

## Research Article

### Quantitation of Allicin in Garlic-based Products: Comparisons among Spectrophotometry, GC and HPLC

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**Abstract:** Spectrophotometric, GC and HPLC methods were used to determinate allicin concentration in garlic-based products (garlic powder, garlic oil and garlic tablets). Allicin was extracted using a mixture of water and ethanol and analyzed by the three methods. The results revealed that the GC method was unsuitable for allicin quantitation because of high coefficient of variations and high temperatures. The spectrophotometric method was the simplest and most effective method for solid garlic-based products. The HPLC method was more accurate for allicin quantitation in all garlic-based products, especially liquids. Furthermore, the HPLC method allowed the simultaneous quantitation of allicin and allicin degradation products such as diallyl sulfide, dimethyl trisulfide, diallyl disulfide and diallyl trisulfide. Based on the spectrophotometric and HPLC method, garlic oil contained the highest allicin concentration, followed by the garlic tablets and garlic powder.

**Keywords:** Allicin, garlic products, gas chromatography, high pressure liquid chromatography, spectrophotometry

## INTRODUCTION

Garlic (*Allium sativum* linn.), a common food condiment, is considered a dietary supplement due to its cholesterol-lowering effects. Garlic-based products have become increasingly popular over the past several years. For individuals who cannot tolerate the consumption of raw garlic, garlic-based products constitute a means of consuming allicin, the main bioactive compound in garlic. Allicin (S- (2-propenyl) 2-propene-1-sulfinothioate), which is also known as diallyl thiosulfinate, is a flavor compound. As shown in Fig. 1, the structure of allicin has a Sulfur Oxide (SO) group (Ali *et al.*, 2000). Allicin is not present in whole garlic. Allicin is instantaneously synthesized when garlic cloves are crushed or damaged and the enzyme alliinase and the compound alliin, which appear to be stored in separate compartments in whole garlic, interact (Bachrach *et al.*, 2011). The synthesis of allicin takes place through a series of reactions detailed in Scheme 1 (Miron *et al.*, 2000). Extracted allicin, however, loses its beneficial properties within hours through its conversion into other sulfur-containing compounds (Sigounas *et al.*, 1997; Hirsch *et al.*, 2000). As shown in Fig. 2, allicin (diallyl thiosulfinate) is formed by attaching an allyl group and an oxygen atom to the sulfur atom of cysteine (Khar *et al.*, 2011).



Fig. 1: Structure of allicin (S- (2-propenyl) 2-propene-1-sulfinothioate or diallyl thiosulfinate)

Heat induces the degradation of allicin into several diallyl sulfide compounds (Lawson and Gardner, 2005): Diallyl Sulfide (DAS), Dimethyl Trisulfide (DAT), Diallyl Disulfide (DADS) and Diallyl Trisulfide (DATS). These compounds are stable and used in the treatment of cancer and bacterial and fungal infections (Song and John, 2001; Tripathi, 2009). Among the diallyl sulfide compounds, DADS is the most potent. A study reported that garlic extract, allyl alcohol and DADS increase oxidative stress and reduce glutathione concentrations in *Candida albicans* (Lemar *et al.*, 2007).

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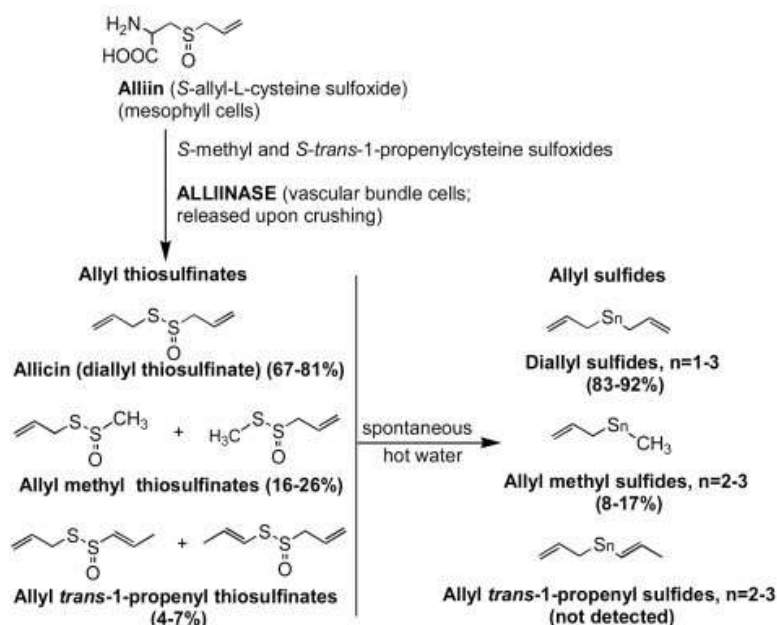
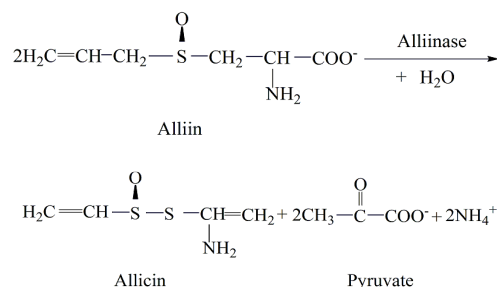


Fig. 2: Structure of degradation products of alliin (Lawson and Gardner, 2005)

Furthermore, alliin and its degradation products are important due to their antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activities (Oommen *et al.*, 2004). Alliin belongs to the family of phytochemicals, which are useful in the treatment and cure of several cancers. According to Banerjee and Maulik (2002), alliin prevents the risk of cardiovascular diseases and cancers and has antimicrobial and antioxidant effects. Additionally, alliin protects against dental caries and periodontitis (Bachrach *et al.*, 2011).

The quantitative determination of alliin and its degradation products is important due to their pharmacologic activities. Currently, quantitative determination methods rely on spectrophotometry, High Pressure Liquid Chromatography (HPLC) and Gas Chromatography (GC) (Han *et al.*, 1995; Miron *et al.*, 2002; Baghalian *et al.*, 2005; His and Wu, 1989). Other methods including GC-Mass Spectrometry (GC-MS) (Mondy *et al.*, 2001), Supercritical Fluid Chromatography Mass Spectrometry (SFC-MS) (Calvey *et al.*, 1994), Ultra-Performance Liquid Chromatography with photo Diode Array Detection (UPLC-DAD) (Khar *et al.*, 2011) have been used. GC-MS, SFC-MS and UPLC-DAD methods can be used for scientific research; however, they are very expensive. Chromatographic methods are reliable, selective and accurate because possible interferences from other sulfur-containing compounds can be easily eliminated. However, the accuracy of alliin determination from these chromatographic methods ultimately depends on the extraction procedures and purification steps. Pharmaceutical preparations of garlic, which is considered one of the safest herbal plant medicines, are currently available in the market (Velisek *et al.*, 1997). Therefore, the quantitative determination of alliin in

garlic-based products/pharmaceutical preparations should be standardized. The purpose of this study was to compare spectrophotometry, GC and HPLC in the quantitation of alliin in garlic oil, garlic powder and garlic tablets:



Scheme 1

## MATERIALS AND METHODS

**Materials:** Eight garlic-based products were purchased from different areas of Shandong province and Jiangsu province in China. This sampling was performed according to information obtained from local agricultural extension offices. The garlic products included garlic tablets, garlic powder and garlic oil. The geographical origins of the garlic-based products are shown in Table 1. DAS, DAT, DADS and DATS were purchased from Sigma Chemical Inc. (USA). L-Cysteine and 5, 5-dithiobis- (2-nitrobenzoic acid) (DTNB) were obtained from Beijing Land Bridge Technology, Co., Ltd. (Beijing, China).

**Alliin extraction:** The garlic tablets were ground. Approximately, 0.5 g of garlic-based product was mixed

Table 1: Geographical origins of the garlic products

Sample number	Region originated	Product	Climate <sup>a</sup>
Gp1	Jinxiang county	Garlic oil	Warm temperate-semi humid
Gp2	Shouguang county	Garlic powder	Warm temperate-semi humid
Gp3	Chengwu county	Garlic powder	Warm temperate-semi humid
Gp4	Yutai county	Garlic powder	Warm temperate-semi humid
Gp5	Guangrao county	Garlic piece	Warm temperate-semi humid
Gp6	Shanghe county	Garlic piece	Warm temperate-semi humid
Gp7	Sheyang county	Garlic piece	Subtropics transition temperate-humid
Gp8	Cangshan county	Garlic piece	Warm temperate-semi humid

with 5 mL of double distilled water in a 100-mL conical glass bottle. Enzymatic hydrolysis was allowed to take place in a 50°C water bath for 60 min. A volume of 15 M ethanol was added and the mixture was placed in a 45°C oscillator water bath at 150 rpm for 90 min. Every 5 min, the bottle containing the mixture was open to release air. The mixture was filtered using filter paper and the filtrate (allicin) was collected.

**Spectrophotometric method:** Reactions were performed at 30°C. Five milliliters of 10 mM L-cysteine in 50 mM Tris-HCL buffer (pH 7.5) was either mixed with 1 mL of double distilled water (blank) or 1 mL of extracted allicin; 1 mL of the resulting solution was diluted to 100 mL. The diluted solution (4.5 mL) was mixed with 1.5 mM DTNB (0.5 mL) in 50 mM Tris-HCL buffer (pH 7.5). Absorbance was measured at 412 nm after 15 min. Allicin was quantified using the following equations:

$$\Delta A_{412} = A_0 - A$$

$$C_{\text{allicin}} (\text{mmol/mL}) = (\Delta A_{412} \times \beta) / (2 \times 14,150)$$

where,

- $\beta$  = The dilution of L-Cysteine
- 14,150 = The molar Extinction coefficient (E) of allicin in water
- $A_0$  = The absorbance reading of water
- A = The absorbance reading of allicin

**HPLC method:** HPLC was performed using a Waters 616 pump and UV-1575/VIS absorbance detector. Compounds were separated on a 250×4 mm i.d.×5 μm LiChrospher 100 RP18 (C18) column (Hewlett-Packard). The mobile phase consisted of methanol-water (70:30, v/v). DAS, DAT, DADS and DATS were used as standards. HPLC was performed at ambient temperature at a flow rate of 0.8 mL/min. Allicin in the eluent was detected at 202 and 210 nm. Allicin was quantified using the following equation:

$$\text{Allicin (\%, w/w)} = \frac{C \times FV \times D \times 100\%}{W (1000 \mu \text{ g/mg})}$$

where,

- C = The allicin concentration (μg/mL) obtained from the standard curve
- FV = The final sample volume (mL)
- D = The dilution of the sample
- W = The sample weight (mg)

**GC method:** GC was performed using an Agilent GC-6890 system equipped with a flame ionization detector. Air and H<sub>2</sub> flow rates were 350 and 50 mL/min, respectively. The flow rate of the carrier gas (N<sub>2</sub>) was set to 1.0 mL/min. The temperature of the injection port and detector were set to 160 and 180°C, respectively. The oven temperature was set to 50°C for 3 min, increased to 160°C at a rate of 10°C/min and held at this temperature for 3 min and finally increased to 230°C at a rate of 30°C/min and held at this temperature for 3 min. The injection volume was 1 μL (splitless mode). A capillary column (SPD-1 fused-silica capillary; 30 m×0.53 mm I.D., 0.01 μm; Supelco, Bellefonte, PA, USA) was used. Allicin was quantified using the following equations:

$$\text{Allicin (\%, w/w)} = \frac{\rho \times A_1 \times V \times V_2 \times 100\%}{mA_0 V_1 \times (10000 \mu \text{ g/mg})}$$

where,

- $\rho$  = The allicin concentration in the standard (μg/mL)
- $A_1$  = The allicin peak area of the standard
- V = The final sample volume (mL)
- $V_1$  = The extraction volume of the sample (mL)
- $V_2$  = The allicin volume in the standard (mL)
- m = The sample weight (g)

**Statistical analyses:** Data analyses were performed using SPSS software. Simple statistics (i.e., mean, maximum and minimum values, standard deviations and coefficient of variation) were used to compare the three different methods. Analysis of variance (ANOVA) and Duncan's multiple range tests were performed. Statistical significance was set at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

Allicin is not available in the market because of its instability. DADS, DATS, DAS, DAT and allyl methyl disulfide are the main degradation products of allicin and allyl methyl thiosulfinates (Vernin *et al.*, 1986; Lawson *et al.*, 1991). In this study, we used DADS, DATS, DAS and DAT as standards. Unlike the HPLC and GC methods, the spectrophotometry method did not require the use of standards. In the GC method (Fig. 3), the standards were separated and identified by three discrete peaks: DAS with a retention time of 3.549 min,

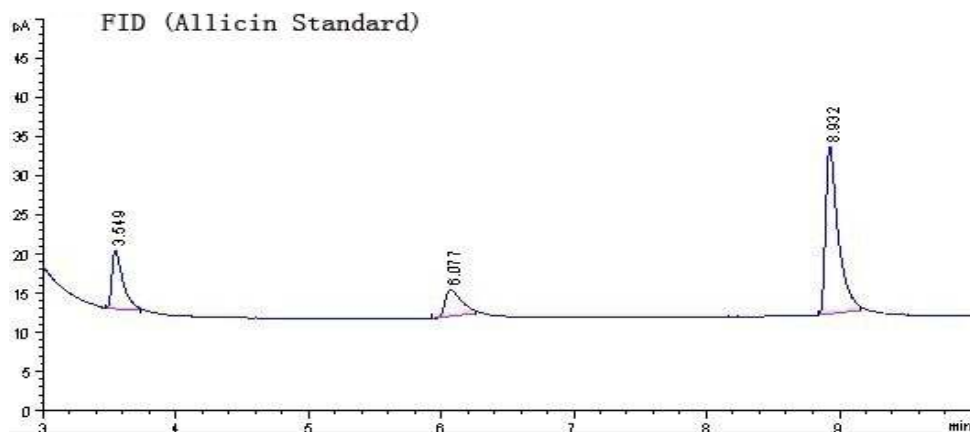
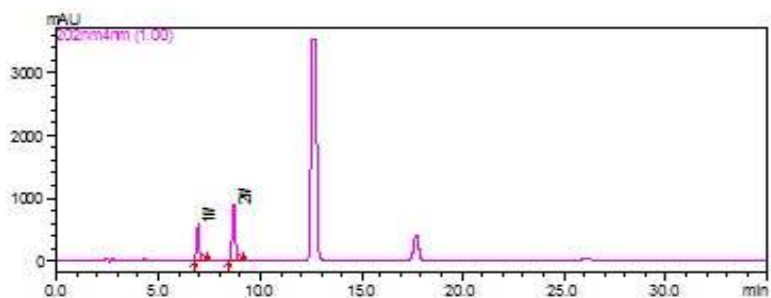
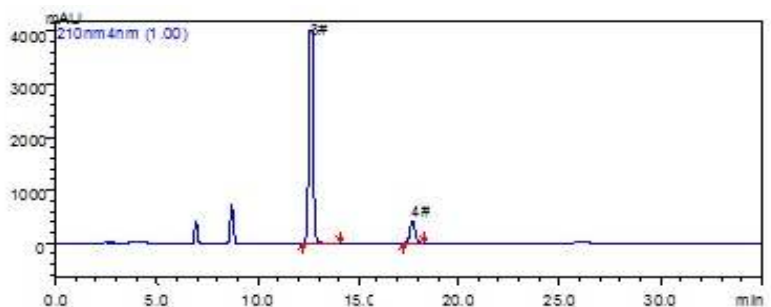


Fig. 3: GC chromatogram of standards  
 DAS: Retention time of 3.549 min; DADS: Retention time of 6.077 min; DATS: Retention time of 8.932 min



(a)



(b)

Fig. 4: HPLC chromatogram of standards. DAS (peak 1; retention time of 6.941 min) and DAT (peak 2; retention time of 8.706 min); (a): were detected at 202 nm. DADS (peak 3; retention time of 12.634 min) and DATS (peak 4; retention time of 17.794 min); (b): were detected at 210 nm

DADS with a retention time of 6.077 min and DATS with a retention time of 8.932 min. However, a DAT peak was not observed. On the other hand, in the HPLC method, all four standards were separated and identified by four distinct peaks (Fig. 4a and b). DAS (retention time of 6.941 min) and DAT (retention time of 8.706 min) had maximum absorbance readings at 202 nm; DADS (retention time of 12.634 min) and DATS (retention time of 17.794 min) had maximum absorbance readings at 210 nm (Fig. 5). The results revealed that the HPLC method was more sensitive than

the GC method; the lowest detection limit of HPLC was lower than that of GC. The average Coefficient of Variation (CV) of the spectrophotometric, GC and HPLC method was 3.44, 8.10 and 4.68, respectively (Table 2). When  $5\% < CV < 10\%$ , the precision of the instrument is adequate (Osman and Chin, 2006). Based on the results, the spectrophotometric and HPLC methods were more precise than the GC method.

The allicin concentration of the garlic-based products was determined by the three methods. In the spectrophotometric method, allicin concentration in the

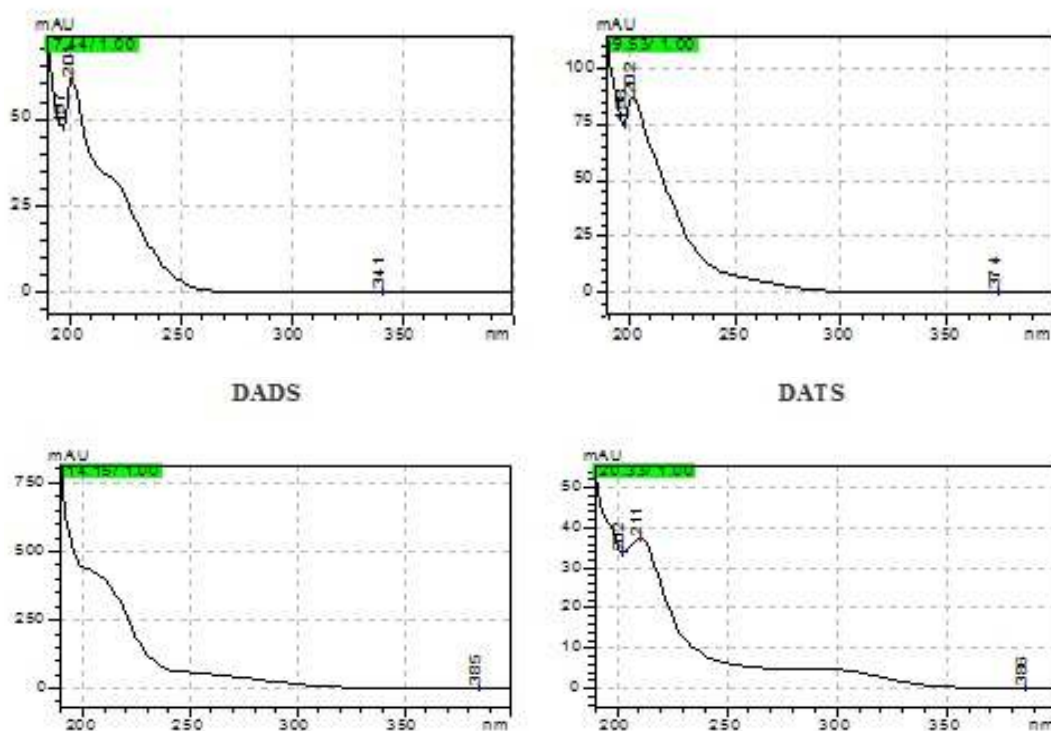


Fig. 5: Ultraviolet spectra of standards. DAS and DAT were detected at 202 nm, DADS and DATS were detected at 210 nm

Table 2: Allicin concentration in the eight garlic products

Allicin (%)	Sp	CV	GC	CV	HPLC	CV
Gp1	61.150	4.82	59.40	8.99	76.39	5.24
Gp2	0.410	3.97	0.36	7.67	0.39	3.80
Gp3	0.480	4.76	0.46	7.94	0.47	6.48
Gp4	0.430	2.46	0.41	6.84	0.43	7.24
Gp5	0.619	4.28	0.56	10.55	0.61	4.16
Gp6	0.570	4.79	0.54	6.23	0.55	2.08
Gp7	0.810	1.06	0.67	12.34	0.68	3.17
Gp8	0.610	1.41	0.58	4.25	0.60	5.25

CV: Coefficient of variation; Sp: Spectrophotometric

products was 0.41-61.15%. Based on the spectrophotometric method, garlic oil contained the highest allicin concentration (61.15%) followed by the garlic tablets (0.65%) and garlic powder (0.44%). According to the GC method, garlic oil had the highest allicin concentration (59.41%); garlic tablets and garlic powder contained 0.59 and 0.41% allicin, respectively. According to the HPLC method, garlic oil contained the highest allicin concentration (76.39%) followed by garlic tablets (0.61%) and garlic powder (0.43%). Regardless of the quantitative method, allicin concentration was significantly different between garlic oil and garlic powder and between garlic oil and garlic tablets. However, there were no significant differences in allicin concentration between garlic powder and garlic tablets ( $p > 0.05$ ). Garlic oil had an allicin concentration of 76.39% based on the HPLC method and of 59.41% based on the GC method. The allicin concentrations in the garlic tablets and powder were higher by the spectrophotometric method than by the GC method (Fig. 6). The spectrophotometric method is simple and provided similar results to those obtained

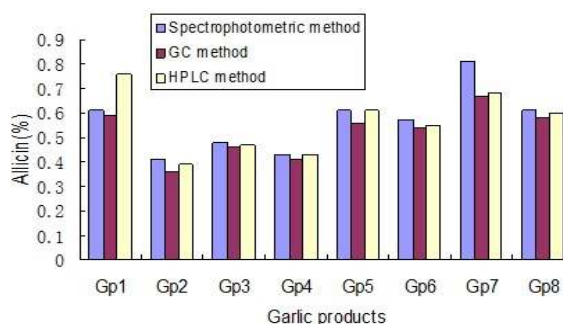


Fig. 6: Allicin concentration in the garlic products based the spectrophotometric, CG and HPLC methods. In Gp1, allicin concentration was multiplied by 100

from the HPLC method. Unlike the GC and HPLC methods, the spectrophotometric method did not require allicin standards for the quantitation of allicin because this method is based on the quantitation of cysteine using the established DTNB/NTB system (Li and Xu, 2007). Compared to the GC and HPLC methods, the

spectrophotometric method was simpler, easier and more efficient. The spectrophotometric method is based on the formation of disulfide compounds from the reaction between sulfhydryl-containing compounds and activated disulfide bonds of thiosulfonates. Therefore, the spectrophotometric method can only provide the concentration of total allicin and not of specific-sulfur containing compounds (Han *et al.*, 1995; Miron *et al.*, 2002). However, the GC and HPLC methods can provide the concentration of allicin and of specific-sulfur containing compounds (Table 3 and 4). The results revealed that the GC method had the lowest efficiency for the quantitation of allicin in garlic-based products. A comparison between the HPLC and GC methods revealed that garlic oil might have been distilled and that the compounds were degraded at high temperature. The allicin concentration in garlic oil by the HPLC method was higher than that by the GC method. Regardless of the method (GC or HPLC), DADS and DATS were the major allicin compounds in the garlic-based products especially in the garlic tablets and garlic powder. On the other hand, the concentrations of DAS and DAT were insignificant (Table 4).

The results revealed that, while allicin can be detected by GC, most of the compound was degraded during chromatography. Therefore, the HPLC method is more sensitive and accurate for the analysis of sulfur-containing compounds in garlic-based products. The HPLC chromatogram was characterized by four peaks with the characteristic UV maxima of DAS, DAT, DADS and DATS. The HPLC chromatograms of garlic oil (Gp1), garlic powder (Gp3) and garlic tablets (Gp7) are shown in Fig. 7a to f. According to the HPLC results, garlic oil consisted of 43.12% DADS, 17.58% DATS, 14.22% DAT and 1.47% DAS (Table 3). A typical commercial preparation of garlic oil contains 26% DADS, 19% DATS, 15% allyl methyl trisulfide and 13% allyl methyl disulfide (Lawson, 1998). Garlic oil from Jinxiang County contained a higher concentration of allicin than commercial garlic oil. According to the British Pharmacopoeia, the minimum allicin concentration required to ensure its pharmaceutical and economical viability is 0.45% in garlic powder (Baghalian *et al.*, 2005). The total allicin concentration in the garlic-based products was higher than required for pharmaceutical purposes, with the exception of Gp2 and Gp4 garlic powder.

Table 3: DAS, DAT, DADS and DATS concentration by the GC method

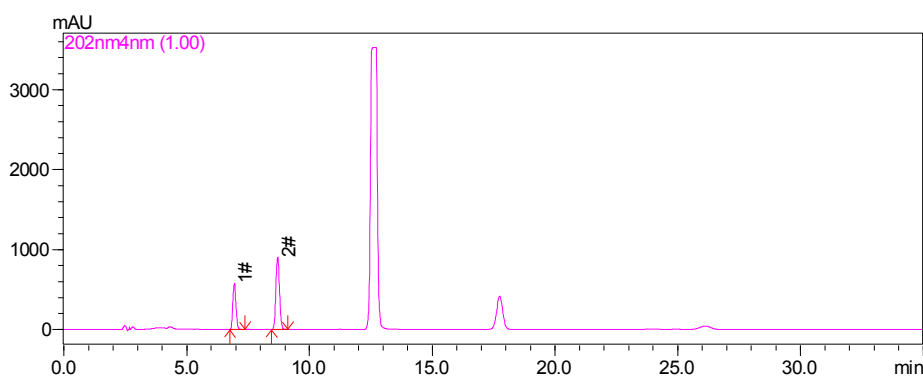
Sample	DAS	DAT	DADS	DATS	TAC (%)
Gp1	5.82	0	11.41	42.20	59.41±0.39
Gp2	0	0	0.16	0.20	0.36±1.20
Gp3	0	0	0.18	0.28	0.46±0.06
Gp4	0	0	0.15	0.26	0.41±0.48
Gp5	0	0	0.27	0.29	0.56±0.97
Gp6	0	0	0.30	0.24	0.54±0.30
Gp7	0	0	0.35	0.32	0.67±0.27
Gp8	0	0	0.31	0.27	0.58±0.48

TAC: Total allicin concentration

Table 4: DAS, DAT, DADS and DATS concentration by HPLC method

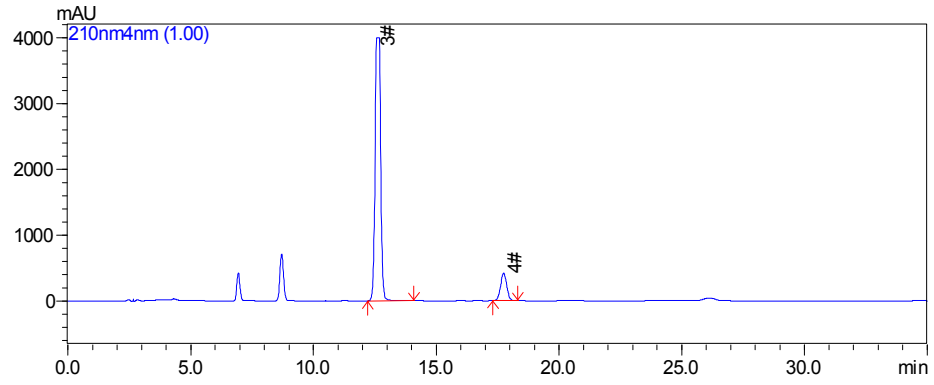
Sample	DAS	DAT	DADS	DATS	TAC (%)
Gp1	1.470	14.220	43.120	17.580	76.39±0.12
Gp2	0.037	0.017	0.270	0.066	0.39±1.01
Gp3	0.047	0.038	0.290	0.095	0.47±0.28
Gp4	0.049	0.032	0.265	0.084	0.43±0.23
Gp5	0.053	0.029	0.445	0.083	0.61±0.52
Gp6	0.069	0.030	0.368	0.083	0.55±2.98
Gp7	0.076	0.030	0.477	0.097	0.68±0.34
Gp8	0.036	0.021	0.436	0.107	0.60±0.16

TAC: Total allicin concentration

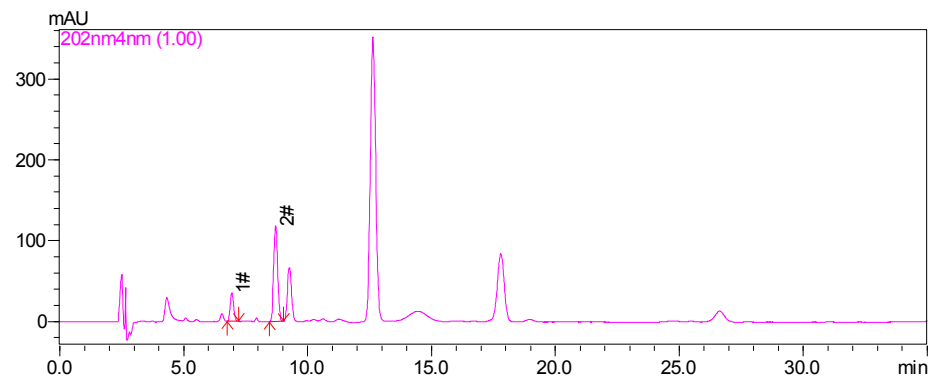


(a)

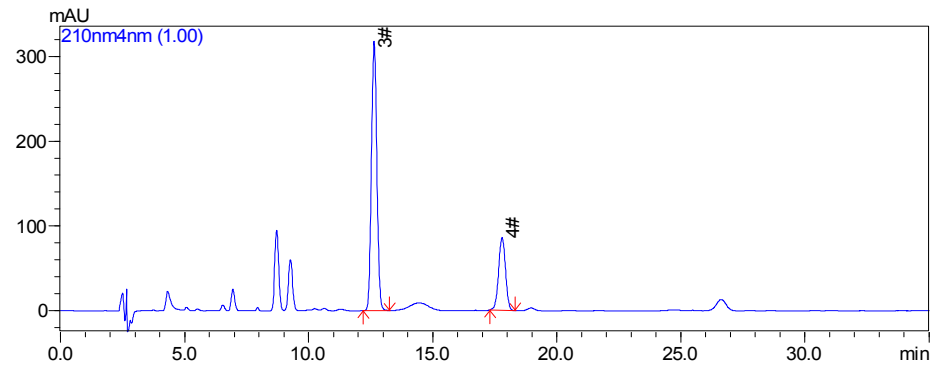




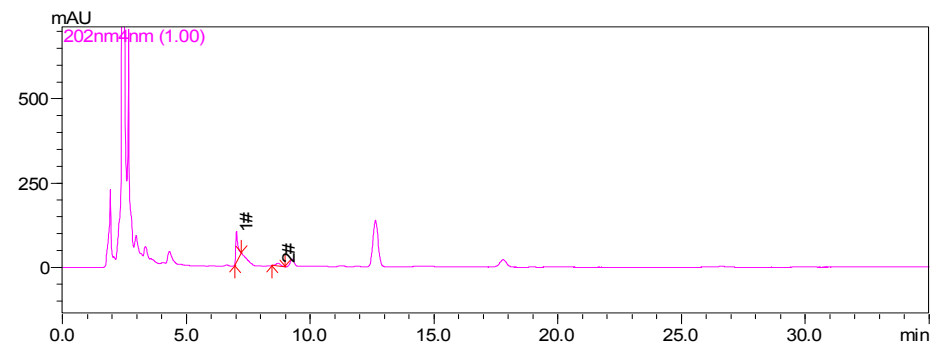
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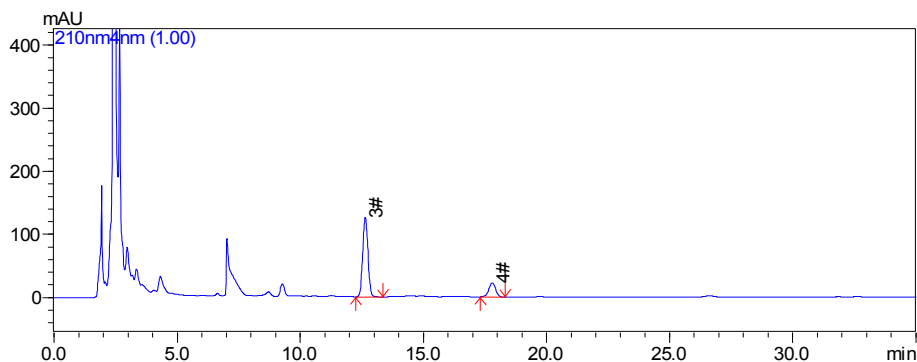
(c)



(d)



(e)



(f)

Fig. 7: HPLC chromatogram of samples; (a): HPLC chromatogram of DAS (peak 1) and DAT (peak 2); These compounds were detected at 202 nm in garlic oil (Gp1); (b): HPLC chromatogram of DADS (peak 3) and DATS (peak 4); These compounds were detected at 210 nm in garlic oil (Gp1); Dilution of garlic oil: 10,000; (c): HPLC chromatogram of DAS (peak 1) and DAT (peak 2); These compounds were detected at 202 nm in garlic powder (Gp3); (d): HPLC chromatogram of DADS (peak 3) and DATS (peak 4); These compounds were detected at 210 nm in garlic powder (Gp3); (e): HPLC chromatogram of DAS (peak 1) and DAT (peak 2); These compounds were detected at 202 nm in garlic tablets (Gp7); (f): HPLC chromatogram of DADS (peak 3) and DATS (peak 4); These compounds were detected at 210 nm in garlic oil (Gp7)

The spectrophotometric method was efficient to measure total allicin concentration. The low costs associated with this quantitation method make it useful to assess the pharmaceutical properties of garlic-based products. For component analysis and international trade, the HPLC method is more adequate. Therefore, the spectrophotometric method can be used to measure total allicin concentration, which is an important index of garlic quality. However, the HPLC method is more applicable for the analysis of allicin. Consequently, laboratories that do not have HPLC instruments can assess allicin concentration in garlic and garlic-based products by the spectrophotometric method.

### CONCLUSION

The results of this study revealed that the GC method was not suitable for the quantitation of allicin, whereas the spectrophotometric method was simpler and suitable for solid garlic products. The HPLC method was more accurate for the quantitation of allicin in all garlic-based products, especially liquid garlic-based products. Regardless of the quantitative method, it could be noticed that allicin content in garlic oil was higher than that of garlic tablets and garlic powder. Based on the spectrophotometric and HPLC method, garlic oil contained the highest allicin concentration (61.15 and 76.39%) followed by the garlic tablets (0.65 and 0.61%) and garlic powder (0.44 and 0.43%), respectively.

### ABBREVIATIONS

GC : Gas chromatography  
 HPLC : High pressure liquid chromatography  
 CV : Coefficient of variation

DAS : Diallyl sulfide  
 DAT : Dimethyl trisulfide  
 DADS : Diallyl disulfide  
 DATS : Diallyl trisulfide

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