

## Note

# Characterization of Volatiles in Garlic and in Exhaled Breath after Garlic Ingestion by On-line Atmospheric Pressure Photoionization Quadrupole Time-of-Flight Mass Spectrometry

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**Through the use of on-line atmospheric pressure photoionization quadrupole time-of-flight mass spectrometry (APPI-QTOFMS), 32 volatiles from garlic were tentatively identified, and 9 metabolites were observed in exhaled breath after garlic ingestion. Methional and 3-methylthiopropionic acid, which may reflect the metabolism of methionine from garlic, were observed in garlic breath for the first time. Variation of metabolites was also monitored within 1 h to show the metabolic processes. The signal intensity of methional, 3-methylthiopropionic acid and *p*-cymene was increased within 35 min and then declined. Other 6 metabolites including allyl mercaptan, allyl methyl sulfide, diallyl sulfide, allyl methyl disulfide, diallyl disulfide and dimethyl selenide reached maxima shortly after ingestion of garlic and declined continuously. Our result indicates that APPI-QTOFMS is potential for investigation of food metabolism in dynamic via exhaled breath.**

Keywords: garlic breath, garlic volatiles, APPI-QTOFMS, metabolites

## Introduction

Garlic has been universally used as an important spice, function food and traditional medicine (Goncagul *et al.*, 2010; Milner, 1996). Ingestion of garlic would cause bad breath, and the odors in exhalation have been studied by many researchers to investigate the metabolic processes (Xiao Jia Cai *et al.*, 1995; Minami *et al.*, 2008; R. T. Rosen *et al.*, 2001; R. T. T. Rosen *et al.*, 2000; Tamaki *et al.*, 2008). According to previous studies, According to available studies, gas chromatography mass spectrometry (GC-MS) is the most commonly used analytical technique. Based on this technique, various components including sulfur-containing compounds and selenium-containing compounds were identified in exhaled breath after ingestion of garlic (Xiao Jia Cai *et al.*, 1995; Minami *et al.*,

2008; R. T. Rosen *et al.*, 2001; R. T. T. Rosen *et al.*, 2000). As an off-line method, GC-MS is often combined with pre-concentration techniques like solid-phase micro-extraction (SPME) to enhance the detection sensitivity, which is relatively time-consuming (Miekisch *et al.*, 2006).

On-line analytical techniques provide a rapid response and avoid sample preparation, which allows real-time measurements and thus enables the investigation of metabolic processes in dynamic. As a prominent on-line technique, proton transfer-reaction mass spectrometry (PTR-MS) has been used to investigate the time dependence of concentrations of 9 metabolites in exhaled breath after eating garlic (Taucher *et al.*, 1996). Munch *et al.* also described the concentration changes of 4 components in garlic

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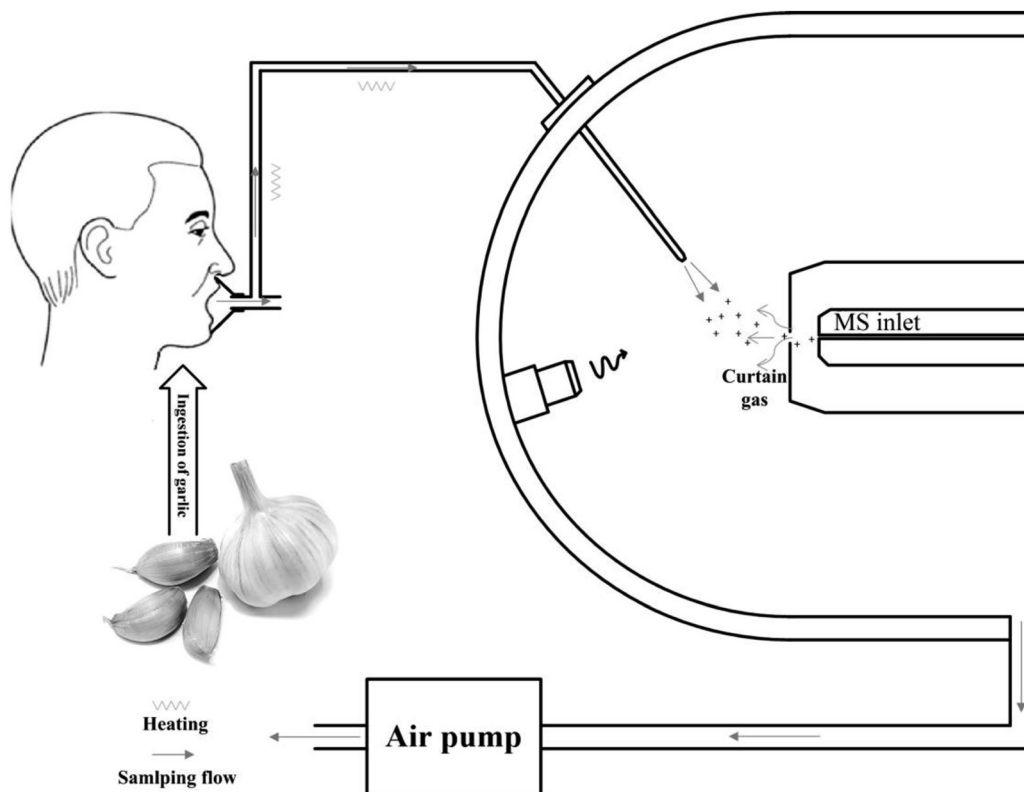


Fig. 1. Schematic diagram of APPI source for on-line analysis of garlic volatiles and garlic breath.

breath by selected ion flow tube-mass spectrometry (SIFT-MS) (Munch *et al.*, 2014). In Chen *et al.*'s study, extractive electrospray ionization mass spectrometry (EESI-MS) was used as an on-line method to identify compounds in garlic breath (Chen *et al.*, 2007), and the result showed that some nonpolar sulfur-containing species cannot be directly ionized by this method. APPI is a common used technique which also allows online analysis, and has never been used for garlic breath analysis. Compared with other techniques used in previous studies, APPI is more appropriate for nonpolar compounds and less susceptible to ionization suppression (Cai *et al.*, 2007). According to available studies, some metabolites observed in garlic breath like diallyl disulfide, allyl methyl disulfide, diallyl sulfide and allyl methyl sulfide are nonpolar sulfur-containing compounds (Chen *et al.*, 2007). Therefore, APPI is suitable for garlic breath analysis, and potential of finding some new metabolites.

In this work, a modified APPI ion source coupling with QTOFMS was used for on-line analysis of metabolites in exhaled breath after garlic ingestion. Volatiles from garlic were also identified as reference to get a better understanding of the processes. The aim of this study is to investigate the metabolism of some garlic components in human body via on-line breath analysis.

## Materials and Methods

**Apparatus** A modified APPI source was used combining with a quadrupole time-of-flight mass spectrometer (MicroTOF-QIII, Bruker Daltonics Inc., Fremont, CA, USA) for on-line MS

analysis. Nebulizer and solvent for spray were not used in ion source to avoid exogenous contaminants from reagents. As shown in Fig. 1, analytes were sucked into the source chamber through an 80 cm long, 1.5 mm inner-diameter PTFE tube with a sampling flow rate of 2 L/min. The PTFE tube was heated to a constant temperature of 100°C to avoid condensation which may lead to compound losses. Neutral molecules were ionized under UV irradiation produced by a Krypton filled vacuum UV lamp with the most intense excitation lines at 10.0/10.6 eV. Curtain gas flow was optimized at 3 L/min.

Mass spectra were recorded in the mass range from  $m/z$  50 to 500 in positive-ion mode with a resolution of 15000 at  $m/z$  200. To assist identification of compounds, collision induced dissociation (CID) analysis was performed with collision energy of  $20 \pm 10$  eV. Room temperature was maintained at 25°C to ensure the stability of mass spectrometer.

**Samples** The garlic was purchased at a local supermarket. A 26-year-old male in our research group volunteered to provide breath samples in this study. To minimize the influence of garlic ingestion from daily diet, the subject was fasted over 10 hours before breath test, and then asked to exhale through a disposable mouthpiece after eating a clove of raw garlic and gargling with 100 mL of water.

**Data processing** Mass spectra of exhaled breath were subtracted by room air spectrum as blank background. For compound identification, search parameters specified a MS tolerance of 6 mDa (~50 ppm), a MS/MS tolerance of 20 mDa, and

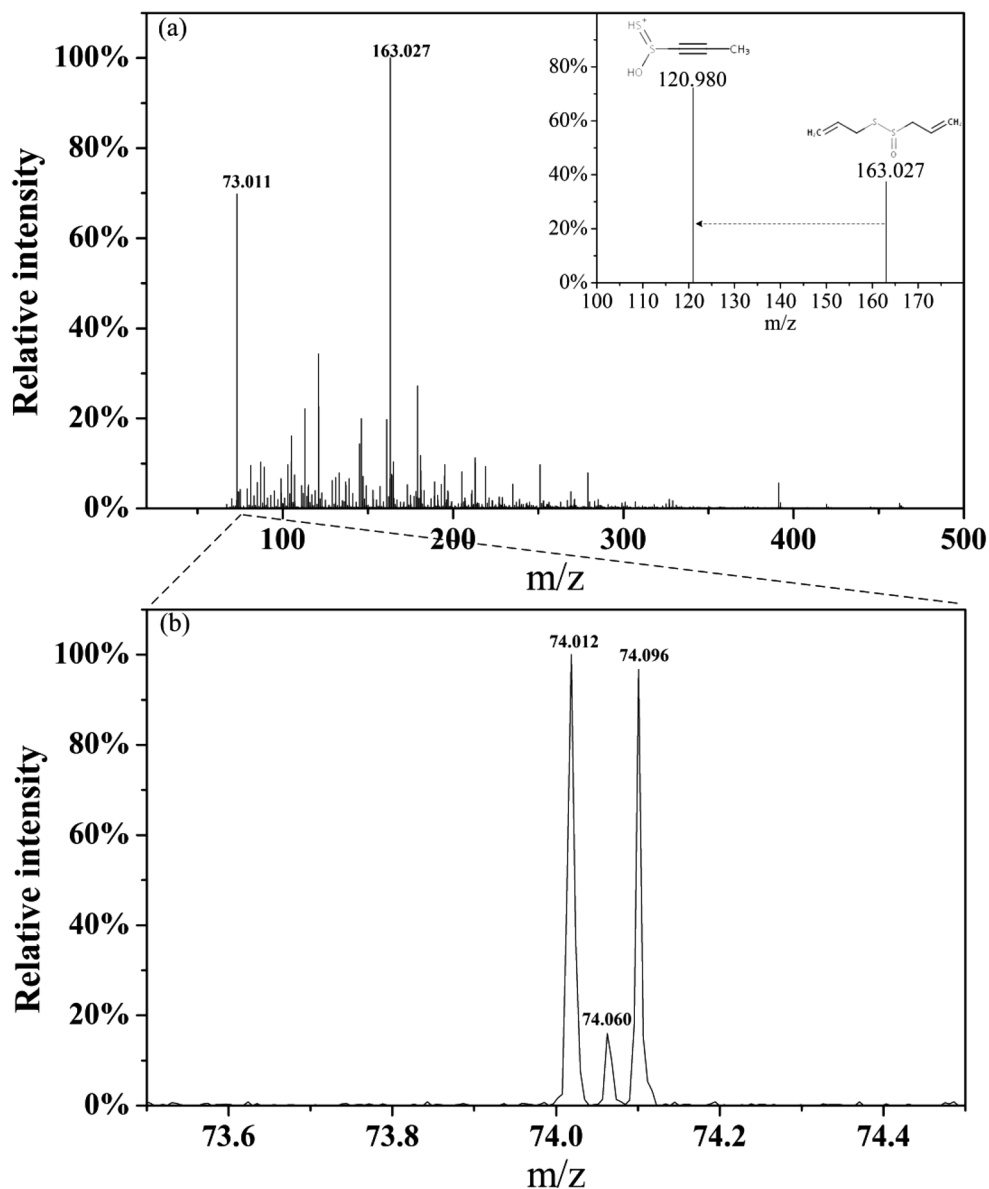


Fig. 2. (a) Typical spectrum of garlic headspace. (b) Partial enlargement of the spectrum.

a mSigma value tolerance of 50. The mSigma value is to show the deviation between the theoretical and the measured isotope pattern (Abu-Reidah *et al.*, 2015). And a lower mSigma value indicates that the measured isotope pattern is more similar to the theoretical isotopic pattern for the proposed ion formula. All raw spectra were acquired by micrOTOFcontrol software, and data analysis was processed in DataAnalysis 4.0 software (Bruker Datonics).

**Statistical analysis** Garlic breath test was repeated 10 times. The data were analyzed using one-way ANOVA followed by LSD test, and expressed as means  $\pm$  SE. Statistics were processed with SPSS 11.5 software.

## Results and Discussion

**Garlic volatiles analysis** Headspace volatiles of crushed garlic were analyzed and a typical spectrum was shown in Fig. 2(a). Within a mass range from  $m/z$  50 to 500, many signals were detected. Since on-line techniques sacrifice the high selectivity

without using chromatography, a high-resolution tandem mass spectrometer was employed to minimize this drawback. As is shown in Fig. 2(b), signals at  $m/z$  74.012, 74.060 and 74.096 can be clearly distinguished. These 3 compounds were assigned to isotopic peak of thioacrolein ( $m/z$  73.011), *N,N*-dimethylformamide and butylamine respectively.

Tentative assignments of these compounds were established basing on the exact formula, ion fragments from CID analysis, open access databases (FoodB database, METLIN Metabolomics Database, Human Metabolome Database, Nist Database) and reviewing of previous studies. For example, the signal at  $m/z$  163.027 with the formula of  $[C_6H_{11}OS_2]^+$  loses  $CH_3-CH=CH_2$  to yield fragments of  $[C_3H_5OS_2]^+$  (inset of Fig. 2(a)). This compound was identified as alliin, which is common in garlic. Through this approach, a total of 32 volatiles from garlic were established and listed in Table 1.

**Breath analysis after garlic ingestion** Breath analysis was

**Table 1.** List of 32 volatiles in headspace of crushed garlic.

| <i>m/z</i>             | $\Delta m/mDa$ | Formula  | mSigma | CID fragments   | Tentative assignments  | Reference reported                  |
|------------------------|----------------|--|--------|---|--|-------------------------------------|
| 73.011 <sup>a</sup>    | 1              | [C <sub>3</sub> H <sub>4</sub> S+H] <sup>+</sup>                               | 3.5    | 44.981[C <sub>2</sub> H <sub>3</sub> S] <sup>+</sup> ,<br>59.037[C <sub>2</sub> H <sub>3</sub> NO] <sup>+</sup> ,   | Thioacrolein   | (Tocmo <i>et al.</i> , 2015)        |
| 74.060                 | 0              | [C <sub>3</sub> H <sub>7</sub> NO+H] <sup>+</sup>                              | 14.9   | 58.031[C <sub>2</sub> H <sub>4</sub> NO] <sup>+</sup> ,<br>44.049[C <sub>2</sub> H <sub>6</sub> N] <sup>+</sup>   | <i>N,N</i> -Dimethylformamide  | -                                   |
| 74.096                 | 0              | [C <sub>4</sub> H <sub>11</sub> N+H] <sup>+</sup>                              | 3.1    | 57.070[C <sub>4</sub> H <sub>9</sub> ] <sup>+</sup>   | Butylamine   | -                                   |
| 75.028 <sup>a</sup>    | 2              | [C <sub>3</sub> H <sub>6</sub> S+H] <sup>+</sup>                               | 47.3   | 41.039[C <sub>3</sub> H <sub>5</sub> S] <sup>+</sup>  | Allyl mercaptan  | (Fanelli <i>et al.</i> , 1998)      |
| 79.055 <sup>a</sup>    | 1              | [C <sub>6</sub> H <sub>6</sub> +H] <sup>+</sup>                                | 24.8   | -   | Benzene  | (Keles <i>et al.</i> , 2014)        |
| 85.009 <sup>a</sup>    | -1             | [C <sub>4</sub> H <sub>4</sub> S+H] <sup>+</sup>                               | 42.7   | 58.996[C <sub>2</sub> H <sub>3</sub> S] <sup>+</sup>  | Thiophene  | (Keles <i>et al.</i> , 2014)        |
| 89.045 <sup>a,b</sup>  | 3              | [C <sub>4</sub> H <sub>8</sub> S+H] <sup>+</sup>                               | 49.5   | -   | Allyl methyl sulfide   | (Yu <i>et al.</i> , 1989)           |
| 91.025 <sup>a</sup>    | 4              | [C <sub>3</sub> H <sub>6</sub> OS+H] <sup>+</sup>                              | 28.4   | -   | 2-Propenesulphenic acid  | (Tocmo <i>et al.</i> , 2015)        |
| 97.066 <sup>a,b</sup>  | 2              | [C <sub>6</sub> H <sub>8</sub> O+H] <sup>+</sup>                               | 27.8   | -   | Dimethylfuran  | (Yu <i>et al.</i> , 1989)           |
| 99.028 <sup>a</sup>    | 2              | [C <sub>5</sub> H <sub>6</sub> S+H] <sup>+</sup>                               | 32.3   | 84.004[C <sub>4</sub> H <sub>4</sub> S] <sup>+</sup> ,<br>73.010[C <sub>3</sub> H <sub>5</sub> S] <sup>+</sup>  | Methylthiophene  | (Keles <i>et al.</i> , 2014)        |
| 104.985 <sup>a</sup>   | 2              | [C <sub>3</sub> H <sub>4</sub> S <sub>2</sub> +H] <sup>+</sup>                 | 49.9   | -   | 1,2-Dithiacyclopent-3-ene  | (Chyau <i>et al.</i> , 1999)        |
| 107.018                | 2              | [C <sub>3</sub> H <sub>6</sub> O <sub>2</sub> S+H] <sup>+</sup>                | 25.1   | 89.006[C <sub>3</sub> H <sub>5</sub> OS] <sup>+</sup>   | Mercaptopropanoic acid   | -                                   |
| 110.971 <sup>a,b</sup> | 0              | [C <sub>2</sub> H <sub>6</sub> Se+H] <sup>+</sup>                              | 45.1   | -   | Dimethyl selenide  | (Cai <i>et al.</i> , 1994)          |
| 111.028 <sup>a</sup>   | 2              | [C <sub>6</sub> H <sub>6</sub> S+H] <sup>+</sup>                               | 38.1   | 85.010[C <sub>4</sub> H <sub>5</sub> S] <sup>+</sup> ,<br>94.996[C <sub>3</sub> H <sub>3</sub> S] <sup>+</sup>  | 2-Vinylthiophene   | (Yu <i>et al.</i> , 1994)           |
| 113.044 <sup>a</sup>   | 2              | [C <sub>6</sub> H <sub>8</sub> S+H] <sup>+</sup>                               | 40.9   | 79.053[C <sub>6</sub> H <sub>7</sub> ] <sup>+</sup> ,<br>75.025[C <sub>3</sub> H <sub>7</sub> S] <sup>+</sup> ,<br>73.009[C <sub>3</sub> H <sub>5</sub> S] <sup>+</sup> ,<br>70.993[C <sub>3</sub> H <sub>3</sub> S] <sup>+</sup>   | 3-Methyl-2-cyclopentene-1-thione   | (Yu <i>et al.</i> , 1989)           |
| 115.059 <sup>a,b</sup> | 1              | [C <sub>6</sub> H <sub>10</sub> S+H] <sup>+</sup>                              | 18.6   | -   | Diallyl sulfide  | (Yu <i>et al.</i> , 1989)           |
| 121.014 <sup>a,b</sup> | 0              | [C <sub>4</sub> H <sub>8</sub> S <sub>2</sub> +H] <sup>+</sup>                 | -      | -   | Allyl methyl disulfide   | (Yu <i>et al.</i> , 1989)           |
| 129.041 <sup>a</sup>   | 4              | [C <sub>6</sub> H <sub>8</sub> OS+H] <sup>+</sup>                              | 36.8   | -   | 2-Methyl-5-(methylthio) furan  | (Andreatta <i>et al.</i> , 2005)    |
| 131.053                | 1              | [C <sub>6</sub> H <sub>10</sub> OS+H] <sup>+</sup>                             | 33.5   | 113.044[C <sub>6</sub> H <sub>9</sub> S] <sup>+</sup> ,<br>91.024[C <sub>3</sub> H <sub>7</sub> OS+H] <sup>+</sup> ,<br>73.012[C <sub>3</sub> H <sub>5</sub> S] <sup>+</sup> ,<br>109.012[C <sub>6</sub> H <sub>5</sub> S] <sup>+</sup> ,<br>65.040[C <sub>5</sub> H <sub>5</sub> S] <sup>+</sup>   | Allyl thiopropionate   | -                                   |
| 136.025 <sup>a</sup>   | 3              | [C <sub>7</sub> H <sub>5</sub> NS+H] <sup>+</sup>                              | -      | -   | Benzothiazole  | (Molina-Calle <i>et al.</i> , 2017) |
| 137.011 <sup>b</sup>   | 2              | [C <sub>4</sub> H <sub>8</sub> OS <sub>2</sub> +H] <sup>+</sup>                | 28.3   | -   | S-Methyl 2-propene-1-sulfinothioate, or S-2-Propenyl methanesulfinothioate | -                                   |
| 141.075 <sup>a,b</sup> | 2              | [C <sub>8</sub> H <sub>12</sub> S+H] <sup>+</sup>                              | 30.5   | -   | Dimethylthiophene  | (Keles <i>et al.</i> , 2014)        |
| 145.017 <sup>a,b</sup> | 3              | [C <sub>6</sub> H <sub>8</sub> S <sub>2</sub> +H] <sup>+</sup>                 | 28.3   | 111.023[C <sub>6</sub> H <sub>7</sub> S] <sup>+</sup> ,<br>104.979[C <sub>3</sub> H <sub>5</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>102.965[C <sub>3</sub> H <sub>3</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>99.026[C <sub>7</sub> H <sub>7</sub> S] <sup>+</sup> ,<br>118.995[C <sub>4</sub> H <sub>7</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>73.010[C <sub>3</sub> H <sub>5</sub> S] <sup>+</sup>  | 2-Vinyl-4H-1,3-dithiine  | (Yu <i>et al.</i> , 1989)           |
| 147.030 <sup>a,b</sup> | 0              | [C <sub>6</sub> H <sub>10</sub> S <sub>2</sub> +H] <sup>+</sup>                | 48.4   | -   | Diallyl Disulfide  | (Yu <i>et al.</i> , 1989)           |
| 157.075 <sup>c</sup>   | 7              | [C <sub>8</sub> H <sub>12</sub> OS+H] <sup>+</sup>                             | 30.0   | -   | 2-[(Isopropylthio)methyl] furan  | -                                   |
| 161.008                | -1             | [C <sub>6</sub> H <sub>8</sub> OS <sub>2</sub> +H] <sup>+</sup>                | 47.8   | 115.020[C <sub>3</sub> H <sub>7</sub> OS] <sup>+</sup> ,<br>113.003[C <sub>3</sub> H <sub>5</sub> OS] <sup>+</sup>  | 2-Methyl-3-(methylthio) furan, or (2-Furanyl)methyl methyl disulfide       | -                                   |
| 161.022 <sup>a</sup>   | 3              | [C <sub>6</sub> H <sub>8</sub> O <sub>3</sub> S+H] <sup>+</sup>                | 32.9   | -   | Thiacremonone  | (Kim <i>et al.</i> , 2012)          |
| 163.027 <sup>a</sup>   | 2              | [C <sub>6</sub> H <sub>10</sub> OS <sub>2</sub> +H] <sup>+</sup>               | 6.6    | 120.977[C <sub>3</sub> H <sub>5</sub> OS <sub>2</sub> ] <sup>+</sup> ,<br>161.007[C <sub>3</sub> H <sub>7</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>138.987[C <sub>3</sub> H <sub>7</sub> O <sub>2</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>137.974[C <sub>3</sub> H <sub>6</sub> O <sub>2</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>136.970[C <sub>3</sub> H <sub>5</sub> O <sub>2</sub> S <sub>2</sub> ] <sup>+</sup> ,<br>120.976[C <sub>3</sub> H <sub>5</sub> OS <sub>2</sub> ] <sup>+</sup> | Allicin  | (Yu <i>et al.</i> , 1989)           |
| 179.023 <sup>b</sup>   | 3              | [C <sub>6</sub> H <sub>10</sub> O <sub>2</sub> S <sub>2</sub> +H] <sup>+</sup> | 5.2    | -   | S-2-Propenyl 2-propene-1-sulfonylthioate                                   | -                                   |
| 211.014 <sup>c</sup>   | 5              | [C <sub>6</sub> H <sub>10</sub> O <sub>4</sub> S <sub>2</sub> +H] <sup>+</sup> | 43.5   | -   | Dimethipin   | -                                   |
| 235.022 <sup>a,b</sup> | -5             | [C <sub>9</sub> H <sub>14</sub> OS <sub>3</sub> +H] <sup>+</sup>               | 25.2   | -   | Ajoene   | (Yu <i>et al.</i> , 1989)           |
| 251.023 <sup>b</sup>   | 0              | [C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> S <sub>3</sub> +H] <sup>+</sup> | 10.9   | -   | 2-Propenyl 3-(2-propenylsulfonyl)-1-propenyl disulfide                     | -                                   |

a. The ion formula corresponding to a compound from garlic reported in previous references.

b. The ion formula corresponding to a compound from garlic found in FooDB database, Metlin database and HMDB database.

c. The ion formula corresponding to a unique compound found in FooDB database, Metlin database and HMDB database.

performed 5 min after ingestion of garlic. Characteristic compounds of garlic breath were analyzed by comparing exhaled breath after garlic ingestion to that before garlic ingestion. As is shown in Table 2, a total of 9 compounds were significantly increased in exhalation. To further investigate the metabolic processes, breath analysis was performed every 10 min within 1 h after consuming garlic. And signal intensity of 9 ions at different time points was monitored as shown in Fig. 3. Based on the variation of ion intensity with time, these 9 ions were divided into two distinctly different groups.

For the first group including  $m/z$  105.041,  $m/z$  121.038 and  $m/z$  135.110, the ion intensity was gradually increased within 35 min after eating garlic and then declined. The signal at  $m/z$  135.110 was assigned to *p*-cymene, which has been detected in previous study as a metabolite of garlic. This compound may be formed from the metabolism of glycosides or even amino acid conjugates in garlic (R. T. T. Rosen *et al.*, 2000). Signals at  $m/z$  105.041 and 121.038 were observed in exhaled breath for the first time as metabolites in garlic breath (Fig. 4). The signal at  $m/z$  105.041 with the formula of  $[C_4H_9OS]^+$ , which yielded fragments of  $[C_3H_6OS]^+$ ,  $[C_4H_8S]^+$ ,  $[C_2H_5S]^+$ ,  $[C_3H_5O]^+$  and  $[CH_3S]^+$ , was identified as methional that can be formed from methionine (Wainwright *et al.*, 1972). Lactic acid bacteria, which is common in digestive tract (Conway *et al.*, 1987; Sookkhee *et al.*, 2001), can also convert methionine to 4-methylthio-2-ketobutanoate and then metabolized to methional (Amárita *et al.*, 2001). The signal at  $m/z$  121.038 with fragments of  $[C_3H_5O_2S]^+$ ,  $[C_4H_7OS]^+$  and  $[C_3H_3OS]^+$  was assigned to 3-methylthiopropionic acid which is also a metabolite of methionine (Blom *et al.*, 1989). Methionine and its derivatives are common components in garlic (Allison *et al.*, 2006; X. J. Cai *et al.*, 1995). Therefore, these 2 compounds may reflect the metabolism of methionine from garlic.

The second group of ion signals including  $m/z$  75.028,  $m/z$  89.045,  $m/z$  110.971,  $m/z$  115.059,  $m/z$  121.014 and  $m/z$  147.030 gave a maximum intensity shortly after ingestion of garlic and declined continuously in the next 50 min. Signals at  $m/z$  75.028,  $m/z$  89.045,  $m/z$  115.059,  $m/z$  121.014 and  $m/z$  147.030 were assigned to allyl mercaptan, allyl methyl sulfide, diallyl sulfide, allyl methyl disulfide and diallyl disulfide respectively. These 5 sulfur-containing compounds were well-understood as metabolites in garlic breath. Available studies have revealed that allicin is metabolized in blood cells to allyl mercaptan, which is then metabolized by methyltransferase with S-adenosylmethionine to allyl methyl sulfide (R. T. Rosen *et al.*, 2001). Both allyl methyl sulfide and allyl methyl disulfide are also postulated to be formed from in vivo methylation by glutathione (R. T. Rosen *et al.*, 2001). Diallyl sulfide and diallyl disulfide can be formed from the decomposition of allicin in stomach acid (R. T. T. Rosen *et al.*, 2000). Munch *et al.*' study showed that the ion intensity of allyl mercaptan, allyl methyl disulfide and diallyl disulfide was declined continuously from 5 min after eating garlic, which agrees well with

**Table 2.** List of 9 metabolites in exhaled breath after garlic ingestion. Ion intensity was presented as mean  $\pm$  SE,  $n=10$ .

| $m/z$   | $\Delta m/mDa$ | Formula              | mSigma | CID fragments   | Tentative assignments      | before garlic ingestion (counts; $n=10$ ) | 5 min after garlic ingestion (counts; $n=10$ ) | Significance | References reported            |
|---------|----------------|----------------------|--------|---|----------------------------|---|--|--------------|--------------------------------|
| 75.028  | 2              | $[C_3H_6S+H]^+$      | 48.1   | 41.039 $[C_3H_5]^+$   | Allyl mercaptan            | 40 $\pm$ 11                               | 1905 $\pm$ 181                                 | $P<0.001$    | (Minami <i>et al.</i> , 1989)  |
| 89.045  | 3              | $[C_4H_8S+H]^+$      | -      | -   | Allyl methyl sulfide       | 47 $\pm$ 8                                | 960 $\pm$ 110                                  | $P<0.001$    | (Taucher <i>et al.</i> , 1996) |
| 105.041 | 4              | $[C_4H_8OS+H]^+$     | 27.7   | 90.011 $[C_3H_6OS]^+$ ,<br>88.032 $[C_4H_8S]^+$ ,<br>61.011 $[C_2H_5S]^+$ ,<br>57.033 $[C_3H_5O]^+$ ,<br>46.995 $[CH_3S]^+$           | Methional                  | 578 $\pm$ 60                              | 8017 $\pm$ 662                                 | $P<0.001$    | -                              |
| 110.971 | 0              | $[C_2H_6Se+H]^+$     | 33.2   | -   | Dimethyl selenide          | 121 $\pm$ 21                              | 1206 $\pm$ 128                                 | $P<0.001$    | (Cai <i>et al.</i> , 1995)     |
| 115.059 | 1              | $[C_6H_{10}S+H]^+$   | 40.0   | 75.025 $[C_3H_7S]^+$ ,<br>73.009 $[C_3H_5S]^+$ ,<br>70.993 $[C_3H_3S]^+$  | Diallyl sulfide            | 134 $\pm$ 18                              | 7214 $\pm$ 580                                 | $P<0.001$    | (Taucher <i>et al.</i> , 1996) |
| 121.014 | -2             | $[C_4H_8S_2+H]^+$    | -      | -   | Allyl methyl disulfide     | 36 $\pm$ 8                                | 967 $\pm$ 111                                  | $P<0.001$    | (Taucher <i>et al.</i> , 1996) |
| 121.038 | 6              | $[C_4H_8O_2S+H]^+$   | 25.5   | 104.999 $[C_3H_5O_2S]^+$ ,<br>103.022 $[C_3H_7OS]^+$ ,<br>86.991 $[C_3H_3OS]^+$ ,<br>120.090 $[C_9H_{12}]^+$ ,<br>93.065 $[C_7H_9]^+$ | 3-Methylthiopropionic acid | 261 $\pm$ 31                              | 2319 $\pm$ 225                                 | $P<0.001$    | -                              |
| 135.110 | 6              | $[C_{10}H_{14}+H]^+$ | 37.3   | 118.995 $[C_4H_7S_2]^+$ ,<br>73.010 $[C_3H_3S]^+$   | <i>p</i> -Cymene           | 834 $\pm$ 92                              | 3729 $\pm$ 294                                 | $P<0.001$    | (Rosen <i>et al.</i> , 2001)   |
| 147.030 | 0              | $[C_6H_{10}S_2+H]^+$ | 46.3   | -   | Diallyl disulfide          | 89 $\pm$ 18                               | 6274 $\pm$ 585                                 | $P<0.001$    | (Minami <i>et al.</i> , 1989)  |

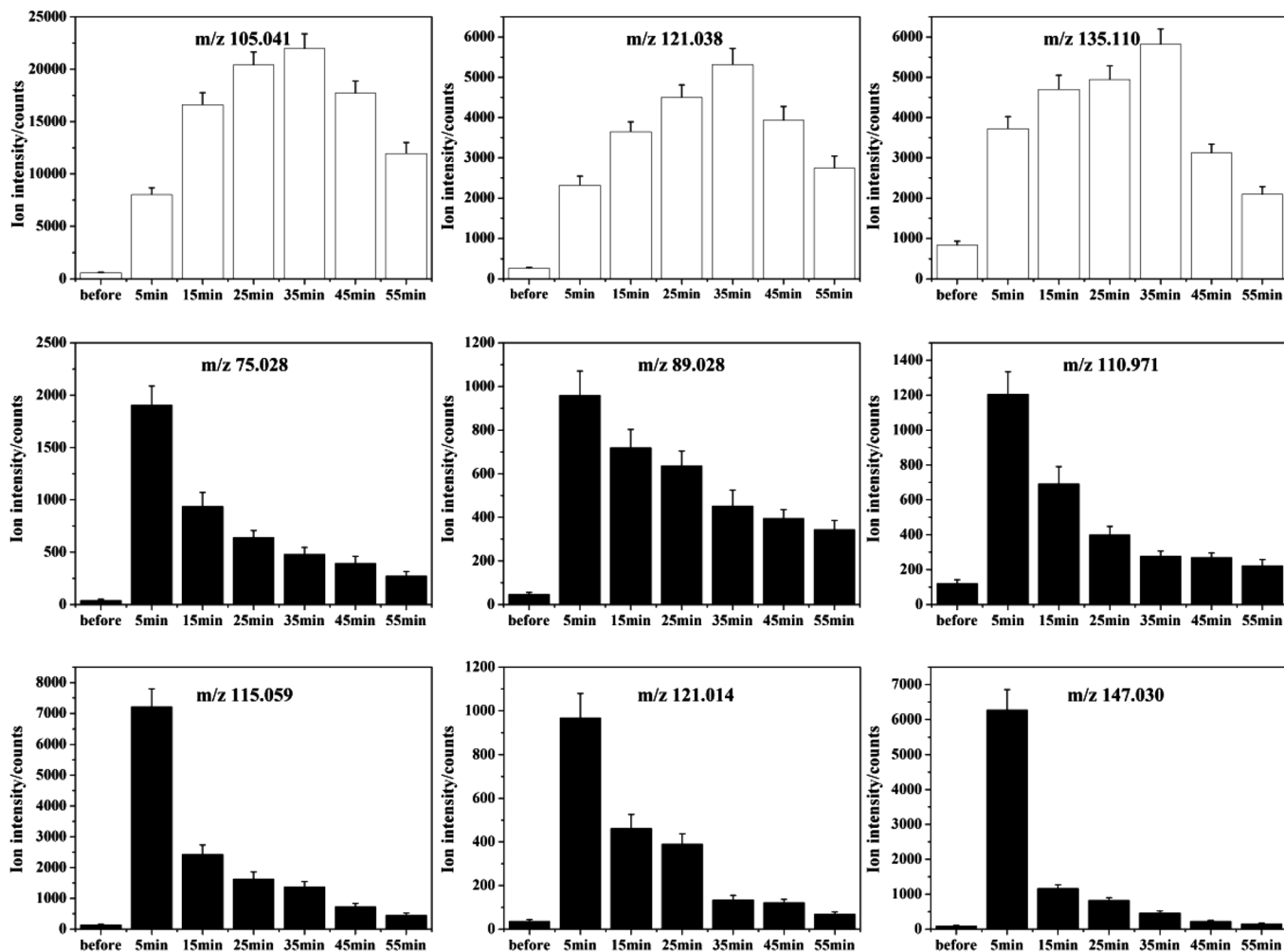


Fig. 3. Variation of  $m/z$  105.041,  $m/z$  121.038,  $m/z$  135.110,  $m/z$  75.028,  $m/z$  89.045,  $m/z$  110.971,  $m/z$  115.059,  $m/z$  121.014,  $m/z$  147.030, in exhaled breath with time after garlic ingestion. Results are shown as mean  $\pm$  SE,  $n=10$ .

our results (Munch *et al.*, 2014). Minami *et al.* investigated the time course of allyl mercaptan and diallyl disulfide concentrations in exhaled breath after eating garlic, and the result is also consistent with ours (Minami *et al.*, 2008). The signal at  $m/z$  110.971 presents specific isotopic patterns (Fig. 5(a)), which indicates a selenium-containing compound. This compound was assigned to dimethyl selenide, and further confirmed by the comparison between on-line breath spectra and simulated standard spectra (Fig. 5(b)). Dimethyl selenide has been observed in exhaled breath after ingestion of garlic by Cai *et al.* (Xiao Jia Cai *et al.*, 1995). This metabolite could result from in vivo methylation of hydrogen selenide and methane selenol that released from selenocysteine and Se-methylselenocysteine, respectively.

It is worth noting that the 6 metabolites in the second group were both observed in garlic headspace and in garlic breath. This phenomenon indicates that these 6 compounds observed in exhaled breath can also be obtained directly from garlic without metabolic processes mentioned above.

In this work, APPI-QTOFMS has been used for online analysis of garlic volatiles and garlic breath. A total of 32 volatiles from garlic were tentatively identified, and 9 metabolites were observed

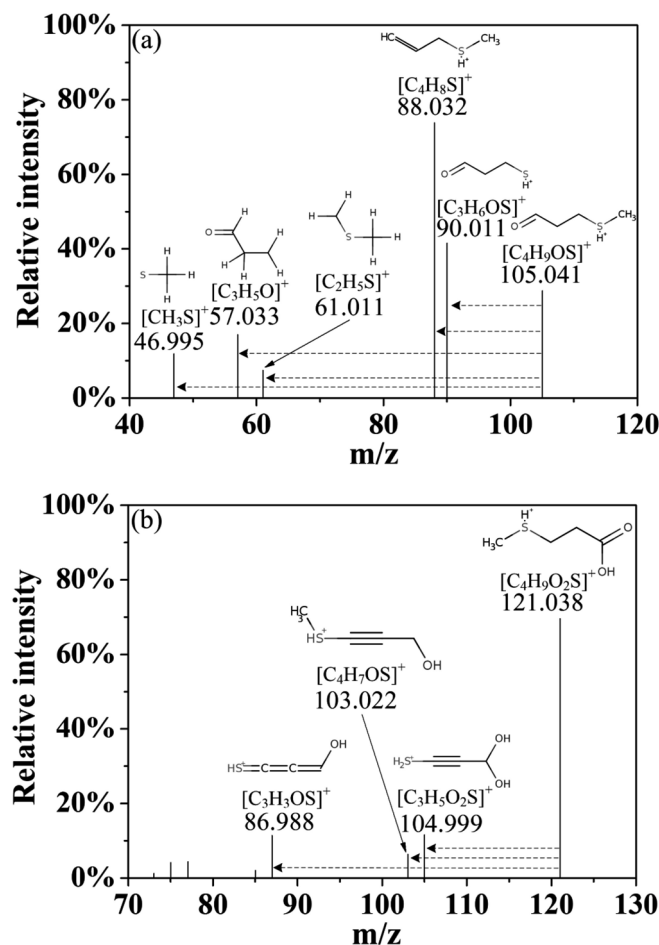
in exhaled breath after ingestion of garlic. Methional and 3-methylthiopropionic acid were observed in garlic breath for the first time, which may reflect the metabolism of methionine from garlic. Our results showed that APPI-QTOFMS is promising method for investigation of food metabolism in dynamic via exhaled breath.

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**Fig. 4.** CID spectra of (a)  $m/z$  105.041 and (b)  $m/z$  121.038. These 2 compounds were identified as methional and 3-methylthiopropionic acid.

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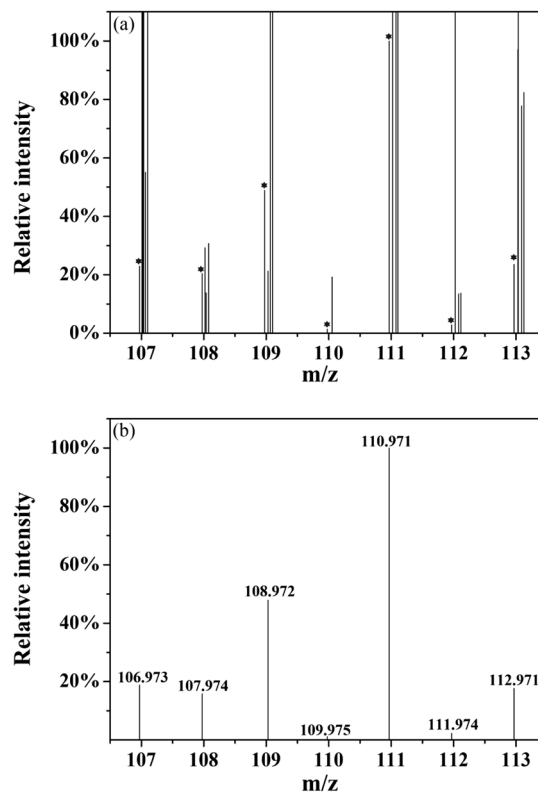
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**Fig. 5.** (a) On-line breath spectra of dimethyl selenide (marked with star). (b) Simulated standard spectra of dimethyl selenide.

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