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The pharmacokinetics of the S35 labeled labeled garlic constituents alliin, allicin and vinyldithiine

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Abstract

Three groups of 3 rats received oral doses (8 mg/kg) of garlic constituents (alliin, allicin and vinyldithiines (2-vinyl-[4H]-1,3-dithiine and 3-vinyl-[4H]-1,2-dithiine)) in the form of an oil macerate of the 35S-labeled substance. The measured activity was referred to 35S-alliin (35S-alliin equivalents). The blood activity levels in each group were monitored for 72 h. For 35S-allicin and the labeled vinyldithiines the excretion with the urine, feces, and exhaled air was also measured. The distribution among the organs (whole-body autoradiography) and the urinary metabolite pattern (thin-layer chromatography) were also determined. For 35S-alliin the blood activity profile differed considerably from those of 35S-allicin and the labeled vinyldithiines: both the absorption and the elimination of the radioactivity were distinctly faster than for the other garlic constituents, maximum blood levels being reached within the first 10 min and elimination from the blood being almost complete after 6 h. For the other garlic constituents the maximum blood levels were not reached until 30-60 min (35S-allicin) or 120 min (vinyldithiines) p.a. and blood levels > 1000 ng-Eq/ml were still present at the end of the study after 72 h. The mean total urinary and fecal excretion after 72 h was 85.5% (35S-allicin) or 92.3% (labeled vinyldithiines) of the dose. The urinary excretion indicates a minimum absorption rate of 65% (35S-allicin) or 73% (vinyldithiines). It is uncertain whether the 19-21% recovered in the feces was unabsorbed substance or had been excreted via the bile or intestinal mucosa. The exhaled air showed only traces of activity although the whole-body autoradiographs, after fairly long exposure (96 h), showed distinct enrichment of activity in the mucosa of the airways and pharynx. The activity is deposite mainly in the cartilage of the vertebral column and ribs. There was no detectable difference in organ distribution between 35S-allicin and the labeled vinyldithiines. All that could be established from the urinary metabolite pattern was that unchanged 35S-allicin and unchanged labeled vinyldithiines are absent. There is therefore extensive metabolization. The metabolites must have a very polar structure with acid functional groups since satisfactory separation was achievable only with acid solvent systems. Conjugates with sulfuric or glucuronic acid were not detectable. These results reveal no differences in pharmacokinetic behavior between 35S-allicin and the labeled vinyldithiines. A final verdict as to whether the metabolites, which may be pharmacologically active, are identical must await further studies designed to identify the metabolites.

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Biological and chemical stability of garlic-derived allicin.

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Source

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Abstract

This study verifies the instability of garlic (Allium sativum L.)-derived allyl 2-propenylthiosulfinate (allicin) in various aqueous and ethanolic solutions as well as in vegetable oil through chemical and biological analyses performed simultaneously. Crushed fresh garlic cloves generated antibacterial activity and chemically detectable allicin, a major antibacterial principle, and both declined on a daily basis in aqueous and ethanolic solutions at room temperature, showing biological and chemical half-lives of about 6 and 11 days, respectively. Allicin was more stable in 20% alcohol than in water, but surprisingly unstable in vegetable oil, with an activity half-life 0.8 h, as estimated from its antibacterial activity toward Escherichia coli, and a chemical half-life of 3.1 h, based on chromatographic quantification. In alcoholic and aqueous extracts, the biological half-life of allicin tended to be longer than the chemical one, suggesting the occurrence of bioactive compounds other than allicin in the extracts.

Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement

Determination of Allicin, *S*-Allylcysteine and Volatile Metabolites of Garlic in Breath, Plasma or Simulated Gastric Fluids[1,2]

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ABSTRACT Various components of garlic and aged garlic extract, including allicin, *S*-allylcysteine (SAC) and volatile metabolites of allicin were determined in breath, plasma and simulated gastric fluids by HPLC, gas chromatography (GC) or HPLC- and GC-mass spectrometry (MS). Data indicate that allicin decomposes in stomach acid to release allyl sulfides, disulfides and other volatiles that are postulated to be metabolized by glutathione and/or *S*-adenosylmethionine to form allyl methyl sulfide. SAC can be absorbed by the body and can be determined in plasma by HPLC or HPLC-MS using atmospheric pressure chemical ionization (APCI)-MS. J. Nutr. 131: 968S–971S, 2001.

KEY WORDS: • *allicin* • *S-allylcysteine* • *liquid chromatography* • *mass spectrometry* • *garlic metabolites*

The fate of phytochemicals in garlic and aged garlic extract (AGE)[5] in the human body is of interest. In a recent article, Lawson (1998) suggested that thiosulfinates, which include allicin, are the only class of compounds that have reasonably proven bioactivity at levels representing normal amounts of garlic clove consumption. This classical assumption relates to theories of modern pharmacy and pharmacognosy in which single entities or, at the most, simple mixtures of bioactive compounds in plants are assumed to be the principle bioactive components. In a nutraceutical or herbal philosophy, garlic or AGE is important as the whole food or herb because these are total entities supplying many sulfur-containing and other phytochemicals, which can have antioxidant and other bioactivity. It is therefore important to develop reliable analytical methodology to determine the fate of garlic phytochemicals in the body.

When crushed raw garlic is consumed, the released allicin decomposes in stomach acid to release a number of volatile compounds including diallyl disulfide (DADS) and diallyl sulfide (DAS). This paper discusses the conditions for the determination of allicin in simulated gastric and intestinal fluids and identifies volatiles released from the mouth after chewing, with time-release studies showing volatiles released from the blood of human subjects over time. The main volatile metabolites, allyl methyl sulfide and allyl methyl disulfide are postulated to be formed from in vivo methylation by the phase II enzyme, glutathione. The analytical technique used was dynamic head-space sampling, specifically short-path thermal desorption gas chromatography-mass spectrometry (GC-MS) of the volatiles from breath. In addition, the analytical methodology (HPLC-MS) utilized for the determination of *S*-allylcysteine (SAC) in plasma after consumption of AGE is presented.

EXPERIMENTAL PROCEDURES

Preparation of simulated gastric fluid and intestinal fluid. Simulated gastric fluid was prepared according to the following procedure of the USP, the National Formulary: 2.0 g NaCl, 3.2 g pepsin and 3.0 mL concentrated HCl. Dilute to 1 L and verify that the pH is 1.2.

Simulated intestinal fluid was prepared according to the following procedure of the USP, the National Formulary: 6.8 g monobasic potassium phosphate, 650 mL H_2O, 190 mL of 0.2 mol/L NaOH and pancreatin mix (10 g).

The above supplies were purchased from Sigma Chemical (St. Louis, MO); HPLC water was from Fisher Scientific (Springfield, NJ).

Preparation of plasma samples for the analysis of SAC by HPLC-MS. The procedure is as follows: *1*) Transfer 100 μL

[1] Presented at the conference "Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement" held November 15–17, 1998 in Newport Beach, CA. The conference was supported by educational grants from Pennsylvania State University, Wakunaga of America, Ltd. and the National Cancer Institute. The proceedings of this conference are published as a supplement to *The Journal of Nutrition*. Guest editors: John Milner, The Pennsylvania State University, University Park, PA and Richard Rivlin, Weill Medical College of Cornell University and Memorial Sloan-Kettering Cancer Center, New York, NY.

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[5] Abbreviations used: ACN, acetonitrile; AGE, aged garlic extract; DADS, diallyl disulfide; DAS, diallyl sulfide; PITC, phenyl isothiocyanate; SAC, *S*-allylcysteine; SIM, selective ion monitoring.

TABLE 1

HPLC and HPLC-mass spectrometry (MS) conditions for the determination of S-allyl cysteine in plasma

Column: Allsphere ODS-2 (Alltech), 150 mm × 4.6 mm, i.d.		
Mobile phase A: 50 mmol/L ammonium acetate		
Mobile phase B: acetonitrile		
Flow rate: 0.9 mL/min for 8 min, then 1.5 mL/min for 7 min		
Time, *min*	%A	%B
0	80	20
8.0	80	20
8.1	20	80
15.0	20	80
Injection volume: 20 μL		
Ionization mode: atmospheric pressure chemical ionization, negative ion		
Masses scanned: m/z 295 and 300 (span 0.3 amu)		
Dwell time: 0.4 s		
Interchannel delay: 0.02 s		
Cone voltage: −10 V		
Source temperature: 150°C		
Probe temperature: 450°C		
Corona discharge: −1.2 kV		

of plasma to a 1.5-mL microcentrifuge tube. *2*) Add 400 μL of internal standard (D5 SAC at 0.1 μg/mL) solution. *3*) Shake centrifuge tube for 20 s. *4*) Centrifuge at 14,000 rpm for 5 min. *5*) Transfer 400 mL to a second 1.5-mL microcentrifuge tube. *6*) Concentrate to dryness by centrifuging under vacuum at 60°C for 60–120 min. *7*) Add 100 mL of phenyl isothiocyanate (PITC) reagent solution (methanol/triethylamine/PITC/H_2O, 7:1:1:1) and let stand for 30 min. *8*) Concentrate to dryness by centrifuging under vacuum at 60°C for 60 min. *9*) Take up residue from Step 8 in 100 mL of H_2O/acetonitrile (ACN) (65:35). *10*) Sonicate for 15 min. *11*) Centrifuge for 15 min at 14,000 rpm. *12*) Analyze supernatant by HPLC-MS.

HPLC conditions for the determination of allicin. A Supelco 25-cm × 4.6-mm i.d., LC-18 column was used on a Varian model 9010 HPLC with a Varian 9065 diode array detector (Sugarland, TX). This was run isocratically using ACN/H_2O at 30:70. Quantitation was at 195 nm.

Allicin was purchased as a standard for the HPLC studies from Plant Bioactives, Orem, UT. The internal standard, diallyl sulfone, was purchased from Parish Chemical, Orem, UT. Dimethyl thiophene was purchased from Sigma Chemical and the deuterated internal standard, D5-SAC was purchased from Isotec, Miamisburg, OH. It was custom synthesized with five deuteriums on the allyl group, and purity was 99%. PITC was purchased from Sigma Chemical as was triethyl amine. Solvents were HPLC grade from Fisher Scientific. HPLC and HPLC-MS conditions for the determination of SAC in plasma are given in **Table 1**.

GC conditions for the determination of breath volatiles. Conditions were as follows: DB-1 methyl silicone capillary column, 0.25-μm film thickness, 60 m × 0.32 mm i.d., J&W scientific (SIS, Ringoes, NJ); programmed from −20 to 280°C at 10°C/min; and helium flow rate of 1 mL/min.

Concentration of breath for volatiles by short-path thermal desorption. Dynamic head-space concentration was accomplished using short-path thermal desorption (Hartman et al. 1993). The equipment was an SIS Model TD-2 SPTD (SIS Corporation), and a GLT (glass-lined stainless steel) desorption tube of 4 mm i.d. packed with 2-cm homogenized adsorbate. The chromosorb was baked at 150°C for 1 h to condition the chromosorb and purge off any volatiles that might interfere with the analysis. The adsorbent trap was spiked with d-8 toluene and d-8 naphthalene for use as internal standards. The initial purge time was set to 3 min, with a desorption temperature of 150°C and a desorption time of 5 min.

Mass spectrometry conditions (volatiles). Mass spectrometry was performed using electron ionization at 70 eV on a Finnigan MAT 8230 mass spectrometer with a Finnigan MAT SS300 data system (Finnigan, San Jose, CA). The mass range scanned was from 35 to 450 amu at a scan rate of 1 s/decade and an interscan time of 0.8 s. The GC-MS interface line temperature and the MS inlet temperature were set to 280°C.

RESULTS AND DISCUSSION

Allicin determinations. An analytical method for the determination of the fate of allicin in stomach and intestinal fluids was developed. The HPLC chromatogram using the conditions described above and showing separation of the internal standard (diallyl sulfone) is shown in **Figure 1**. Diallyl sulfone was chosen as a standard because of its structural similarity to allicin and because it separates well from the analyte of interest. In addition, this internal standard is commercially available as a high purity analytical standard, and both compounds strongly absorbed at the wavelength used (195 nm). A calibration curve was prepared with the two purchased standards. Diallyl sulfone is also chemically stable,

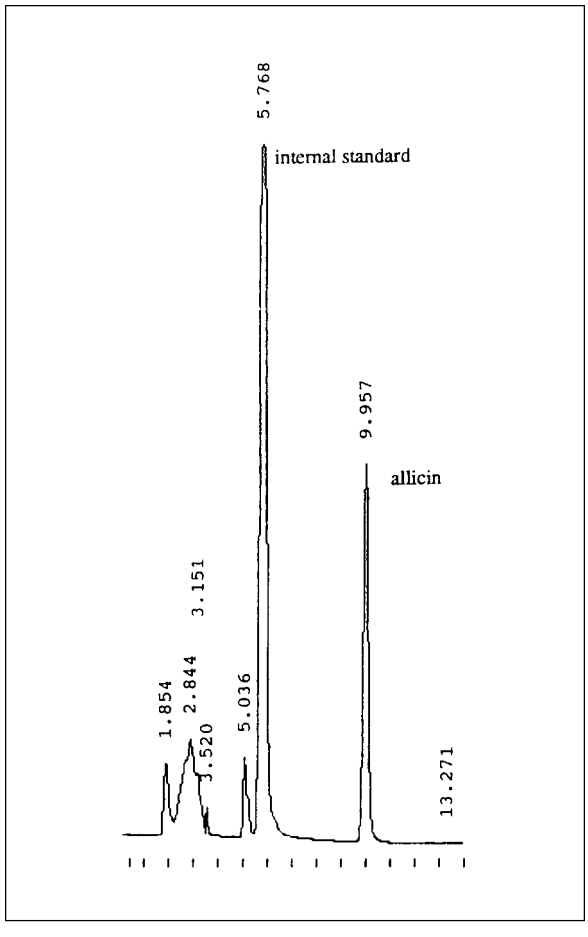

FIGURE 1 HPLC chromatogram showing allicin (retention time, 9.95 min) and diallyl sufone internal standard (retention time, 5.77 min). Conditions: C-18 column, 195 nm, acetonitrile (ACN):H_2O at 30:70.

and stock solutions were prepared and stored without concern for breakdown.

A commercially standardized enteric-coated tablet and a gelatin capsule were purchased. The gelatin capsule consisted of a two-part hard shell, which contained garlic; the enteric coat consisted of a polymer of methacrylic acid, talc and ethyl citrate. The commercial garlic preparation used was in the form of a dried powder with an ability to generate ~7000 ppm of allicin when hydrated. The results in **Figure 2** show that allicin is either not formed at high levels in the simulated stomach acid or decomposes at low pH. A further study showed that allicin is stable at the higher pH found in the intestine but decomposes readily at a stomach pH of 1.2 to ~5% of initial levels after 30 min.

Volatile breath analyses. Volatile breath analyses were carried out by short-path thermal desorption GC-MS. This dynamic head-space concentration technique (Hartman et al. 1993) was used to monitor the volatiles released from the breath from periods of a few minutes to ~4 h after consumption of ~10 g of raw garlic. Volunteers expelled a liter of air into a Teflon or tedlar bag fitted with a shut-off valve. The contents of the bag were then drawn with a vacuum pump through a glass-lined stainless steel tube (4-mm i.d.) packed with either tenax or chromosorb resin. The chromosorb was baked at 150°C for 1 h to condition the chromosorb and purge off any volatiles that might interfere with the analysis. The adsorbent trap was spiked with an internal standard of dimethyl thiophene.

Interestingly, major volatile compounds such as DADS and DAS were not the major compounds found in the breath (**Fig. 3**); rather, the major volatile was allyl methyl sulfide, which may have been formed from action of glutathione on the DADS and DAS or on other components containing the C_3H_5-S-moiety. Lawson (1998) believes that allicin is metabolized in the blood cells to allyl mercaptan, which is then metabolized by an enzyme, methyltransferase, with *S*-adenosylmethionine to allyl methyl sulfide. H_2S was observed as a trace breath component but could not be quantitated at such low levels. It is of interest to note that analysis of the breath of subjects consuming garlic formulations containing zinc (low odor garlic) did not show H_2S as a breath volatile; however, GC-MS did show the other components

Also evident in the breath after consumption of raw garlic were limonene and *p*-cymene. This is most unusual in that

ALLICIN IN GASTRIC FLUID (GELATIN CAPSULE)	225 PPM
ALLICIN IN INTESTINAL FLUID (ENTERIC COATED)	6900 PPM
ALLICIN IN GASTRIC FLUID (ENTERIC COATED)	200 PPM

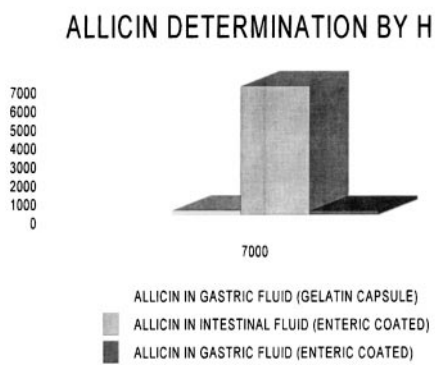

FIGURE 2 Allicin values in simulated gastric and intestinal fluids by HPLC (analysis time, 30 min after introduction).

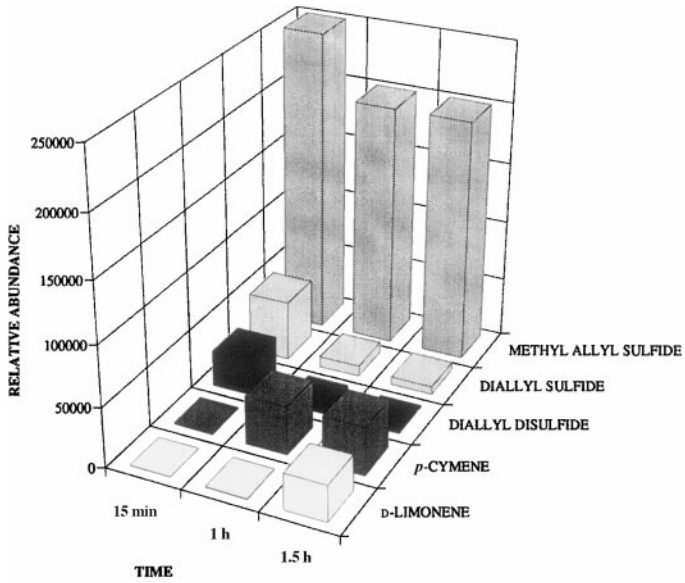

FIGURE 3 Bar graph and data showing release of volatiles and metabolites from breath after consumption of raw garlic.

these components are not observed in garlic or in the first few minutes after consumption of garlic. It is postulated that these components are formed from metabolism of conjugates that are present in garlic. These precursors may be glycosides or even amino acid/peptides/protein conjugates which, upon digestion and metabolism, were released to the blood and subsequently the breath. These are not artifacts. This was replicated in many subjects and shown to be reproducible.

SAC determinations. SAC was determined in plasma by a modification of the method of Nagae et al. (1994). Conditions are listed in the experimental section. HPLC-MS was used instead of HPLC and the internal standard used was a deuterated analog of SAC (D5 SAC, deuteriums positioned on the allyl group) purchased from Isotec. This synthetic standard has a molecular weight of 166 compared with that of SAC (161), and the PITC derivatives elute at almost the same retention time on the HPLC. The mass spectrometer can differentiate between the two species by molecular weight. Use of a stable isotopically labeled internal standard also controls recovery because any losses in the preparation step, which causes losses to SAC, will also cause loss of the internal standard because the chemistry is identical. Recovery then, by definition, becomes 100%. This was checked with spiking experiments and recoveries ranged from 98 to 100%.

Figure 4 shows the chromatogram of the derivatized analogs of SAC and its internal standard as isolated from human plasma after consumption of AGE. The molecular weights of the derivatized SAC and the deuterated derivatized SAC are 295 and 300, respectively. These ions are monitored by selective ion monitoring (SIM) by the HPLC-mass spectrometer. This mode increases sensitivity over the scanning mode and is generally used for quantitation of components and metabolites in plasma, especially in the pharmaceutical industry. Essentially, the mass spectrometer is set to alternate between the two masses of 295 and 300, with equal dwell time. The time to monitor each peak is ~10 -20 s. Conversely, in the scanning mode, the mass spectrometer is set to monitor an entire spectrum, e.g., between masses 35 and 350 in 1 s. The difference is that in the scanning mode each mass is focused on the electron multiplier for 1/315 of a second. This constitutes a mass spectrum. The SIM mode, in which each mass is focused

on the electron multiplier for seconds rather than fractions of a millisecond as in the scanning mode, is much more sensitive. This method is in use at present to monitor SAC in the blood of people consuming AGE. The selected ion chromatogram obtained from human plasma is shown in **Figure 5**. In the matrix, the internal standard elutes at 4.82 min and the SAC is observed at 4.92 min. This difference of retention time relates to absolute absorption differences between the deuterated standard and the nondeturated analyte. It should be noted that although the absolute retention time decreases in plasma vs. a standard (Figure 4) injection, the relative retention time remains steady. The true retention time in plasma was confirmed with spiking experiments. SAC levels in blood reach levels of 800 ppb after consumption of three AGE capsules (Steiner, personal communication).

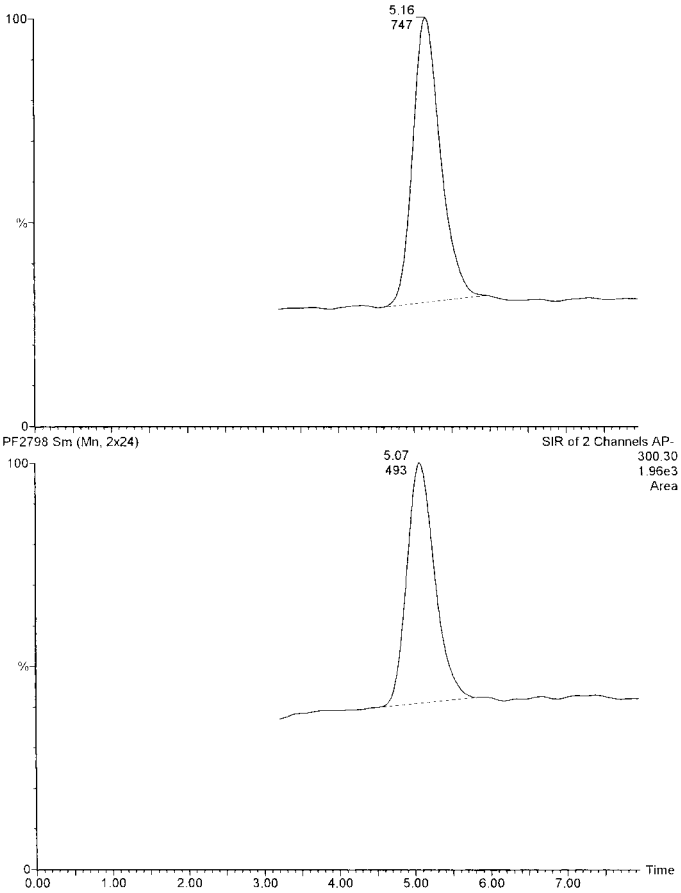

FIGURE 4 Selected ion monitoring traces of deuterated *S*-allylcysteine (*lower trace* at *m/z* 300) and *S*-allylcysteine (*upper trace* at *m/z* 295) standards; elution time, 5.07 and 5.16 min, respectively.

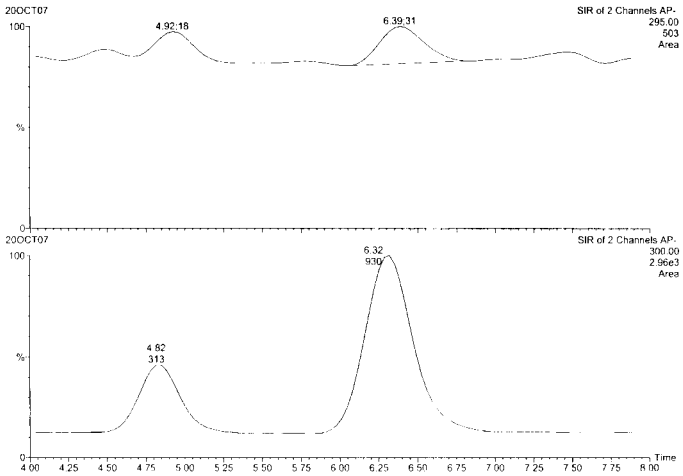

FIGURE 5 Selected ion monitoring traces of deuterated *S*-allylcysteine (*lower trace* at *m/z* 300) and *S*-allylcysteine (upper trace at *m/z* 295) from human plasma; elution time, 4.82 and 4.92 min, respectively. The number following the retention time is the area counts.

Summary

Analytical methodology has been developed for the determination of the active principles of garlic and AGE in breath, plasma or simulated gastric fluids. This methodology includes GC-MS for the determination of volatiles and HPLC or HPLC-MS for the determination of nonvolatiles, including allicin and SAC. The specificity of mass spectrometry has been used for identification of these most important species.

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Table 3.8 Fate of Undiluted Allicin at Room Temperature

Time	Allicin	Ajoene	1,3-VDT	1,2-VDT	AAS_2	AAS_3
Isolated garlic thiosulfinates (87% allicin)						
0 h	100	0	0	0	0	0
24 h	32	17	21	7	0	0.5
48 h	6	13	12	7	0	1.5
Synthesized allicin (98%)						
0 h	100	0	0	0	0	0
24 h	19	6	7	4	7	3
48 h	3	5	4	3	6	3

Values represent weight %, as determined by C18-HPLC. Zero time began when samples were extracted and the solvent evaporated. The amount of unidentified compounds was estimated from the difference between compounds and the initial weight, and by assigning the extinction coefficient of diallyl disulfide to each methods of which approximately agreed. Abbreviations: VDT, vinyldithiin; AAS_2, diallyl disulfide; AAS_3 LD, Wang ZJ, unpublished data, 1994.

3.2.6.3.2 Transformation of Undiluted Allicin

Pure allicin is an oily liquid, but this is its most unstable form, and as such, it has been reported to disappear almost completely in less than 20 hours at room temperature (Brodnitz et al., 1971). This original gas chromatography study reported that the allicin is transformed primarily to diallyl disulfide (66%), diallyl sulfide (14%), and diallyl trisulfide (9%). However, using non-destructive HPLC analysis, contrary results have been found (Lawson & Wang, 1994). Table 3.8 shows the results of incubating undiluted allicin, either synthetic or isolated as total thiosulfinates from garlic, in closed vials for 1–2 days. Most of the allicin had decayed in 24 hours; however, the dominant identifiable transformation products were the vinyldithiins and ajoene. Diallyl disulfide was also abundant with synthetic allicin, but was not found with allicin isolated from garlic, indicating that other thiosulfinates present have an influence. The majority of the transformation products, however, especially at 48 hours, were unidentified compounds, of which there were about 40. Incubation in a nitrogen atmosphere rather than in air did not change the results. The reason for the great instability of pure allicin is simply that it easily reacts with itself. As mentioned previously, allicin's stability increases greatly with dilution, especially in solvents capable of hydrogen bonding.

3.2.6.3.3 Thiosulfinate [illegible] Organic Solvents: Viny[illegible]

Upon incubation in [illegible] thiosulfinates undergo [illegible] tions than in water (Fig. [illegible] the distribution of [illegible] formed depends upon [illegible] vent and other factors. [illegible] vents (e.g., hexane, ether, [illegible] etable oil), allicin and [illegible] nate rapidly form mainly [illegible] vinyl-4*H*-1,3-dithiin) [illegible] (3-vinyl-4*H*-1,2-dithiin [illegible] amounts of *ajoene* (*E,Z*-4,5,9 1,6,11-triene 9-oxide [illegible] *fides* (18%) (Voigt & Wolf, 1990a; Lawson et al., 19[illegible] ins were first discovered [illegible] tion products of allicin [illegible] artifacts during the gas [illegible] allicin (Brodnitz et al., [illegible] shown to be the dominant [illegible] found in commercial garlic [illegible] maceration of crushed garlic [illegible] (Voigt & Wolf, 1990). [illegible] misidentified the structure [illegible] vinyldithiin, but this has [illegible] ed (Block et al., 1984).

Ajoene was originally [illegible] incubation of chopped garlic [illegible] a new compound with [illegible] activity toward inhibition of [illegible] aggregation (Apitz-Castro [illegible] Shortly thereafter, the [illegible]