Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 20, No. 5, 442-447, 2007

Determination of diallyl disulfide in garlic by reversed-phase high performance liquid chromatography

Xiaolong Wan, Yulia Polyakova and Kyung Ho Row★

Center for Advanced Bioseparation Technology and Dept. of Chem. Eng., Inha University, 253 Yonghyun-Dong, Nam-Ku, Incheon 402-751, South Korea

(Received July 16, 2007; Accepted August 28, 2007)

역상 고성능 액체 크로마토그래피를 이용한 마늘에서 diallyl disulfide의 분석

만효룡 · Yulia Polyakova · 노경호★

초정밀생물분리기술연구센터, 화공과, 인하대학교

(2007. 7. 16. 접수. 2007. 8. 28. 승인)

요 약: 마늘(*Allium sativum* L.)의 특성은 organosulfur 화합물이다. 마늘에 포함된 diallyl disulfide(DADS)를 분석하기 위한, 간단하고 신속한 시료준비와 분석방법을 제시하였다. 모든 마늘시료들은 메탄올, 벤젠, 또는 테트라하이드로퓨란과 같은 용매로 추출하여 분석하였다. 실험결과에 의하면, 메탄올로 추출한 시료가 가장 우수하였다. 분석조건으로는 이동상은 메탄올과 물로 구성되고 기울기법을 적용하였다. 분말가루로 된 마늘 1 g에 0.61 mg DADS를 추출하였다. 기존 분석방법에 비해 우수하여 마늘관련 연구에 도움이 될 것이다.

Abstract: The properties of garlic (*Allium sativum* L.) are attributed to organosulfur compounds. In this paper, an analytical technique with a rapid and simple sample preparation procedures for determination of diallyl disulfide (DADS) in garlic was reported. The DADS was simply extracted with various solvents (methanol, benzene or tetrahydrofuran) from garlic and prepared for HPLC analysis. From the results, the methanol was select as an optimal extraction solvent. The mobile phase was composed from methanol and water, and the gradient elution mode was applied. 0.61 mg of DADS per g garlic powder can be extracted with methanol. This work offers some advantages over the currently accepted techniques and would be useful for chemical and biological studies of garlic and its products.

Key words: garlic, dially disulfide, RP-HPLC, extraction

★ Corresponding author
Phone : +82-(0)32-860-7470 Fax : +82-(0)32-872-0959
E-mail : rowkho@inha.ac.kr

1. 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.[2,3] Many studies on animals showed its protective effects against chemically induced toxicity and against carcinogenesis.[2] The modulation of the metabolism of carcinogens by DADS was considered one of the possible mechanisms of its protective effect against the occurrence of cancer.[3] However, once garlic is cut, chopped or crushed, the clove's membrane disrupts and S-allylcysteine sulfoxide is transformed enzymatically into allicin by allinase.[4] The main component of the volatile oil are sulfur compounds especially allicin, diallyl sulfide (DAS), DADS, diallyl trisulfide (DATS). DADS is one of the major volatile degradative compounds of garlic formed from allicin.[5] Therefore, it becomes important to control sample preparation to minimize artificial errors caused by their chemical characters. Moreover, it has been reported that contents of organosulfur compounds in garlic change during cultivation[6, 7] and storage.[8] Therefore, an analytical method for the determination of DADS, in a garlic sample is required for evaluating the quality of garlic. It has been previously reported that organosulfur compounds in garlic were analyzed using reversed-phase high performance liquid chromatography (RP-HPLC)[9-18] gas chromatography[19] thin layer chromatography[20] and biosensors.[21] 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.[20-23] 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 and rapid analytical technique using sample preparation procedure and subsequent HPLC techniques to determine DADS in garlic qualitatively and quantitatively.

2. Experimental

2.1. Reagents

The garlic was purchased from local markets in Korea. The standard of DADS (analytical grade) was obtained commercially from Sigma (St. Louis, MO, USA). The methanol and tetrahydrofuran (THF) for HPLC were purchased from Duksan Pure Chemical Co. (Ansan, Korea). The benzene (extra pure) was bought from Oriental Chemical industries (Korea). The distilled water was filtered with a vacuum pump (Division of Millipore, Waters, USA) and a filter (HA-0.45, Division of Millipore, Waters, USA) prior to use.

2.2. Apparatus

An instrument with an analytical HPLC system was used an M930D solvent delivery module equipped with a M930D solvent delivery pump (Young-In Co, Korea), a UV M720 absorbance detector (Young-In Scientific Co., Korea), and a Reodyne injector (Cotati, CA, USA) valve with a 20 μL sample loop. Experiments were performed with a commercially available column Platinum ESP C_{18} (150 × 4.6 mm i.d. and 5 μm particles) from Alltech Associates Inc. (Waukegan Road, IL, USA). Chromate software (Ver. 3.0 Interface Eng., Korea) on a PC was used as a data acquisition system. BCHI heating bath B490 (Flawil, Switzerland), BCHI evaporator R200 (Flawil, Switzerland) and aspirator A35 (Tokyo, Japan) were used for sample concentration. Ultrasonic bath was used for homo-genization. 1200L single quadrupole gas chromatography (GC) and mass spectrometry (MS) system with 3800GC (Varian, USA) were used for qualitative DADS determination.

2.3. Sample preparation

Garlic powder was prepared as follows: fresh

garlic was peeled and 500 g of peeled garlic was dried using a dryer at 50 °C for 24 hrs. The resulting was ground into powder with a mortar and pestle and stored at 4 °C until analysis. 2.0 g powder of homogenized with 100 mL of extractant, in an ultrasonic bath at room temperature for 5 min. After mix it round at room temperature for 30 min, and then was enriched to 1 mL solution by concentration system. Distillation was performed with vacuum at 35 °C. As the stability of DADS is very temperature-dependent, it is important to carry out the assay as quickly as possible, so the sample solutions were stored at 4 °C before injection.

2.4. HPLC analysis

In this work, flow rate was 1 mL/min, wavelength was UV 210 nm, injection volume was 3 μL and mobile phases were composed of water and methanol. Gradient elution mode was applied. The program used throughout the experiments is described below. The mobile phase composition of the reservoir A was water, while that of the reservoir B was methanol. In first 15 min, the mobile phase compositions of reservoirs A was linearly decreased from 95 to 0 vol. %, and the mobile phase composition of reservoir B was linearly increased from 5 to 100 vol. %, then hold last stage (water 0 vol. %, methanol 100 vol. %) in 3 min, at last return beginning stage (water 95 vol. %, methanol 5 vol. %) in 2 min. The HPLC parameters (retention time and peak area) reported in this study were the averages of at least three determinations. Evaluation of the results of the chromatographic experiment was carried out by mathematical statistic techniques. The relative error of a single measurement did not exceed at 5 %. Within-day and between-day precision expressed by relative standard deviation was less than 5 %. All chromatographic procedures were performed at an

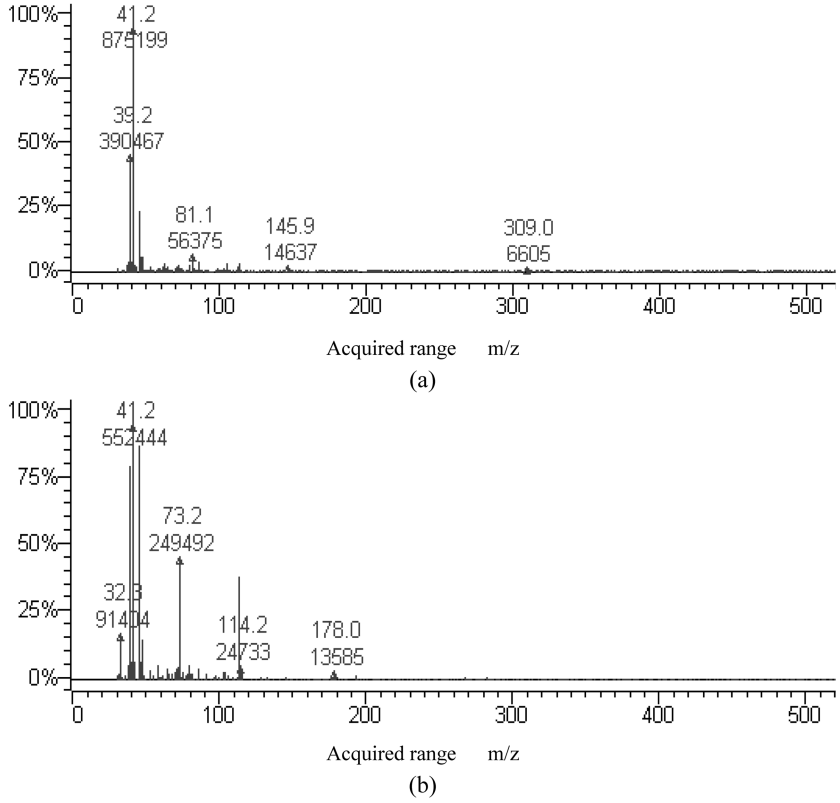

Fig. 1. GC-MS of effluent of peaks from methanol extract.

ambient temperature.

2.5. GC-MS analysis

Column VF-5MS was utilized. GC oven was programmed: 40 °C 2 min, 10 °C /min to 300 °C, 20 min at 300 °C. The injection temperature was kept on 250 °C (splitless mode). Flow rate of the carrier gas (helium) was 25 cm/s. MS detector was operated at 194 °C, and ionization energy was 70 eV. Scan range was from 0 to 520 m/z at scan rate of 0.9 1/s.

3. Results and Discussion

The sample preparation procedures used in the work were relatively simple; and extracts were injected. HPLC experiment was conducted to separate DADS using C_{18} column. Surprisingly, it was found that two peaks were disclosed at injection of the purchased DADS standard solution. Thus, the additional procedures of the identification of the peaks of standard had to be made. The effluents of peaks supposed as DADS from methanol extract were collected and they were injected into GC with MS for detection. The results were shown in *Fig.* 1. Molecular weight of DADS is 146.28. The target molecular ion was finding in the first eluted peak (*Fig.* 1(a)). The second collected peak hasn't DADS molecular ion signal (*Fig.* 1(b)). When looked at in more detail, from *Fig.* 1(b) did not find DADS, but we considered that this effluent was most comprised of DAS and DATS. It is more realistic to suppose that the purchased DADS standard was not enough pure. From these experiments it was concluded that the first peak was validated as DADS.

Moreover, in order to calculate the amount of DADS extracted from the garlic, a calibration curve needs to be drawn. In order to assess the extent of the linear relationship between the known concentrations of DADS (X) and the corresponding absorbance values (Y), the (r^2) was calculated. By plotting of concentration vs. peak area, the regression equation of the calibration curve was Y=8367.3X + 19661. A value of $r^2 = 0.998$ indicates a high positive correlation. This is illustrated in *Fig.* 2.

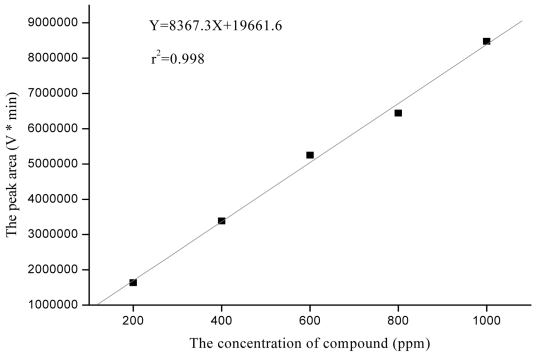

Fig. 2. The calibration curve of the DADS.

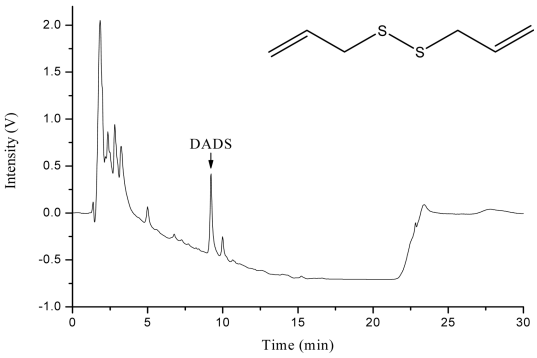

Fig. 3. Chromatogram of DADS in methanol extract.

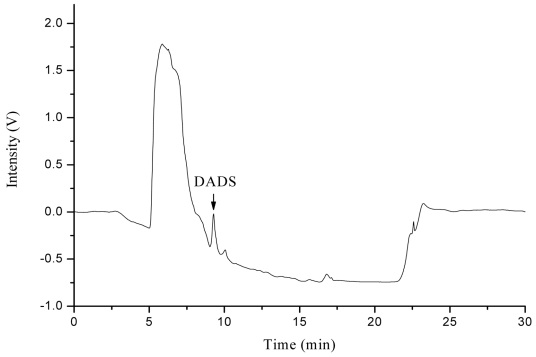

Fig. 4. Chromatogram of DADS in benzene extract.

Figs. 3-5 are shown chromatograms of determination of DADS in three differences extracts. In our work, the goal was to generate a convenient technique for rapidly but accurately determining the DADS in garlic. Methanol, benzene or THF were selected as the extracting solvents because they readily dissolve the target DADS but will not easily dissolve other potentially interfering compounds.

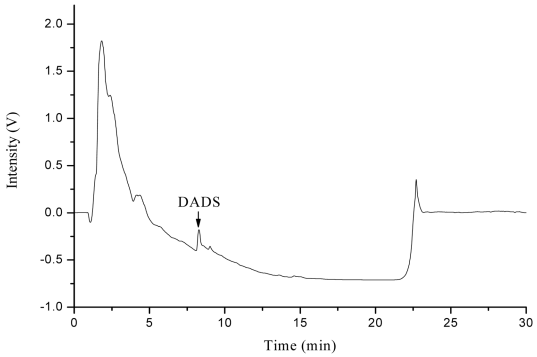

Fig. 5. Chromatogram of DADS in THF extract.

Table 1. The extraction amounts of DADS from garlic with three different solvents

Solvent	Amount of DADS mg/g (DADS/garlic)
Methanol	0.61
Benzene	0.33
THF	0.19

Without any doubt, the effects of extraction conditions have influence on extraction efficiency.

The extraction amounts of DADS from garlic with three used solvents are shown in *Table* 1. Contrasted with three results, methanol as the extraction solvent was optimized and 0.61 mg of DADS per g garlic can be extracted. It is worthy of note that presented DADS amounts are the average values, obtained after three experiments. In this work of three different in the polarity of extractant they were used. The best results of the extraction of DADS were achieved with the polar solvent - methanol. Nonpolar benzene extracts practically two times less substance than methyl alcohol. THF, as substance with the middle polarity, it showed the worst results with the extraction of DADS. It was obviously found that the nature of extractant markedly affect on the extraction performance and as a consequence on the amount of extractible DADS.

4. Conclusions

In summary, we used a simple rapid analytical technique for the separation and determination of DADS in garlic. The experimental results showed that the optimized extraction solvent was methanol and 0.61 mg of DADS per g garlic can be extracted. In addition, this method was validated with respect to reproducibility. Thus, this technique will be useful for chemical and biological studies of garlic and its products.

Acknowledgments

The authors are grateful for the financial support of the Center for Advanced Bioseparation Technology, Inha University.

Reference

1. K. Rahman, *Ageing Res. ReV.,* **2**, 39-56 (2003).
2. C. Ip, D. J. Lisk and G. S. Stoewsand, *Nutr Cancer*, **17**, 279-286 (1992).
3. B. S. Reddy, C. V. Rao, A. Rivenson and G. Kelloff, *Cancer Res.*, **53**, 3493-3498 (1993).
4. E. Block, *Sci. Am.*, **252**, 94-99 (1985).
5. E. Block, *Angew Chem. Int. Ed. Eng.*, **31**, 1135-1178 (1992).
6. Y. Ueda, H. Kawajiri, N. Miyamura and R. Miyajima, *Nippon Shokuhin Kogyo Gakkaishi,* **38**, 429-434 (1991).
7. H. Matsuura, M. Inagaki, K. Maeshige, N. Ide, Y. Kajimura and Y. Itakura, *Planta Med.*, **62**, 70-71 (1996).
8. L. D. Lawson, Z. J. Wang and B. G. Hughes, *J. Nat. Prod.*, **54**, 436-444 (1991).
9. S. J. Ziegler and O. Sticher, *Planta Med.*, **55**, 372-378 (1989).
10. M. M. Eckner, O. Sticher and B. Meier, *J. Chromatogr.*, **625**, 183-190 (1992).
11. J. Imai, N. Ide, S. Nagae, T. Moriguchi, H. Mastuura, and Y. Itakura, *Planta Med.*, **60**, 417-420 (1994).
12. I. Krest, J. Glodek and M. Keusgen, *J. Agric. Food Chem.*, **48**, 3753-3760 (2000).
13. K. S. Yoo and L. M. Pike, *Sci. Hortic.*, *72*, 1-10 (1998).
14. D. J. Thomas and K. L. Parkin, *J. Agric. Food Chem.*, *42*, 1632-1638 (1994).
15. Y. Ueda, M. Sakaguchi, K. Hirayama, R. Miyajima and A. Kimizuka, *Agric. Biol. Chem.* **54**, 163-169 (1990).
16. I. Arnault, J. P. Christides, N. Mandon, T. Haffner, R. Kahane and J. Auger, *J. Chromatogr. A*, **991**, 69-75

(2003).
17. L. D. Lawson and C. D. Gardner, *J. Agric. Food Chem.*, **53**, 6254-6261 (2005).
18. I. Arnault, T. Haffner, M. H. Siess, A. Vollmar, R. Kahane and J. Auger, *J. Pharm. Biomed. Anal.*, **37**, 963-970 (2005).
19. R. Kubec, M. Svobodova and J. Velisek, *J. Chromatogr. A*, **862**, 85-94 (1999).
20. J. E. Lancaster and K. E. Kelly, *J. Sci. Food Agric.*, **34**, 1229-1235 (1983).
21. M. Keusgen, M. Junger, I. Krest and M. Schoning, *J. Biosens. Bioelectron.* **18**, 805-812 (2003).
22. R. L. M. Synge and J. C. Wood, *Biochem. J.* **64**, 252-259 (1956).
23. M. Ichikawa, N. Ide, J. Yoshida, H. Yamaguchi and K. ONO, *J. Agric. Food Chem.* **54**, 1535-1540 (2006).