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Attempt to Detect Garlic Allyl Sulphides from Saliva after Consumption of Garlic Tablets Using GC-MS

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Abstract

Allicin is an organosulfur compound of *Allium sativum* (garlic) with anti-fungal and antimicrobial properties. However, to date, neither allicin nor its metabolites has been detected in human organs or bodily fluids following oral consumption of garlic. Bioavailability has only been demonstrated using breath studies. Enteric-coated formulations are designed to pass through the acidic stomach environment; hence detection of garlic compounds in saliva following consumption of enteric-coated garlic tablets would provide evidence of bioavailability in a body secretion. An attempt was made to identify metabolites dially disulphide and allyl methyl sulphide using Gas Chromatography Mass Spectrometry (GC-MS). We completed a single participant mass spectrometry study using 13 time points over 24 hours following consumption of 20 enteric-coated garlic tablets. There was no detection of allicin derived sulfides at any time point. In summary, it is possible that these highly volatile compounds may be more readily detectable using a solid phase micro-extraction GC-MS, or headspace analysis methodology, although the less volatile allyl methyl sulfone and allyl methyl sulfoxide may be the main metabolites present.

Keywords: *Allium sativum*; GC-MS; Allicin bioavailability; Garlic; Diallyl disulphide; Allyl methyl sulphide

Abbreviations

DADS: Diallyl disulphide; DAS: Diallyl Sulphide; DATS: Diallyl Trisulphide; DMTS: Dimethyl Trisulphide AM: Allyl Mercaptan; AMDS: Allyl mMethyl Disulphide; AMS: Allyl Methyl Sulphide; AMSO: Allyl Methyl Sulfoxide; AMSO2: Allyl Methyl Sulfone; AMTS: Allyl Methyl Trisulphide; GC-MS: Gas Chromatography Mass Spectrometry

Introduction

Garlic produces volatile sulfur compounds, to which many anti-fungal and antimicrobial properties have been attributed. The authors were interested in the main volatile sulphur compound, allicin, and its potential as a systemic anti-fungal agent. However, the presence of allicin or its metabolites in human fluids has not been reported [1,2].

During the digestive process, allicin quickly metabolizes into other sulphur compounds [3-5]. Thus far, bioavailability has only been measurable by breath studies, which have detected the presence of metabolites AM, AMS and DADS after oral consumption [5,6]. Only traces of allicin were found in blood after being incubated for five minutes [7]. In another *in vivo* study, DADS was spiked into rat's blood and identified using GC-MS [8].

Previously in animals, organosulphur compounds from garlic have been detected using GCMS [9,10]. In rats, DADS, AM, AMSO and AMSO2 were found in stomach, liver, plasma and urine after consumption of 200 mg/kg of body weight. Time-dependent tissue concentrations of DADS were highest within 24 hours and not detectable by 72 hours. In plasma, DADS appeared transiently for 20 minutes then was undetectable. Only trace amounts of AMS were detectable in urine on Day 2, while AMSO and AMSO2 were excreted for a longer time periods, up to 48 hours [10]. Breath AMS has been used to measure bioavailability of garlic thiosulfinates [5].

In this study, an attempt was made to extract and detect allicin transformation products, AMS and DADS, from saliva following ingestion of enteric-coated garlic tablets.

Materials and Methods

Ethics approval was obtained from University of Melbourne Human Research Ethics Committee (ID 1033568.1).

Garlic allyl sulphide standards were purchased from MP Biomedicals Australia for validation and refinement of methods. 25 mg of each of the following standards was purchased: allyl disulphide (purity 91.2%; molecular weight 146.27 g/mol), allyl trisulphide (molecular weight 178.3 g/mol) and allyl methyl sulphide (molecular weight 88.17 g/mol). Standards were diluted in the concentration range of 0.50-50 µg/mL in hexane (w/v) for use as points for a linear standard curve, and to determine limits of detection. Additional testing was performed using steam-distilled garlic oil, diluted in hexane to 0.0005% v/v, as a source of a large variety of allyl sulphides.

Using an Agilent 7890a Gas Chromatograph coupled to a 5975C mass selective detector, cryogenic Gas Chromatography (GC) conditions were refined using standards and essential oils. Samples were separated using a 30 m Varian Factor Four VF-5 ms column with a 10m ezi-guard column attached. Column dimensions were 250 µm inner diameters with 0.25 µm film thickness. Oven ramp parameters used were -20°C held for 2 minutes, 10°C/min to 200°C, 35°C/min to

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300°C held for 1 minute. Total run time was 27.85 min. Scan range was 30-300 amu, scan rate 15.26 scans/sec. Ionisation voltage was 70eV.

A number of common ions were selected for the allicin transformation products, these were: m/z 41, 45, 73, 88, 111 and 144.

Allyl disulfide and diallyl trisulfide were tested using a concentration range of 0.50-50 µg/Ml (w/v) in hexane. Samples were run on the cryogenic SIM method [11] and areas quantified for the expected peak.

Garlicin™ tablets, each containing 350 mg garlic powder and yielding 3200 µg of allicin (label claim), with batch number 591678 (expiry date 10/2011), were purchased from an online distributor. This brand has been reported to give high allicin bioavailability [12]. As the time for AMS to reach maximum breath concentrations (tmax) varies from individual to individual [12], estimates were made for the sole participant of this study. Consumption of three or more Garlicin™ tablets commonly causes temporary abdominal discomfort upon disintegration. By recording the time delay between tablet consumption and abdominal discomfort or garlic breath/taste experienced in five tests, a mean tablet disintegration time of 5.5 hours was calculated. Following consumption of 20 Garlicin™ tablets at 0800, saliva samples were collected every hour for 12 hours, and a further sample at 0800h the following day. The samples were collected in 0.5 ml Eppindorf tubes and placed immediately in the freezer and stored at -80°C until processing. Samples were extracted into hexane at a ratio of 3:1 hexane: saliva to precipitate salivary proteins.

Results and Discussion

A chromatogram of steam-distilled garlic oil, containing a variety of allyl sulphides, is shown in Figure 1a. An example standard curve for allyl disulfide is shown in Figure 2. No allyl sulphides matching known standards were detected in the saliva at any of the time points (Figure 1b).

The method used had a sensitivity of 0.013 umol for AMS or 0.010 umol DADS mL^{-1} saliva. The detection of AMS or DADS would have been possible only if at least 10-50% of the consumed allicin had been metabolized to these compounds. Equal availability in saliva as in the total body water or blood would also have been necessary for detection of these compounds. Organosulphur compounds from garlic have never been detected in human organs or bodily fluids following oral consumption. Detection of garlic compounds in saliva following ingestion of enteric-coated tablets may, with methodological refinement, provide an alternative to breathe studies to prove bioavailability by enabling direct detection of organosulphur compounds in body fluids following consumption. In this small study, standards for allyl sulphides from *Allium sativum* were detected by GC-MS when injected alone and when spiked into saliva (not shown), which enabled full spectral identification of these compounds. However, the sensitivity of the method outlined was not sufficient for detection of physiological levels of allicin metabolites AMS and DADS in saliva after consumption of large amounts of garlic tablets. The presence of electrolytes in saliva may have affected the solubility of AMS. Measuring peroxidases in saliva [13] for kinetics of oxidation of AMS to AMSO and AMSO2 may have clarified breakdown products present. It is possible that these highly volatile compounds may be more readily detectable using a solid phase micro-extraction GC-MS, or headspace analysis methodology [14-16]. Nevertheless, it appears clear that AMS and DADS are not significantly found in saliva after consuming large amounts of garlic, although the presence of allicin metabolites AMSO and AMSO2 needs yet to be evaluated.

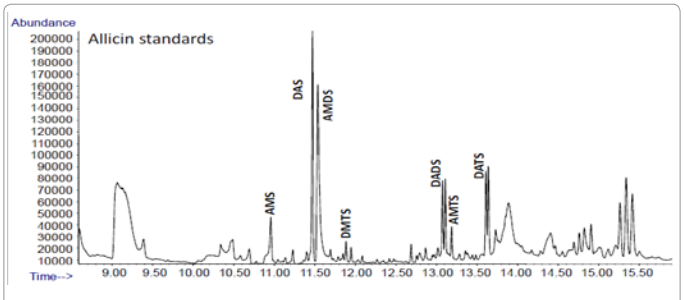

Figure 1a: Chromatogram of allicin transformation products from standards.

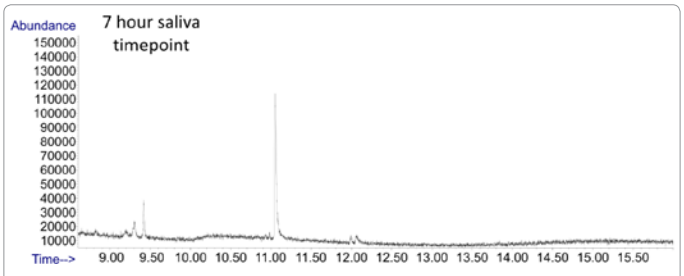

Figure 1b: Example chromatogram for 7 hour saliva sample post garlic tablet ingestion.

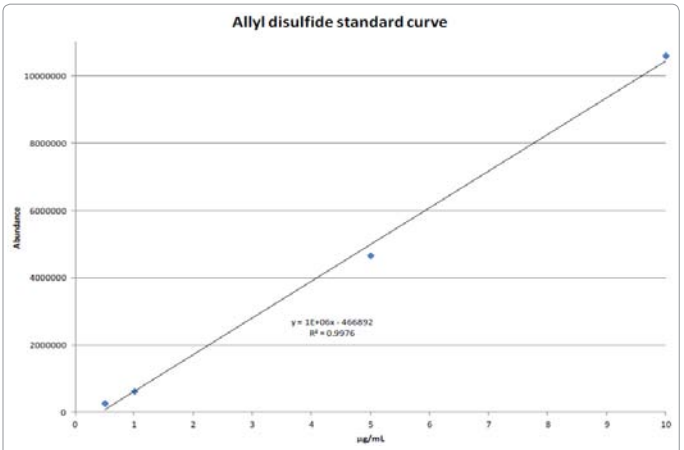

Figure 2: Standard curve for allyl disulfide.

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References

1. Caporaso N, Smith SM, Eng RH (1983) Antifungal activity in human urine and serum after ingestion of garlic (*Allium sativum*). Antimicrob Agents Chemother 23: 700-702.

2. Lawson LD, Ransom DK, Hughes BG (1992) Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial garlic products. Thromb Res 65: 141-156.

3. Taucher J, Hansel A, Jordan A, Lindinger W (1996) Analysis of compounds in human breath after ingestion of garlic using proton-transfer-reaction mass spectrometry. J Agric Food Chem 44: 3778-3782.

4. Lawson LD, Wang ZJ (1993) Pre-hepatic fate of the organosulfur compounds derived from garlic (*Allium sativum*). Planta Med 59: A688.

5. Minami T, Boku T, Inada K, Morita M, Okazaki Y (1989) Odour components of human breath after the ingestion of grated raw garlic. J Food Sci 54: 763-764.

6. Freeman F, Kodera Y (1995) Garlic chemistry-stability of *S*-(2-Propenyl) 2-Propene-1- Sulfinothioate (Allicin) in blood, solvents and simulated physiological fluids. J Agric Food Chem 43: 2332-2338.

7. Sun X, Guo T, He J, Zhao M, Yan M, et al. (2006) Determination of the concentration of diallyl trisulfide in rat whole blood using gas chromatography with electron-capture detection and identification of its major metabolite with gas chromatography mass spectrometry. Yakugaku Zasshi 126: 521-527.

8. Abu-Lafi S, Dembicki JW, Goldshlag P, Hanu A, LÌr O, et al. (2004) The use of the Cryogenicâ™ GC/MS and on-column injection for study of organosulfur compounds of the *Allium sativum.* J Food Comp Anal 17: 235-245.

9. Yu TH, Wu CM, Liou YC (1989) Volatile compounds from garlic. J Agric Food Chem 37: 725-730.

10. Germain E, Auger J, Ginies C, Siess MH, Teyssier C (2002) In vivo metabolism of diallyl disulphide in the rat: identification of two new metabolites. Xenobiotica 32: 1127-1138.

11. Patterson DG Jr, Hampton L, Lapeza CR Jr, Belser WT, Green V, et al. (1987) High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal Chem 59: 2000-2005.

12. Lawson LD, Gardner CD (2005) Composition, stability, and bioavailability of garlic products used in a clinical trial. J Agric Food Chem 53: 6254-6261.

13. Banerjee RK, Datta AG (1986) Salivary peroxidases. Mol Cell Biochem 70: 21-29.

14. Jung MJ, Shin YJ, Oh SY, Kim NS, Kim K, et al. (2006) Headspace hanging drop liquid phase microextraction and gas chromatography-mass spectrometry for the analysis of flavours from clove buds. Bull. Korean Chem Soc 27: 231-236.

15. Chin ST, Nazimah SAH, Quek SY, Man YBC, Rahman RA, et al. (2007) Analysis of volatile compounds from Malaysian durians (Durio zibethinus) using headspace SPME coupled to fast GC-MS. J. Food Compos Anal 20: 31-44.

16. Rosen RT, Hiserodt RD, Fukuda EK, Ruiz RJ, Zhou Z, et al. (2001) Determination of allicin, S-allylcysteine and volatile metabolites of garlic in breath, plasma or simulated gastric fluids. J Nutr 131: 968S-971S.