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Research Article

QUALITATIVE ANALYSIS OF VARIOUS COMPONENTS OF *ALLIUM SATIVUM* (FRESH GARLIC BULB), EXTRACTED IN DIFFERENT ORGANIC SOLVENTS USING GC- HIGH RESOLUTION MASS SPECTROPHOTOMETER (JMS-700).

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Abstract

The work was planned for the precise determination of the mass to charge ratio of various components of *Allium sativum*, since it is known to be very important source of different phytonutrients used for treatment of various diseases, such as diabetes, cardiovascular problem and other disorders. In this studies Chromatographic data were generated and detected them by high resolution mass spectrophotometer, mass to charge ratio were taken in to consideration to produce better picture of its components from high resolution mass determination of its components such as Allicin, Allin and other components. Five different solvents of Analytical grades were used, in order to find the precise molecular mass of the components. Isolation and quantification of sulfur compounds from garlic extract was extensively studied, we have focus our attention towards qualitative analysis of various sulfur compounds from different garlic extracts. The purpose of study was to make sure the impact of various solvents on extraction of garlic's components in order to get the appropriate mass fragments of each compound extracted and obtained during analytical process. Generally it was observed in previous research on *Allium sativum*; the components of it were extracted using absolute ethanol, or 80% (Aq) ethanol[1]. In present studies fresh bulb of garlic bought from the local market of Riyadh Saudi Arabia, Garlic were chopped and dissolved immediately in various organic solvent, of different polarities such as Chloroform, Ethanol (96%), Ethyl Acetate, Hexane and methanol,to see the variation of components on extraction, which may result to produce the appropriate High resolution mass spectra .All extracts were run through GC-HRMS, the instruments used in these studies were of Agilent Technologies GC 7890A, and MStationJeol JMS-700, using GC Column HP-5 30mx ID0.320mm x 0.25µm film, These instruments are highly sophisticated in term of better separation and fine resolution and produces mass with high precision.

Keywords: *Allium sativum* (Garlic), GC-HRMS, JMS-700, organic solvents Allin and Allicin,

Introduction

Allium sativum (Garlic) had well known medicinal importance since centuries, it is being using for various treatment of human diseases, it has an antibiotic affect against various pathogens such as, streptococcus and staphylococcus bacteria etc.[2], it is also known for the treatment of hysteria and epilepsy and other medical conditions, but most widely known effect on cardio vascular system which reduce the parameters of the risk of atherosclerosis: total cholesterol, LDL, triglycerides, oxidized LDL[3].Allin is chemically (S)-3-(2-Propenylsulfinyl) - L-alanine but other synonyms also used, it is the one of the component Allium Sativum which converted to the Allicin by an enzyme Allinase[4].Since it was qualitative analysis so selection of different solvent was chosen on the basis of their different polar nature, some are non- polar and others are having of variable polarity. In this studies concept was to separate and find out molecular ion peak of the components of garlic, using highly sophisticated analytical technique of high resolution mass Spectrophotometer JMS-700 (Jeol)coupled with Agilent GC 7890A, different parameters were used to optimize

the instrument and identify the molecular mass of the Components of Allium Sativum. Previously these components of Allium Sativum also had been studied using liquidChromatographic and GC-MS methods [5,6,10].As far as the components of garlic are concern studies showed that a total of 16 non proteins organosulphur compoundshave been found in whole cloves and 23 in crushed cloves[7]. Variousresearchers have studies garlic fresh bulb from differentprospectof, the conversion ofcomponents byenzymes and also the impact on some components of garlic during cooking process[8].Theinstability of garlic (Allium sativum L.)-derivedallyl 2-propenylthiosulfinate (Allicin m/z 162) in various aqueous and ethanolic solutions as well as in vegetable oil through chemical and biological analyses performed simultaneously was already studied[9]. It has been reported earlier that two sulfur cyclic compound 3-vinyl-4H-1,2-dithiin (m/z 144) and 2-vinyl-4H-1,3-dithiin (m/z 144) were found in extract of garlic[10]. It is also studied earlier that Allinase is the trigger factor which convert the Allin in to Allicin[11].In this studies different organic solvent were used to find out the better response of different compounds of Allium Sativum such as S-Allyl,Prop-2-ene,1-Sulfothioate(m/z 162) , 1,2-Diallyl disulfane (m/z146),1,3-diallyltrisulfane (m/z 178), 1-allyl,3-methyltrisulfane (m/z 152) and others by using High resolution capillary column and high resolution Mass spectrophotometer. It's been well known that GC-MS has immense value in the study of compound of moderate stability such as those found in allium Sativum i.e. thiosulfinates from garlic as postulated (Block et.al.1993).

Experimental

5g of fresh garlic bulb were taken (bought from Local Store at Riyadh Saudi Arabia) and chopped them individually for each sample prepared, and transfers in to separate 100ml volumetric flask immediately. Five different organic solvents of both polar and non- polar in nature were used, viz: Chloroform, Ethanol, Ethyl Acetate, Hexane andmethanol, all were of analytical grade. Transferred 60ml of each solvent separately in to the respective labeled 100ml volumetric flask and soaked them for five days shook them intermittently, than solvents were filled up to the mark in to respective volumetric flask, shook well and filtered the solution using 0.45µm HVLP filter of Millipore. Analysis was done with GC-HRMS, high resolution mode keeping resolution at 5000, samples were analyzed at room temperature, with HRMS using EI ion source. Agilent GC system 7890A used was equipped with HP-5 capillary column 30m x 320 µm ID and 0.25 µm film thickness and run with following GC program, initial temperature of 80 degree with rise of 15^0C per minutes to 280 degree ^{0}C and injector temperature was kept180^0C with split less, and flow was set to 3ml/min. Jeol JMS700 HRMS(High resolution mass spectrophotometer) was connected with Agilent GC system 7890, calibration of HRMS system was done using PFK (Perflourokerosene). EI mode was used for the measurement, by keeping the following instrumental conditions, HRMS was programmed with ionization energy of 70eV, keeping 250 degree Celsius of Ionization temperature. The range of scan was set from 50-500 m/z with MF linear mode, the full range scan time was set to 1.0 sec with appropriate detector setting and the solvent delay was set to 3 minutes.All samples were analyzed keeping high resolution of 5000

Results and Discussion

This section was taken in consideration from two prospective, first discussion from chromatographic point of view, secondly from the prospect of High resolution mass spectrometric data. Chromatographic data reveals that multiple compounds were extracted through all five solvents,Chloroform, Ethanol, Ethyl Acetate, Hexane and Methanol,The extraction of compounds varies solvent to solvent, However two common compounds extracted and detected in all five solvents were 1,2-Diallyledisulfane (m/z146.02,) and 1,3-Diallyltrisulfane (m/z 178). Two compounds of Allium sativum 2-vinyl-4H-1,3-dithiin (m/z 144) and 3-Vinyl-4-H-1,2-dithiin (m/z 144), are isomers converted from Allicin(Block1985,1992).were extracted in Chloroform, Ethyl Acetate and hexane.1-allyl,3-methyl trisulfane (m/z152) extracted in ethanol and methanol. 1,4-Dimethyl tetrasulfane (m/z 157) was extracted in chloroform and hexane. whereas the major compound of Allium Sativum S-Allyl Prop-2-ene,1-Sulfinothioate(m/z 162 Allicin) was extracted in Ethanol and in Ethyl acetate. Table#1 showed the retention times of each compound eluted through GC with respect to m/z. From GC-HRMS prospects it was observed that previously no publication have been seen which deals with HRMS analysis of different solvents extracts of this type. Since the fragmentation obtained by this techniques may be different or less from those observed with other mass spectrophotometric methods, therefore identification of sulfur compounds are easy among mixture while performed analysis by GC-HRMS.

Since above discussed chromatographic data already mentioned the extraction of various sulfur compounds in different solvents(see table-1) so in high resolution mass spectrometric discussion fragments of each component were presents in below spectrums was discussed individually. The Mass spectra of Hexane extract showed a characteristic two peak of m/z 144 for 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin. The base peak of 2-vinyl-4H-1,3-dithiin in this spectra appears to be 72

Table. 1 Retention times of compounds with respect to their Molecular mass ion peaksin different solvents extracts run by GC-HRMS

	Retention time given in minutes					
m/z	**Name of Compounds**	$CHCl_3$	Ethanol	Methanol	Eth.Acet.	Hexane
144	**2-vinyl-4H-1,3-dithiin**	4.79	ND	ND	4.76	4.75
144	**3-vinyl-4H-1,2-dithiin**	4.01	ND	ND	5.00	4.98
146	**1,2-Diallylsulfane**	3.74	3.73	3.64	3.69	3.66
152	**1-allyl-3-methyltrisulfane**	ND	4.32	4.22	ND	ND
157	**1,4-Dimethyltetrasulfane**	7.40	ND	ND	ND	7.4
162	**S-Allyl, prop-2-ene,1-sulfinothioate**	ND	4.93	ND	5.63	ND
178	**1,3-Diallyltrisulfane**	6.02	5.81	5.77	5.78	6.01
			ND= Not detected			

Figure. 1 Mass spectra of Hexane extract

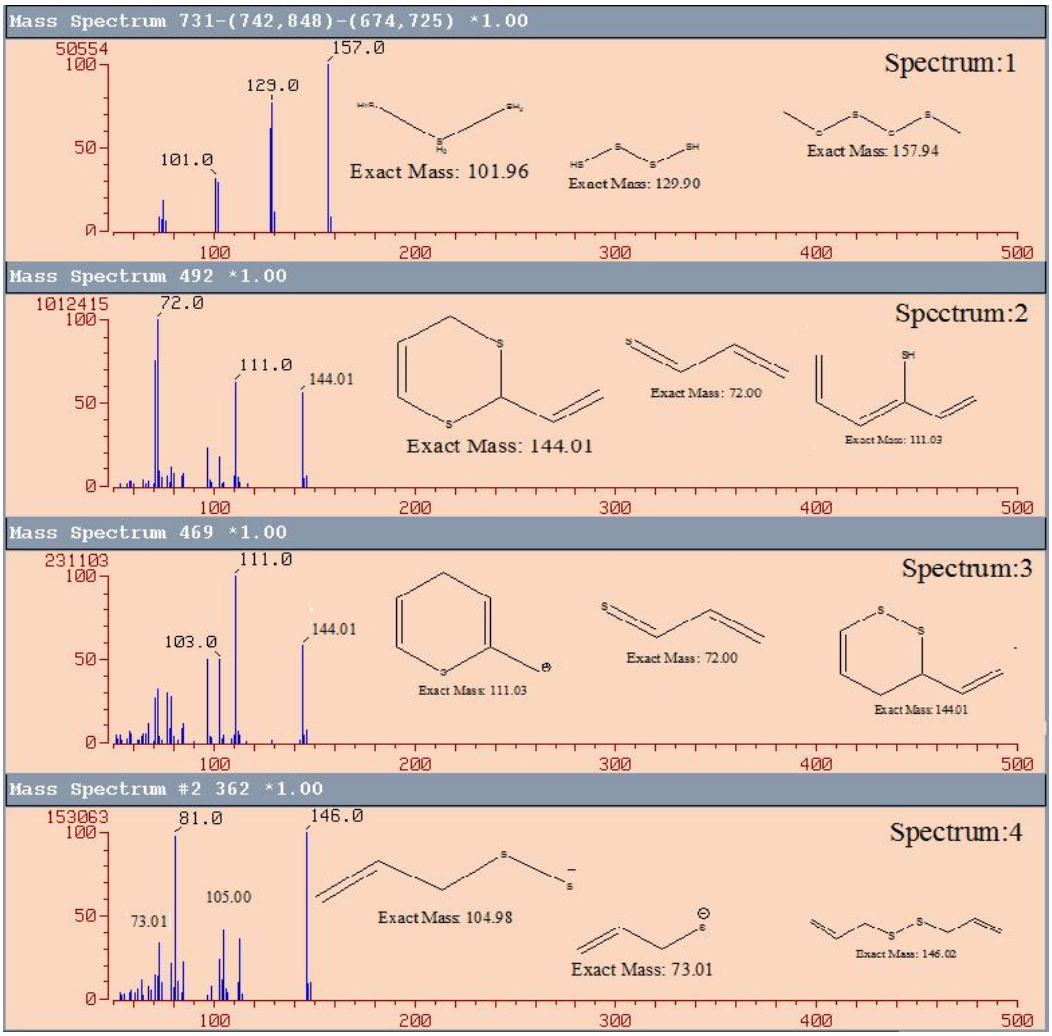
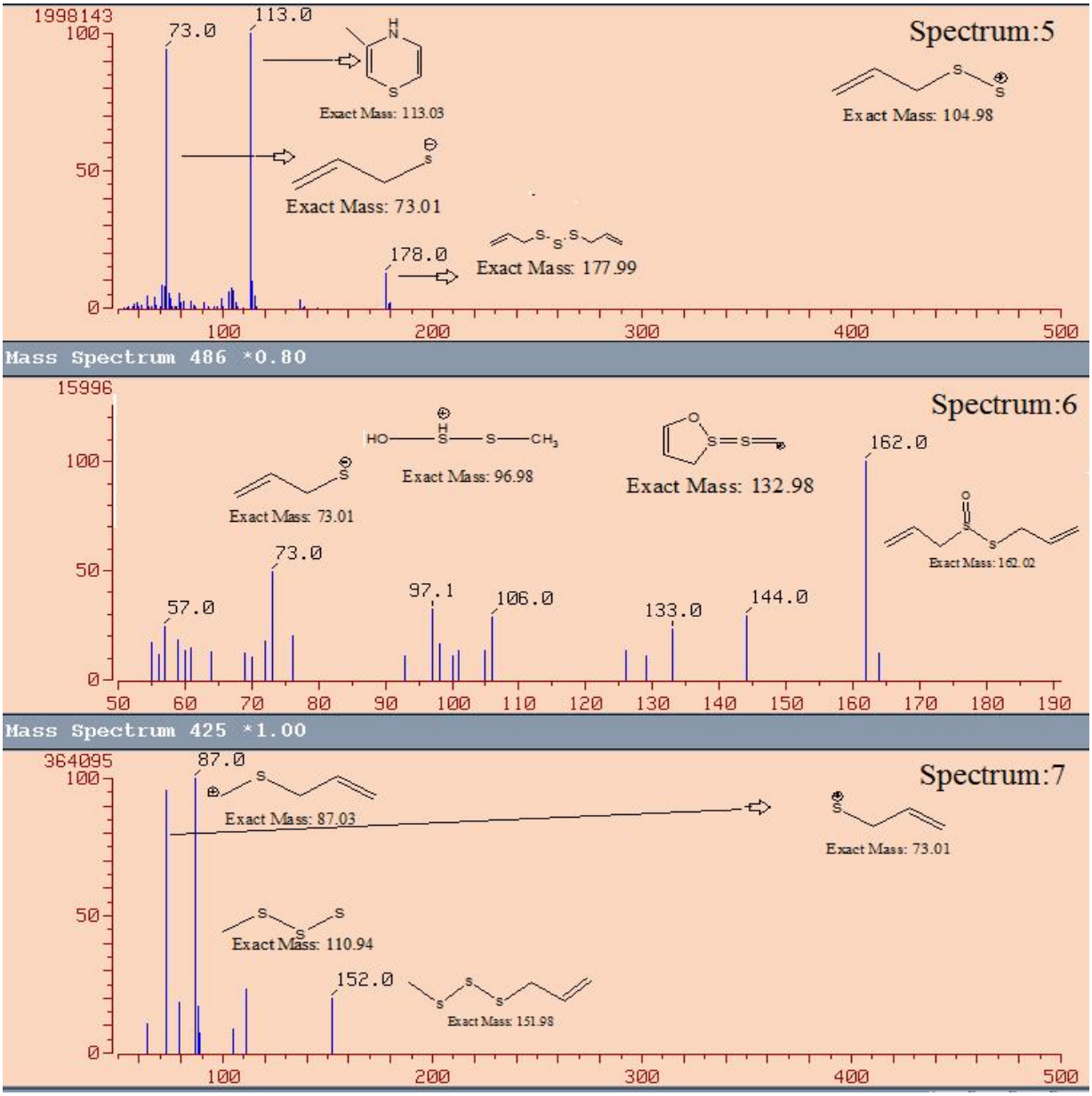


Figure. 2 analysis of MS spectrumsobtained from Hexane, Ethyl Acetate, Chloroform, Methanol and Ethanol extracts



[M-72] which is attributed to loss of propenethiol (H_2C=HC-CH=S) as shown in spectrum:2,whereas the base peak in 3-vinyl-4H-1,2-dithiin appears to be m/z 111 as shown in spectrum 3.

The analysis of MS spectrumsobtained from Hexane, Ethyl Acetate, Chloroform, Methanol and Ethanol extracts, the presence of some molecules are evident in all five extracts studied. 1,2- diallyldisulfane appear to be present in all five extracts with m/z peak 146 and concerted loss of $[C_5H_6]^+$produces(m/z81) confirm the presence of molecule, whereasallyldisulfane (m/z105),is also in accordance with the proposed molecule. see spectrum:4, Ethanol and methanolextract exhibit a peak of m/z 152, this peak corresponds to 1- allyl-3-methyltrisulfane. Fragmentation pattern also confirm the presence of this compound in which loss of allylsulfide (m/z 73)and trisulfane (m/z. 111.0) exhibits a strong peak See Spectrum: 7.Hexane and chloroform extracts shown a peak of m/z 157, which is assigned as 1,4-dimethyl tetra sulfane. Fragmentation pattern confirm the loss of tetrasulfane and trisulfane, which attributes to the peak m/z 129 and m/z 102see Spectrum: 1. Allicin appears to be present in ethanol and ethyl acetate extracts while Allin is found in all five extracts.

This can be explain by the fact that Allicin is unstable and decomposes to other strong smelling sulfur compounds. Allicin and Allin are confirmed by their characteristic fragmentationpattern due to presence of sulfur as its main component see spectrum: 5 and spectrum: 6 respectively, both these component had been discussed in detail previously[12]. Attached Mass spectrumshowed all the fragments discussed respectively.

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