalliin) among samples prepared with solvents used in this experiment. Furthermore, there were no significant differences in the contents

Table 1. Effects of Hydrochloric Acid and Methanol Concentrations on Extraction Efficiency of Organosulfur Compounds in Garlic Powder

concentration		content (mg/g)						
HCl (N)	methanol (%)	alliin	isoalliin	methiin	cycloalliin	GSAC	GSPC	GSMC
0.001	80	18.7 (93)[a]	0.93 (89)	1.17 (92)	0.80 (100)	3.47 (97)	4.89 (97)	0.17 (82)
	90	19.0 (94)	1.00 (96)	1.12 (87)	0.80 (99)	2.49 (70)	3.60 (71)	0.12 (60)
	95	15.1 (75)	0.86 (83)	0.78 (61)	0.51 (63)	1.43 (40)	2.08 (41)	0.07 (36)
0.01	80	19.2 (95)	0.93 (89)	1.23 (97)	0.77 (95)	3.55 (99)	4.99 (99)	0.20 (97)
	90	20.2 (100)	1.04 (100)	1.28 (100)	0.81 (100)	3.57 (100)	5.06 (100)	0.20 (100)
	95	18.1 (89)	1.00 (96)	1.00 (78)	0.65 (81)	2.59 (73)	3.67 (73)	0.15 (73)
0.1	80	20.0 (99)	1.01 (97)	1.29 (101)	0.66 (81)	3.43 (96)	4.82 (95)	0.20 (96)
	90	20.2 (100)	1.06 (102)	1.28 (100)	0.77 (95)	3.33 (93)	4.83 (96)	0.18 (88)
	95	20.2 (100)	1.07 (102)	1.18 (92)	0.80 (99)	2.98 (83)	4.51 (89)	0.17 (82)
	50	9.3 (46)	0.13 (13)	1.00 (78)	0.72 (89)	3.49 (98)	5.02 (99)	0.18 (87)

[a] Relative value (%) against content of 90% methanol solution containing 0.01 N HCl is given in parentheses.

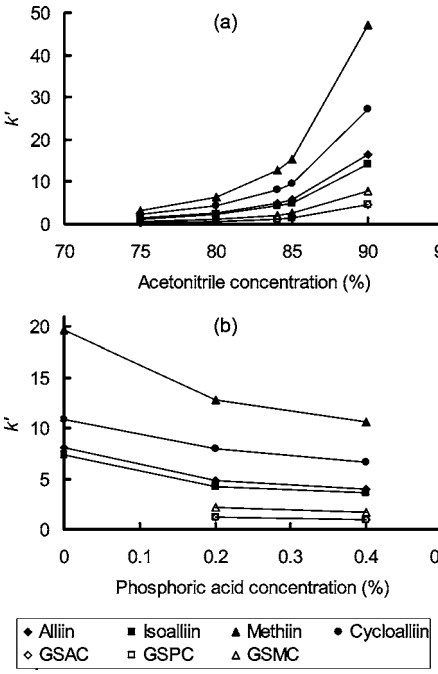


Figure 2. Effects of acetonitrile (**a**) and phosphoric acid (**b**) concentrations in the mobile phase on the capacity factor (k') of organosulfur compounds. Conditions: column, Shodex Asahipak NH2P-50 2D (150 mm × 2 mm); flow rate, 0.2 mL/min; wavelength, 210 nm; column temperature, 25 °C; mobile phase (**a**), acetonitrile/water (75:25, 80:20, 84:16, 85:15, or 90:10, v/v) containing 0.2% phosphoric acid; mobile phase (**b**), acetonitrile/water (84:16, v/v) containing phosphoric acid (0, 0.2, or 0.4%).

of seven organosulfur compounds extracted with a range of 88−92% methanol in the presence of 0.01 N HCl (data not shown). In addition, the GSAC content of the sample extracted with a 90% methanol solution containing 0.01 N HCl was as high as that with 50% methanol, which was previously used as the extraction solvent for GSAC assay in garlic as listed in the USP (*13*) (**Table 1**). The remaining rate of organosulfur compounds in the sample and reference solutions prepared with a 90% methanol solution containing 0.01 N HCl was >96% even after storage for 48 h at room temperature (data not shown). It suggested that the sample preparation minimized the artificial errors caused by the instability of analytes.

Chromatography. Preliminary experiments were conducted to separate organosulfur compounds by NP chromatography using an aminopropyl-bonded column. **Figure 2a** shows the effects of acetonitrile concentrations in the mobile phase on the capacity factor, k' value (t_0, 1.9 min) of organosulfur compounds. Retention times of all compounds were found to be dependent on acetonitrile concentration, and peaks of all compounds except GSAC and GSPC were completely separated when the acetonitrile concentration was >84%. Peak resolution (R_S) values between isoalliin and alliin increased at higher concentrations of acetonitrile, and R_S values of 80, 84, 85, and 90% acetonitrile were 0.9, 1.6, 1.7, and 2.5, respectively. Therefore, 84% acetonitrile was selected as the optimum concentration to shorten the analytical time. **Figure 2b** shows the effects of various phosphoric acid concentrations in the mobile phase on k' values of organosulfur compounds. Peaks of the γ-glutamyl peptides (GSMC, GSAC, and GSPC) did not appear on the chromatogram in the absence of phosphoric acid. There might be an ionic interaction between negative charges of γ-glutamyl peptides and amines on the surface of the stationary phase support particles. However, all peaks appeared on the chromatogram when 0.2 or 0.4% phosphoric acid was added to the mobile phase, and these might result from suppression of ionization of γ-glutamyl peptides. Peaks of all compounds except GSAC and GSPC showed good separation; R_S values between isoalliin and alliin with 0.2 and 0.4% phosphoric acid were 1.6 and 1.1, respectively. Therefore, 0.2% phosphoric acid was selected as the optimum concentration in the mobile phase. **Figure 3a** shows a typical chromatogram of sample prepared with garlic from Hokkaido, Japan. However, GSAC and GSPC were not separated using NP HPLC techniques with mobile phase as described above.

Further experiments were conducted to separate GSAC and GSPC by RP chromatography. Garlic samples were analyzed with HPLC conditions, which were adopted for GSAC assay in garlic listed in the USP (*13*). A typical chromatogram for garlic from Hokkaido, Japan, is shown in **Figure 4**. The peaks of GSAC and GSPC were separated completely; therefore, these chromatographic conditions were chosen for separation of GSAC and GSPC.

Validation. The proposed quantitative methods for seven organosulfur compounds in garlic and its powder product were validated with regard to specificity, linearity, detection limit, accuracy, and precision.

Specificity was demonstrated by analyzing garlic samples obtained from four different culture areas and garlic powder. All peaks for organosulfur compounds in chromatograms were completely separated from peaks for other constituents in garlic (**Figures 3a** and **4**) and were identified by comparing retention times and UV spectra of samples with those of reference compounds. Moreover, no peaks for alliin, isoalliin, and methiin were found in the chromatogram of garlic sample homogenized with water, as shown in **Figure 3b**, due to the activity of

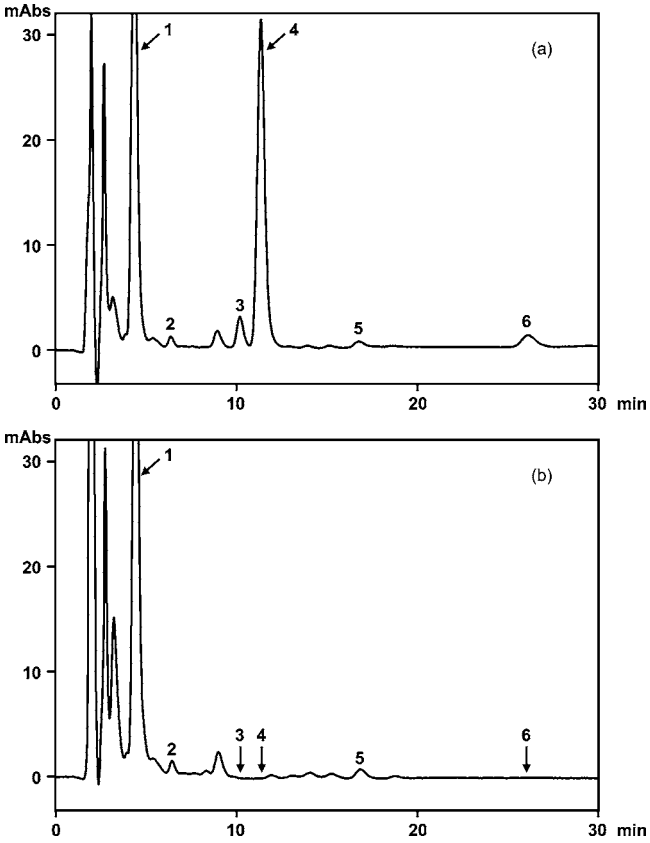


Figure 3. Typical normal-phase HPLC chromatogram of garlic clove (**a**) and homogenized garlic clove with water (**b**). Peaks: 1, GSAC and GSPC; 2, GSMC; 3, isoalliin; 4, alliin; 5, cycloalliin; 6, methiin. Garlic clove (Hokkaido, Japan) was (**a**) prepared according to the method described in the text or (**b**) homogenized with an equal volume of water and then prepared according to the method described in the text. Conditions: column, Shodex Asahipak NH2P-50 2D (150 mm × 2 mm); flow rate, 0.2 mL/min; wavelength, 210 nm; column temperature, 25 °C; mobile phase, acetonitrile/water (84:16, v/v) containing 0.2% phosphoric acid.

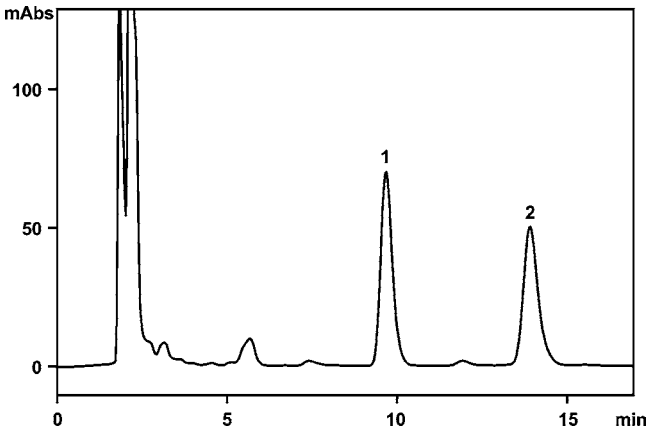


Figure 4. Typical reversed-phase HPLC chromatogram of garlic clove. Peaks: 1, GSAC; 2, GSPC. Garlic clove (Hokkaido, Japan) was prepared according to the method described in the text. Conditions: column, Symmetry C18 (150 mm × 4.6 mm); flow rate, 0.8 mL/min; wavelength, 205 nm; column temperature, 25 °C; mobile phase, 50 mM phosphate buffer (pH 2.6)/methanol (85:15, v/v).

alliinase, which is the conversion enzyme for sulfoxides except for cycloalliin to thiosulfinates, in garlic (*7*, *26*).

Linearity was examined using a standard mixture solution of organosulfur compounds. Linearity relationships between concentrations of organosulfur compounds and corresponding peak areas were obtained, and regression equations and correlation coefficients are shown in **Table 2**. Linearity response ranges were 4.4−6000, 4.1−821, 9.7−970, 4−1000, 0.5−1015, 1.6−816, and 5.2−521 μg/mL for alliin, isoalliin, methiin, cycloalliin, GSAC, GSPC, and GSMC, respectively. Correlation coefficients (r) of all of these compounds were >0.999.

Detection limit was estimated on the basis of a signal-to-noise ratio of 3. Detection limits were 0.9, 0.8, 2.4, 1.0, 0.2, 0.4, and 1.0 μg/mL for alliin, isoalliin, methiin, cycloalliin, GSAC, GSPC, and GSMC, respectively (**Table 2**).

Accuracy of the analytical method was studied by recovery experiments (n = 3) using garlic powder. Recoveries were determined by adding two different concentrations of each organosulfur compound to garlic powder, and the results are listed in **Table 3**. Recoveries of 97.1−102.3% were obtained with relative standard deviation (RSD) values lower than 3.5%.

Table 2. Linearity Response Range and Detection Limits

compound	regression eq[a]	correlation coefficient	linear range (μg/mL)	detection limit[b] (μg/mL)
alliin	$y = 5606x + 6768$	0.999	4.4–6000	0.9
isoalliin	$y = 5438x + 2714$	0.999	4.1–821	0.8
methiin	$y = 3241x - 7850$	0.999	9.7–970	2.4
cycloalliin	$y = 2797x - 5765$	0.999	4.0–1000	1.0
GSAC	$y = 13889x - 150$	0.999	0.5–1015	0.2
GSPC	$y = 11734x + 3486$	0.999	1.6–816	0.4
GSMC	$y = 2874x + 1723$	0.999	5.2–521	1.0

[a] In the regression equation, x is the concentration of compound (μg/mL) and y is the peak area (mAbs × s). [b] Detection limits are estimated on the basis of a signal-to-noise ratio of 3.

Table 3. Recoveries of Organosulfur Compounds Spiked to Garlic Powder (n = 3)

compound	original amount (mg/g)	added amount (mg/g)	found amount (mg/g)	recovery (%)	RSD (%)
alliin	19.8	10.1	30.0	101.6	1.5
		20.1	39.6	98.6	2.6
isoalliin	0.967	0.557	1.536	102.3	3.0
		1.113	2.062	98.4	2.8
methiin	1.157	0.588	1.745	100.1	2.2
		1.175	2.323	99.2	1.6
cycloalliin	0.795	0.228	1.019	98.7	3.5
		0.455	1.256	101.3	1.4
GSAC	3.54	1.80	5.30	97.7	1.3
		3.60	7.09	97.1	0.9
GSPC	5.02	1.68	6.70	100.6	0.6
		3.35	8.39	100.6	0.9
GSMC	0.231	0.107	0.339	101.2	3.4
		0.214	0.441	98.0	2.5

Table 4. Intra- and Interday Precision and Reproducibility of Injection

	RSD (%)		
compound	intraday (n = 6)	interday (n = 3)	reproducibility of injection (n = 6)
alliin	1.3	1.2	0.9
isoalliin	2.0	3.9	1.3
methiin	2.5	3.7	1.5
cycloalliin	2.6	2.3	1.3
GSAC	1.0	1.8	1.0
GSPC	1.0	0.8	0.9
GSMC	2.1	4.6	1.4

Table 5. Contents of Organosulfur Compounds in Garlic Clove

source	content[a] (mg/g of dry wt)						
	alliin	isoalliin	methiin	cycloalliin	GSAC	GSPC	GSMC
Hokkaido, Japan	12.44 ± 2.00	0.14 ± 0.03	1.52 ± 0.67	0.70 ± 0.27	14.87 ± 2.11	11.74 ± 1.34	0.78 ± 0.17
Hiroshima, Japan	12.68 ± 1.77	0.72 ± 0.12	2.18 ± 0.08	0.80 ± 0.11	14.51 ± 1.73	15.12 ± 0.51	0.90 ± 0.10
United States	20.70 ± 2.33	1.77 ± 0.35	1.61 ± 0.34	1.18 ± 0.24	14.84 ± 2.80	8.52 ± 0.50	0.79 ± 0.16
China	24.05 ± 3.68	2.33 ± 0.15	2.14 ± 0.41	0.87 ± 0.02	5.43 ± 0.29	7.93 ± 0.49	1.27 ± 0.12

[a] Values are means ± SD ($n = 5$).

Table 4 gives RSD values of intra- and interday precision and reproducibility of injection. Intra- and interday precisions were investigated by analyzing six separately prepared samples daily, and for 3 days, respectively, using garlic powder. In both situations, RSD values were lower than 2.6 and 4.6%, respectively. Reproducibility of injection was evaluated by six replicate injections of a sample solution of garlic powder. RSD values of all organosulfur compounds were <1.5%.

Our data demonstrated this method was quite suitable for the quantitative analysis of alliin, isoalliin, methiin, cycloalliin, GSAC, GSPC, and GSMC in garlic and its powder products.

Contents of Organosulfur Compounds in Garlic Clove. Contents (corrected for moisture) of organosulfur compounds in garlic cloves obtained from four different culture areas are shown in **Table 5**. We found that contents of all compounds were different among culture areas. Especially, isoalliin content in garlic from China was ~17 times higher than that from Hokkaido, Japan. These results may be due to origin, variety, cultivation (*10, 11*), and storage conditions (*12*).

In summary, we developed and validated a simple, rapid, and precise analytical method for the determination of alliin, isoalliin, methiin, cycloalliin, GSAC, GSPC, and GSMC in garlic clove and its powder using a one-step sample preparation procedure and NP and RP HPLC techniques. Validation of this analytical method showed good specificity, linearity ($r > 0.999$), detection limit (0.2−2.4 μg/mL), recovery (97.1−102.3%), intraday precision (RSD < 2.6%), and interday precision (RSD < 4.6%). This newly developed analytical method offers some advantages over the currently accepted techniques including specificity, linearity, accuracy, precision, speed, and ease of use and would be useful for chemical and biological studies of garlic and its preparations.

LITERATURE CITED

(1) Rahman, K. Garlic and aging: new insights into an old remedy. *Ageing Res. Rev.* **2003**, *2*, 39−56.

(2) Lawson, L. D. Bioactive organosulfur compounds of garlic and garlic products. Role in reducing blood lipids. In *Human Medical Agents from Plants*; ACS Symposium Series 534; Kinghorn, A. D., Balandrin, M. F., Eds.; American Chemical Society: Washington, DC, 1993; pp 306−330.

(3) Agarwal, K. C. Therapeutic actions of garlic constituents. *Med. Res. Rev.* **1996**, *16*, 111−124.

(4) Yanagita, Y.; Han, S. Y.; Wang, Y. M.; Tsuruta, Y.; Anno, T. Cycloalliin, a cyclic sulfur imino acid, reduces serum triglycerol in rats. *Nutrition* **2003**, *19*, 140−143.

(5) Sendl, A.; Elbl, G.; Steinke, B.; Redl, K.; Breu, W.; Wagner, H. Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*. *Planta Med.* **1992**, *58*, 1−7.

(6) Yeh, Y. Y.; Liu, L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. *J. Nutr.* **2001**, *131*, 989S−993S.

(7) Block, E. The organosulfur chemistry of the genus *Allium*—implications for the organic chemistry of sulfur. *Angew Chem., Int. Ed. Engl.* **1992**, *31*, 1135−1178.

(8) Lancaster, J. E.; Shaw, M. L. γ-Glutamyl peptides in the biosynthesis of *S*-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium*. *Phytochemistry* **1989**, *28*, 455−460.

(9) Lawson, L. D. The composition and chemistry of garlic cloves and processed garlic. In *Garlic: The Science and Therapeutic Application of Allium sativum L. and Related Species*; Koch, H. P., Lawson, L. D., Eds.; Williams & Wilkins: Baltimore, MD, 1996; pp 37−107.

(10) Ueda, Y.; Kawajiri, H.; Miyamura, N.; Miyajima, R. Content of some sulfur-containing compounds and free amino acids in various strains of garlic. *Nippon Shokuhin Kogyo Gakkaishi* **1991**, *38*, 429−434.

(11) Matsuura, H.; Inagaki, M.; Maeshige, K.; Ide, N.; Kajimura, Y.; Itakura, Y. Changes in contents of γ-glutamyl peptides and fructan during growth of *Allium sativum*. *Planta Med.* **1996**, *62*, 70−71.

(12) Lawson, L. D.; Wang, Z. J.; Hughes, B. G. γ-Glutamyl-*S*-alkylcysteines in garlic and other *Allium* spp.: precursors of age-dependent *trans*-1-propenyl thiosulfinates. *J. Nat. Prod.* **1991**, *54*, 436−444.

(13) United States Pharmacopeial Convention. Garlic. In *United States Pharmacopoeia 27*; United States Pharmacopeial Convention, Inc.: Rockville, MD, 2004; pp 1997−1999.

(14) Ziegler, S. J.; Sticher, O. HPLC of *S*-alk(en)yl-L-cysteine derivatives in garlic including quantitative determination of (+)-*S*-allyl-L-cysteine sulfoxide (alliin). *Planta Med.* **1989**, *55*, 372−378.

(15) Eckner, M. M.; Sticher, O.; Meier, B. Reversed-phase high-performance liquid chromatography of *S*-alk(en)yl-L-cysteine derivatives in *Allium sativum* including the determination of (+)-*S*-allyl-L-cysteine sulphoxide, γ-L-glutamyl-*S*-allyl-L-cysteine and γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine. *J. Chromatogr.* **1992**, *625*, 183−190.

(16) Imai, J.; Ide, N.; Nagae, S.; Moriguchi, T.; Mastuura, H.; Itakura, Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med.* **1994**, *60*, 417−420.

(17) Krest, I.; Glodek, J.; Keusgen, M. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food Chem.* **2000**, *48*, 3753−3760.

(18) Yoo, K. S.; Pike, L. M. Determination of flavor precursor compound *S*-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci. Hortic.* **1998**, *72*, 1−10.

(19) Thomas, D. J.; Parkin, K. L. Quantification of alk(en)yl-L-cysteine sulfoxides and related amino acids in Alliums by high-performance liquid chromatography. *J. Agric. Food Chem.* **1994**, *42*, 1632−1638.

(20) Ueda, Y.; Sakaguchi, M.; Hirayama, K.; Miyajima, R.; Kimizuka, A. Characteristic flavor constituents in water extract of garlic. *Agric. Biol. Chem.* **1990**, *54*, 163−169.

(21) Arnault, I.; Christides, J. P.; Mandon, N.; Haffner, T.; Kahane, R.; Auger, J. High-performance ion-pair chromatography method for simultaneous analysis of alliin, deoxyalliin, allicin and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. *J. Chromatogr. A* **2003**, *991*, 69−75.

(22) Lawson, L. D.; Gardner, C. D. Composition, stability, and bioavailability of garlic products used in a clinical trial. *J. Agric. Food Chem.* **2005**, *53*, 6254−6261.

(23) Arnault, I.; Haffner, T.; Siess, M. H.; Vollmar, A.; Kahane, R.; Auger, J. Analytical method for appreciation of garlic therapeutic potential and for validation of a new formulation. *J. Pharm. Biomed. Anal*. **2005**, *37*, 963−970.
(24) Kubec, R.; Svobodova, M.; Velisek, J. Gas chromatographic determination of *S*-alk(en)ylcysteine sulfoxides. *J. Chromatogr. A* **1999**, *862*, 85−94.
(25) Lancaster, J. E.; Kelly, K. E. Quantitative analysis of the *S*-alk(en)yl-L-cysteine sulphoxides in onion (*Allium cepa* L.). *J. Sci. Food Agric.* **1983**, *34*, 1229−1235.
(26) Keusgen, M.; Junger, M.; Krest, I.; Schoning, M. Development of a biosensor specific for cysteine sulfoxides. *J. Biosens. Bioelectron.* **2003**, *18*, 805−812.
(27) Synge, R. L. M.; Wood, J. C. (+)-(*S*-Methyl-L-cysteine *S*-oxide) in cabbage. *Biochem. J.* **1956**, *64*, 252−259.
(28) Sakai, K.; Yoneda, N. Convenient synthesis of 1,4-thiazane-3-carboxylic acid derivatives. *Chem. Pharm. Bull*. **1981**, *29*, 1554−1560.
(29) Shen, C.; Parkin, K. L. In vitro biogeneration of pure thiosulfinates and propanethial-*S*-oxide. *J. Agric. Food Chem*. **2000**, *48*, 6254−6260.
(30) Lawson, L. D.; Hughes, B. G. Characterization of the formation of allicin and other thiosulfinates from garlic. *Planta Med.* **1992**, *58*, 345−350.

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