J. of university of anbar for pure science : Vol.4:NO.2 : 2010

ISSN: 1991-8941

Determination of Alliin and Allicin in different types Garlic using High Performance Liquid Chromatography

Mohammad J. Abdul Ghani
University of Sallah Aldin - Collage of Basic Education
Received: 10/1/2010 Accepted: 28/4/2010

Abstract: Alliin and Allicin products were measured and determined by ion-pair reversed-phase liquid chromatography (RP-LC) with UV detection at 210 nm. These two Compounds were extracted from various types of garlic with methanol / ethyl acetate and chromatographed on octadecyl silane column [ODS C18 (250 x 4.6 mm id)] with gradient elution from 0.01M phosphate buffer (PH=2.5) with 5M heptanslfonic acid (mobile phaseA) to 0.01M phosphate buffer (PH=2.5) acetonitrile(1:1) (mobile phase B). Allicin was eluted after Alliin. The results observed show that the concentration differs between the different types of garlic. The aqueous Iraqi garlic extract has the highest concentration of Alliin and Allicin (17.9 ppm, 0.9%),(23.94 ppm, 1.2%) respectively. But the lowest concentration of allicin was found in French garlic extract (0.56 ppm, 0.03%) while the lowest level of Alliin was (4.3 ppm, 0.22%) in Chinese garlic extract.

Key words: Determination .Alliin . Allicin . Garlic . HPLC

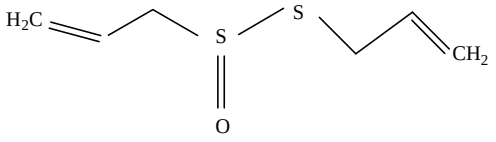
Introduction

Garlic (Allium Sativum), like othter plants, has an exquisite defense system composed of as many different components boosting human immune system. In order to protect itself from insects and fungi, garlic enzymatically produces Allicin when injured.

Allin (S-allylcysteine sulfoxide, percent composition: C 40.66%, H 6.26%, N 7.90%, O 27.08% and S 18.09%) is constructed of an allyl group, a sulfoxide group, and the amino acid cysteine (contains SH rather than S=0). Alliin is biosynthesized from its parent ompound, S-Allyl cysteine (deoxyalliin). [1]

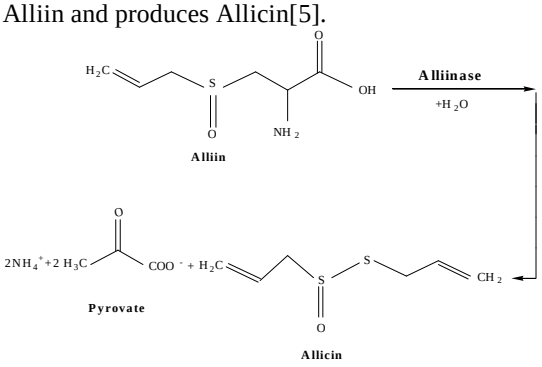
Allin is quite stable in the absence of active alliinase, and it can also be found in cooked garlic. It has been demonstrated as antioxidant activity. [2]

Allicin is known as 2-propene-1-sulfinothioic acid S-2-propenyl ester, thio-2-propene-1-sulfinic acid S-allyl ester, diellyl disulfide-oxide, diallyl thiosulfinate [3] percent composition: C 44.4%, H 6.21%, 0 9.86%, and S 39.52%.



Allicin

Allicin was discovered in 1944 by Cavallito et al., who first noted its potent antimicrobial activity [4]. Allicin is produced by an enzymatic reaction when raw garlic is either crushed or injured. The enzyme allinase combined with Alliin and produces Allicin[5].



Allicin has been reported to possess numerous

biological and biochemical activities. They include beside antibectrial effects[4], reduction of serum cholesterol and triglycerides[6], inhibition of platet aggregation[7].

Because of its instability, allicin is not commercially available and can be conveniently synthesized by oxidation of diallyl disulfide with acidic hydrogen peroxide and purified using Si-TLC[8].

Allicin can also be isolated from dichloromethane extract of garlic homogenated by C18-TLC. Asimple and sensitive method for quantitation of this compound is still not available. Indirect quantitation of allicin by conversion of either dially disulfide or allyl mercaptan followed by gas chromatographic (GC) analysis[9]. These GC and HPLC methods all require allicin as an external standard [8,9]. Han J.et al [10]described a spectrophotometric method for quantitative determination of Allcin [11,12] based on, that one molecule of Allicin reacts rapidly with two molecules of cysteine to form two molecules of S-allyl mercaptocysteine(SAMC). The mechanism of this reaction is not known.

Unlike GC and HPLC methods[8,9], Han J. et al [10] method does not require an allicin standard to quantify allicin and can be conveniently used to measure the total concentration of thiosulfinates in garlic extract

Experimental

1. Adjustment of ODS column:

For any chromatographic column to be maximally effective at retarding a sample molecule and more stability may be adjustment. Therefore, they are flushing with methanol or acetonitrile once aweek under 0.1ml/min. Any non polar compounds, which remain on the reversed phase column, are easily removed by flushing with methanol or acetonitrile once a day. Columns should not be back flushed unless indicated in the column manual, nor should they be stored in buffer, such as phosphate buffer, that promotes microbial growth.

2. Sample preparation:

Samples rarely come in a form that can be injected directly into the instrument; some form of sample preparation usually is required.

In this research, sample preparation includes any manipulation of the sample prior to analysis, involving techniques such as weighing, dilution, concentration, filtration, centrifugation, derivatization, and chromatography.

3. Sample preparation for seperationof Allicin extract [13]:

Frozen fresh garlic cloves (20 g) of each sample were pleaded, chopped, blended with absolute ethanol:ethylether (1:1) into blender, and extracted twice with (10)ml of cool mixture about {10 min) for each extraction. The extracts were dried over anhydrous sodium sulfate and filtered. The extract was immediately subjected to HPLC.

4. Simultaneous qualitative and quantitative determination of Alliin and Allicin by HPLC [14]:

The isolated components were analyzed by ion-pair reversed- phase liquid chromatography with UV detection, using an octadecyl silane column with gradient elution.The operation conditions are listed in table (1).

Results and discussion

Determination of Allicin In aqueous garlic extract:

Asimple and rapid HPLC method suitable for routine analysis of Alliin and Allicin, was developed by Arnault l. et al.[15] using eluent containing an ion-pairing reagent (Heptane sulfonate) and a (150*3) mm column. Allicin was eluted after Alliin and the synthetic reference compounds were characterized by the same chromatographic method using diod-array UV detector.

Addition of hydrochloric acid solution to garlic extract will ihibit the formation of Allicin, and in addition to this, adding of sulfite can be determined, without interference of Allicin by reversed phase ion-pairing liquid chromatography with post-column detection [16].

Mochizuki E.N. et al.[17] reported, that Allicin and Alliin in garlic were determined simultaneously by ion-pair reversed liquid chromatography with diod array UV detection. In these articles Alliin is extacted from garlic and applies as external standard after purification by ion-exchange chromatography.

The method that consists of using an octadecyl silane (OSD) column with gradient elution from 0.01M phosphate buffer (pH 2.5) with 5mM heptane sulphonic acid (A) to 0.01M phosphate buffer (pH 2.5)-acetonitrile [(1:1),(B)] can be used to analyze fresh garlic preparations, and health foods.

The limits of detection were between 1.7 and 9.40 ng for allyl methyl sulfide and dimethyl disulfide, respectively, and percentage recovery rates of aqueous garlic extracts ranged from 74.4% for the first to 90.3% for dipropyl disulphide, using GC and MS [18].

By Applying adeveloped liquid chromatography technique based on florescent detection of 9-fluorenyl methyl chloroformate derivatives, Methyl-L-Cystein sulfoxide and 2-propyl-L-

Cystein sulfoxide were determined in garlic with detection limits less down to 2.5mg/100g fresh weight [19].

The major sulphoxide component that is found in garlic was (+)-S-allyl-Cystein sulphoxide (>95%) can be determined by HPLC on two spherisorb columns (OSD) in series with elution of extracted garlic, allinase was inhibited by addition of 10mM of hydroxyl amine during extraction and eluted with 2M ammonium hydroxide through an Amberlite IR-120 anion exchange column [20].

Kasuga S. et al.[21] found, in Japanese garlic, that raw garlic juice contained 0.162% allicin but no Alliin, heated garlic juice contained 0.266% Alliin and 0.001% Allicin, dehydrated garlic juice contained 0.462% Alliin but no Allicin and aged garlic extract contained 0.003% alliin but no Allicin.

In our work we applied Mochizuki E.N. et al. [17] method to determine simultaneous Alliin and Allicin by ion-pair reversed liquid chromatography using UV detection under the conditions listed in table (1).

In table(2), the retention times, area, and concentration of Alliin and Allicin in standard, Iraqi, Iranian, Lebanes, French, and Chinese garlic extracts are listed.

It is seen that Iraqi aqueous garlic extract is high in Alliin (17.9 ppm, 0.9%), and Allicin(23.94 ppm, 1.2%) concentrations.

Figures (3-7) are showing the chromatograms of Alliin and Allicin for the studied types galic.

The optimum conditions for separation of standard Alliin and Allicin were applied as shown in typical chromatograms in figures (1 and 2).

The results confirm that main bioactive component in garlic is Allicin.

The predominates active components in all types of garlic were Alliin andAllicin but the Iraqi garlic extract have the highest concentration of these components.

High Performance Liquid Chromatography is ideal method for separation and measurement of active ingredients Allicin and Alliin.

References:

1. Lawson L.D., Garlic: Review of its medical effects and indicated active compounds. In Lawson L.D., and Bauer R. (eds). Phytomedicines of Eurrope: Chemistry and Biological Activity. Washington, DC: American Chemical Society 176-209 (1998) Historical perspective on the use of garlic. Rivlin, R.S., Historical perspective on the use of garlic, J. Nutr., 131(3S), 951S-4S(2001).
2. Rabinkov et al., Biochim Biophys Acta, 1379(2):233-234, Kourounaskis, P.N., Rekka, E.A. Res Commun Chem Pathol Pharmacol, 74(2:249-52(1991).
3. Allicin. The Merk Index. 1999 by Merk&& Co Inc, Whitehouse Station, NJ, USA, Published on CD-ROM by Chapman&& Hall/CRC.
4. Cavallito C.J. and Bbailey J.H., Allicin, the antibacterial principle of Allium sativum.I. isolation physical properties and antibacterial action, J. Am. Chem.Soc., 66, 1950-1(1944).
5. Freeman F. and Kkodera Y. Garlic chemistry: stability of S-(2-propenyl)-2-propene-1-sulphinothioate(Allicin) in blood, solvents, and simulated physiological fluids,J. Agric. Food Chem., 43, 2332-8(1995).
6. Augusti K. and Mathew P.T., Lipid lowering effect of Allicin (diallyldisulfide-oxide) on long term feeding on normal rats, Experienta, 30, 468-70(1994), Han J.C. Lawson L.D., Han G., and Han P., Biochem., 157-60(1995).
7. Lawson L.D., Ransom D.K., and Huges B.G., Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial products, Thromb Res., 65, 141-56,(1992).
8. Lawson L.D., Wood S. G., and Hughes B. G., Planta Med., 57, 263-70 (1991). Release of volatile compounds from microwave heating of garlic juice with 2,4 decadienanals.J. Food chem, 64, 531-35 (1999).
9. Koch J., Berger L., Vieregge – Reiter C., Planta Med., 55, 327-31(1989).
10. Han J. C. Lawson L. D., Han G., and Han P., Anal. Biochem., 225, 157-60(1995). Ccomparative study of extraction techniques for determination of garlic flavor components by gas chromatography - mass spectrometry, Anal. Bioanal. Chem. 377, 749-56 (2003).
11. Cavallito C. J., Buck J.s., and Suter C. M., Allicin antibacterial principle of Allium sativum II. Determination of the structure, J. Am. Soc., 66, 1952-4 (1944).
12. Lawson L. D., and Wang Z. J., Planta Med. 59, 4688-9 (1993). Aspectrophotometric method for quantitative determination of Allicin and total garlic thiosulfinates, Analytical Biochemistry 225, 157-60(1995).

13. Chyau C-C, Mau J-J, Release of volatile compounds from microwave heating of garlic juice 2,4- decadienals, Food Chemistry, 64,531-5(1999).
14. Mochizuki E. N., Yyamamoto T., Horie M., Ikai Y., and Nakazawa H. Electroforetic identification of garlic and garlic products, Journal of AOAC International, 80(5), 1052-6(1997).
15. Arnult I., Christides J.P., Mandon N., Haffner T., Kahane R., and J. High performance ion- pair chromatography method for simultaneous analysis of Alliin, deoxyalliin, and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. J. Chromatogr. A, 991(1), 69-75(2003)(Abstract, Pub. Med.).
16. Perfetti G. A.and Diachenko G. W, Journal of AOAC International, 86(3), 544-50(2003)(Abstract Medline .).
17. Mochizuki E. N., Yamamoto T., Horie M., Ikai Y., and Nakazawa H., Liquid chromatographic determination of Alliin in garlic and garlic products. J. chromatography 455:271-7(1988) journal of AOAC international, 80(5), 1052-6(1997)(Abstract Midline).
18. Martinlagos R. A., Serrano M.F.O., and Lopez M.D.R., Food Chem., 531(1), 91-3(1995)(Abstract Midline).
19. Thomas D. J.and Parkin K., Agric. Food Chem., 42(8), 1632-8(1994).
20. EwaRDS s. j., Musker D., Collin H. A., and Britton G., Phytochemical Analysis, 5(1), 4-9(1994).
21. Kasuga S., Uda N., Kyo E., Ushijima M., Morihara N., and Itakura Y. Immunomodulation and antitumer activities of aged garlic extract phytomedicine 5: 259-67(1998) J. Nutr., 131S(3s, 1080S-4S(2001).

Table (1) : the HPLC gradient conditions of separation of Allicin and Alliin

Mobile phase (A) at pH 2.5 by phosphoric acid	(10mM)Pot.diyydrogen phosphate +(5mM) 1-heptane Sulphonic acid		
Mobile phase (13) at pH 2.5 by phosphoric acid	[(10mM)Pot.diyydrogen phosphate +Acetonitrile (AcN0)](1:1)(v:v)		
Flow rate	1.0mL.Min-1		Injector
Detection :210nm	Temperature:40Co		Volume 50ML
Time(min)	0.01	20	25
Mobile phase (B)%	0	100	stop

Table (2):Retention times, areas, and concentrations of Alliin and allicin in standard,Iraqi, Iranian, Lebanese, and Chinese garlic extracts.

	Alliin				Allicin			
Garlic Extract	tR min	Area	Conc .ppm	Conc.%	tR min	Area	Conc .ppm	Conc.%
Standerd	3.080	337105	2.50	0.00025	3.700	569480	2.50	0.00025
Iraqi	3.197	2413007	17.40	0.900	3.425	5452475	23.94	1.200
Iranian	3.190	1776597	13.18	0.660	3.590	1316251	5.78	0.290
Lebanese	3.210	788764	5.85	0.293	3.420	1523505	6.69	0.335
French	3.080	681753	5.10	0.260	3.700	126612	0.56	0.030
Chinese	3.160	579170	4.30	0.22	3.27	1333754	5.90	0.290

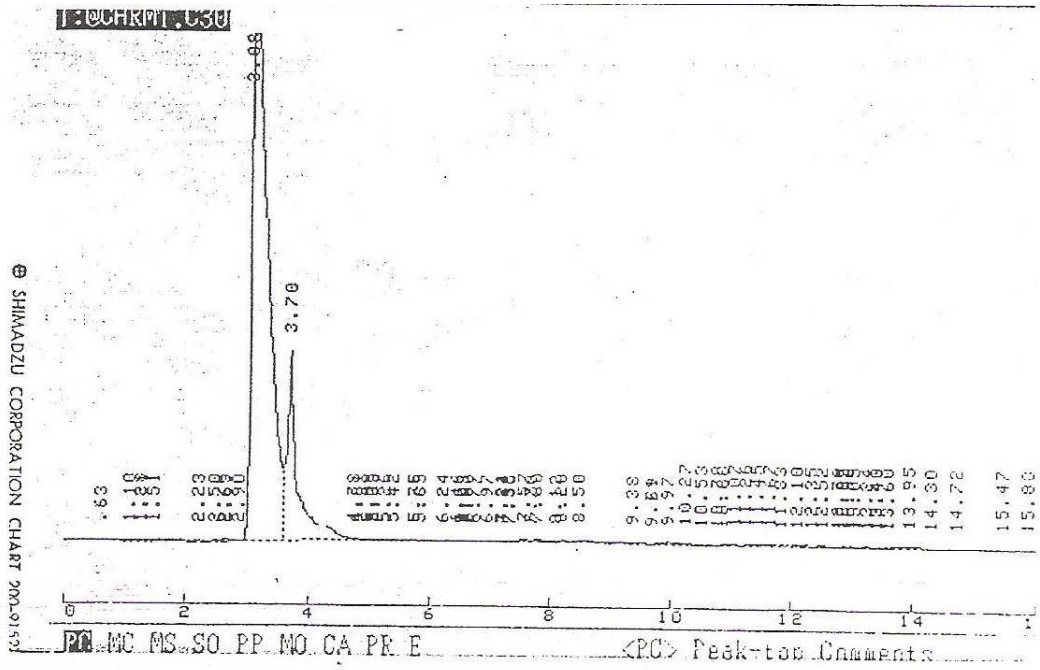


Fig (1) : Chromatogram of standard alliin(3.08) and allicin(3.70).

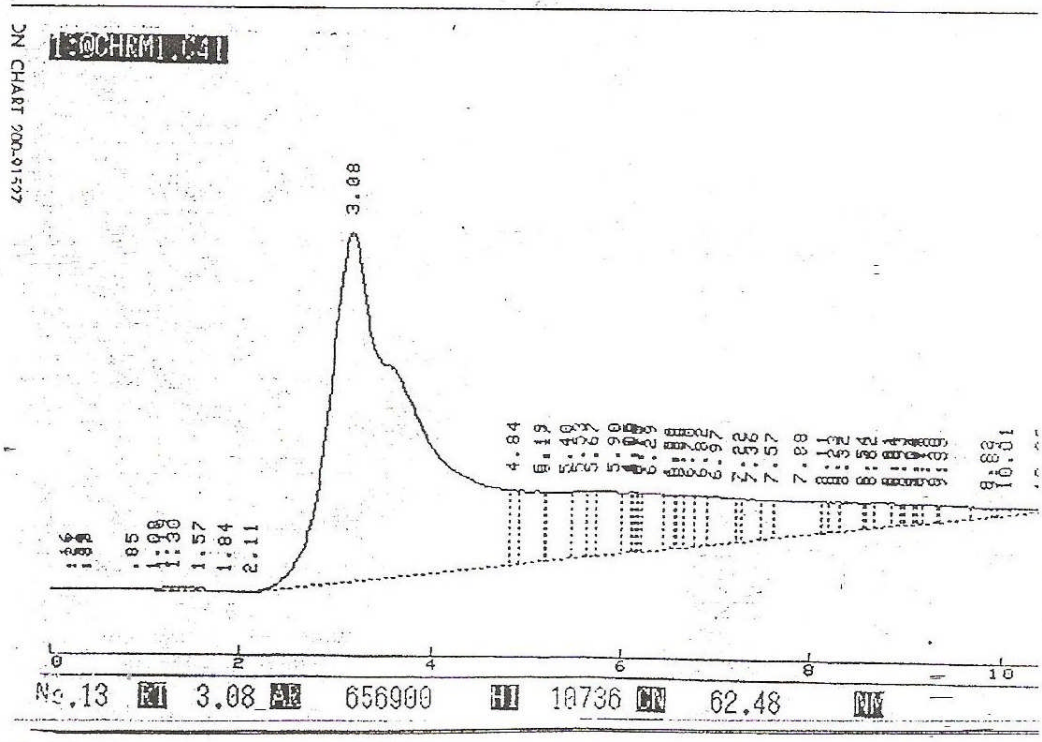


Fig (2) : Chromatogram of standard alliin(3.08).

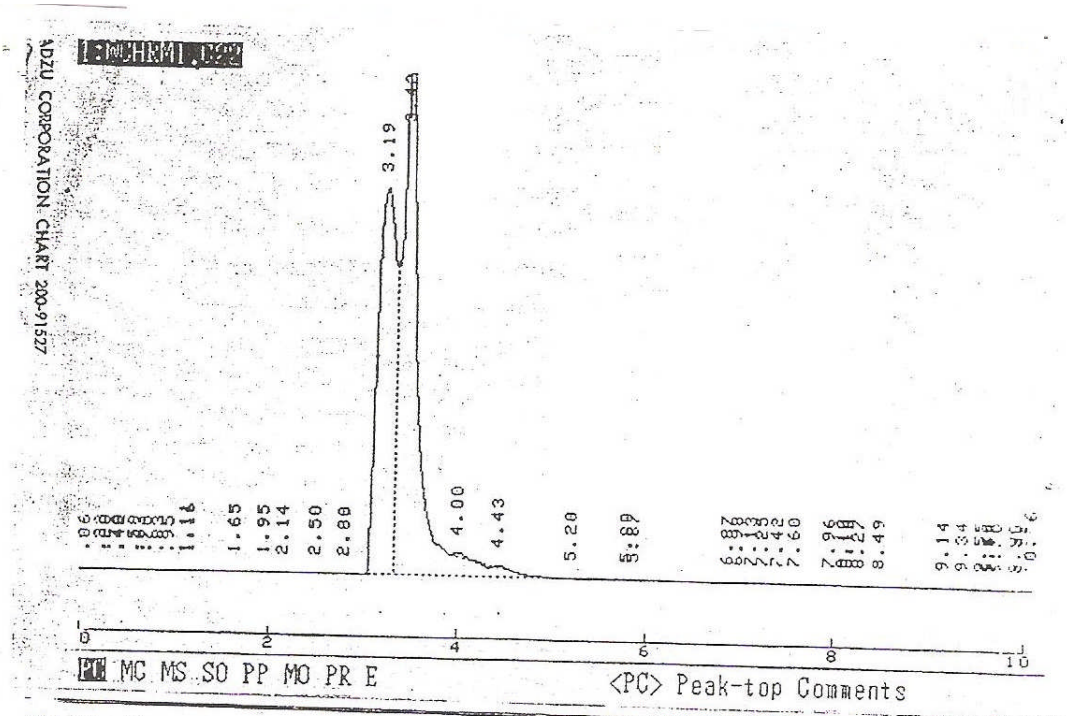


Fig (3) : Chromatogram of alliin (3.19) and allicin(3.42) in fresh Iraqi garlic extract.

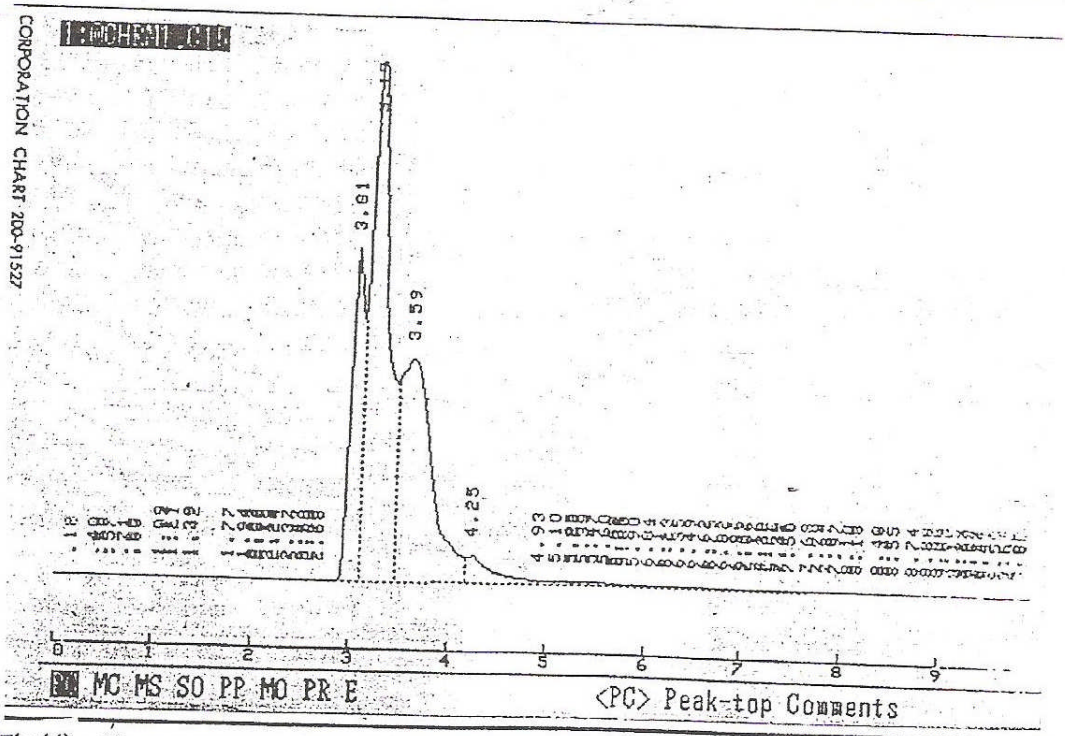


Fig (4) : Chromatogram of alliin(3.01) and allicin (3.59) in fresh Iranian garlic extract.

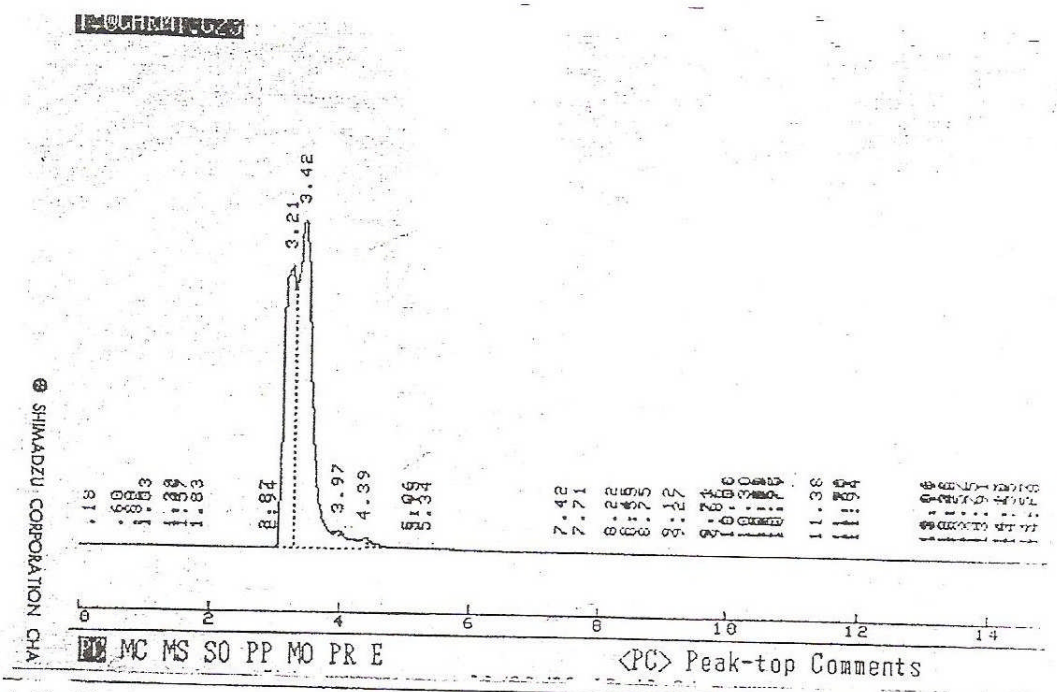


Fig (5) : Chromatogram of alliin (3.21) and allicin (3.42) in fresh Lebanese garlic extract.

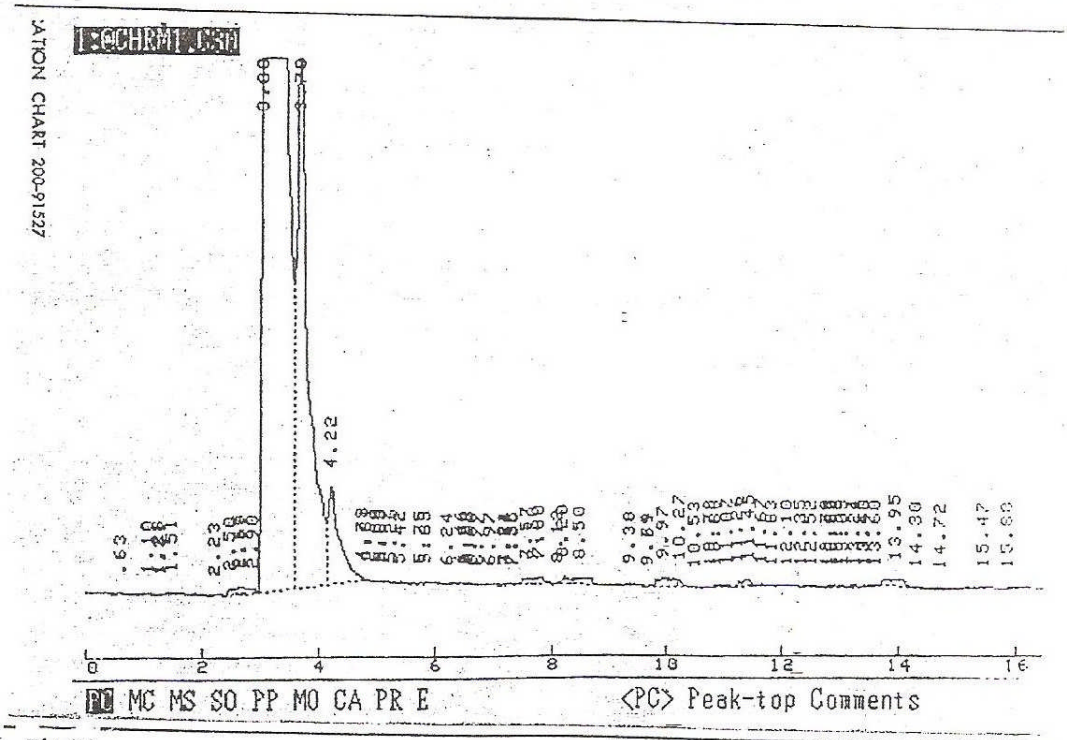


Fig (6) : Chromatogram of alliin (3.08) and allicin (3.70) in fresh French garlic extract.

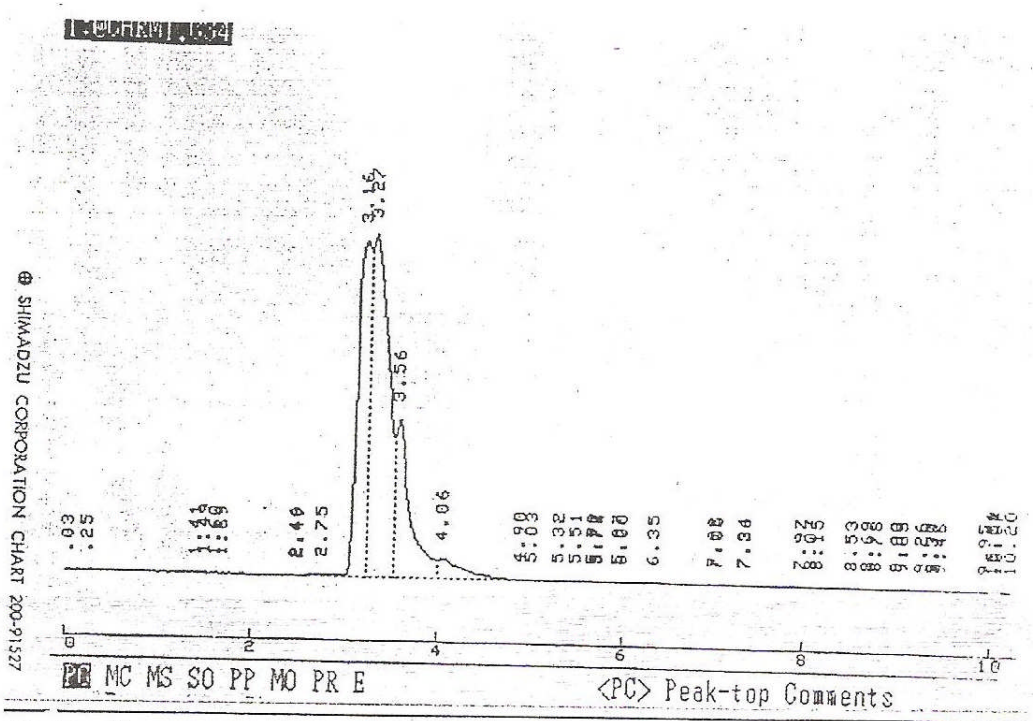


Fig (7) : Chromatogram of alliin (3.16) and allicin (3.27) in fresh Chinese garlic extract.

تعيين مركبات(Alliin) و (Allicin) في انواع مختلفة من الثوم باستخدام HPLC

محمد جميل عبد الغنى
E.mail: scianb@yahoo.com

الخلاصة:تم قياس و تعيين المركبين (Alliin) و (Allicin) بتقنية كروماتوغرافيا السائل ذي المزدوج الايونى – الطور المعكوس بكاشف الاشعة فوق البنفسجية عند طول موجى (210 nm) . تم استخلاص هذين المركبين من انواع مختلفة من الثوم بواسطة Methanol/Ethyl acetate و تم قياس الكروماتوغرافيا باستخدام عمود [ODS C18 (250 x 4.6 mm id)] مع استرجاع تدريجى من المحلول المنظم 5M heptanslfonic acid و 0.01M phosphate buffer (PH=2.5) كطور متحرك (mobile A phaseA) الى المحلول المنظم 0.01M phosphate buffer (PH=2.5) acetonitrile(1:1) كطور متحرك (mobile phase B) حيث تم استرجاع (Allicin) بعد (Alliin). اظهرت النتائج ان التراكيز تختلف باختلاف نوع الثوم ،حيث تبين ان تركيز ال(Alliin) و (Allicin) يكون الاعلى فى مستخلص الثوم العراقى (23.94 ppm, 1.2%),(17.9 ppm, 0.9%) على التوالى بينما اعطى مستخلص الثوم الفرنسى اقل نسبة من Allicin (0.56 ppm, 0.03%) فى حين اعطى المستخلص الصينى من (4.3 ppm, 0.22%) Alliin