RESEARCH PAPER

Modification of an atmospheric pressure photoionization source for online analysis of exhaled breath coupled with quadrupole time-of-flight mass spectrometry

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Abstract

Breath analysis is a promising method for metabolomics studies and clinical diagnosis, as it enables the observation of metabolites in a convenient and noninvasive way. In this work, an atmospheric pressure photoionization (APPI) source was modified for online analysis of exhaled breath by coupling with quadrupole time-of-flight mass spectrometry (QTOFMS). Three parameters, namely, the capillary voltage, the sampling flow and the curtain gas flow of the APPI source, were optimized. Five healthy volunteers, three males and two females, were enrolled to test the performance of modified APPI-QTOFMS by analyzing their exhaled breath. A total of 21 compounds were tentatively identified, and four metabolites, namely, dimethyl selenoxide, δ-valerolactam, hydroxymandelic acid and palmitic amide were detected in the exhaled breath for the first time. The result shows that modified APPI-QTOFMS can be used for the online study of exhaled breath.

Keywords Breath analysis · Modified APPI · QTOFMS

Introduction

Breath analysis has become an increasingly attractive method for metabolomics studies, since it provides abundant information in a simple and noninvasive way [1, 2]. Human exhaled breath contains various components [3], most of which are variable and found in trace concentrations, requiring noninvasive and rapid measurement techniques [4]. To date, various analytical techniques including mass spectrometry, spectroscopy and sensors have been used for breath analysis [5, 6]. Compared with other techniques, mass spectrometry enables non-targeted screening, which is more suitable for exploration

of new metabolites in exhaled breath. Gas chromatography mass/spectrometry (GC-MS), sometimes coupled with preconcentration techniques such as solid-phase micro-extraction (SPME) or needle trap extraction [7], has traditionally been used in breath gas analysis [8, 9]. However, GC-MS is limited to offline analysis, which is relatively time-consuming. By contrast, online breath analysis provides immediate data of exhalation without sample pretreatment. Proton transfer reaction mass spectrometry (PTR-MS) and selected ion flow tube mass spectrometry (SIFT-MS) are prominent online techniques [10], which enable online analysis of volatile organic compounds (VOCs) in exhaled breath. Atmospheric pressure ionization techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have also been applied to online breath analysis in combination with mass spectrometry [11]. In the first decade of the twenty-first century, techniques based on electrospray ionization mass spectrometry (ESI-MS), such as secondary electrospray ionization mass spectrometry (SESI-MS) and extractive electrospray ionization mass spectrometry (EESI-MS), were introduced for real-time breath analysis [12, 13].

Compared with ESI and APCI, atmospheric pressure photoionization (APPI) is less susceptible to ionization suppression, and more appropriate for nonpolar compounds [14, 15]. It offers an alternative method of ionization for MS. The

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traditional APPI source is for liquid samples. The solvent containing the analytes is sprayed and vaporized by a heated nebulizer at a temperature of 400 °C. Dopants such as acetone and toluene are universally used in APPI to enhance ionization efficiency [16].

For breath analysis, the analytes are in gaseous form, with no need for vaporization, while the high content of water vapor in exhaled breath can act as a solvent during the APPI measurement. In addition, acetone in exhaled breath can act as dopant during ionization. Therefore, a commercial APPI source was modified according to the characteristics of the exhaled breath sample in this report. The heated nebulizer in the APPI source was removed, and the solvent for spray was not used in the ion source in order to avoid exogenous contaminants from reagents. Three parameters including the capillary voltage, the sampling flow and the curtain gas flow of the APPI source were optimized by coupling with quadrupole time-of-flight mass spectrometry (QTOFMS). Compounds in exhaled breath were tentatively identified, and some metabolites were observed in the exhaled breath for the first time. The results indicate that modification of the APPI source is a promising new method for online breath test by coupling with mass spectrometry.

Experimental section

Modification of the APPI source A modified APPI source combined with a quadrupole time-of-flight mass spectrometer (MicrOTOF-Q III, Bruker Daltonics Inc., Fremont, CA, USA) was used for breath analysis. The heated nebulizer in the APPI source was removed, and the subjects were required to breathe toward the ion source chamber via a T-tube as shown in Fig. 1. The exhaled breath was then sucked into the chamber from the bypass through a polytetrafluoroethylene (PTFE) tube 80 cm long, with an inner diameter of 1.5 mm. In order to avoid the condensation of exhaled breath, the PTFE tube was heated to a constant temperature of 100 °C. Neutral molecules were ionized by a krypton-filled vacuum UV lamp with the most intense excitation lines at 10.0 and 10.6 eV. A gas flow controller was employed upstream of the air pump to control the total pumping flow rate.

The mass spectra were recorded in a mass range of m/z 20–1000 in positive-ion detection mode. To assist in the elimination of fragments and identification of compounds, collision-induced dissociation (CID) analysis was performed, with collision energy of 20 ± 10 eV. The room temperature in the lab was maintained at 25 °C to ensure the stability the of mass spectrometer.
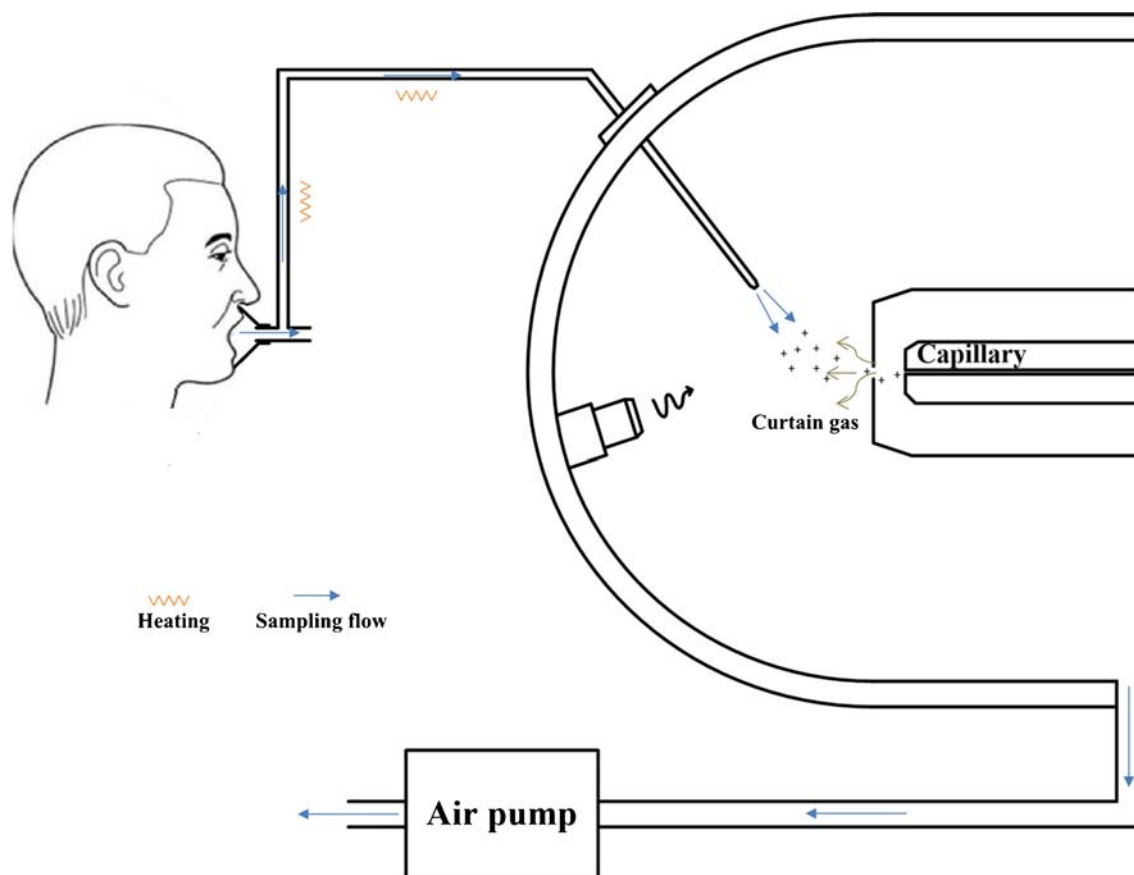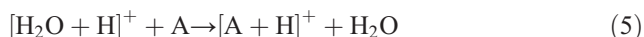

Fig. 1 Schematic of APPI source for detection of exhaled breath

Ionization mechanism In the breath detection experiment, most ion signals are protonated ions. Initially, the analyte (A) is ionized by UV photon energy to form the radical cation ($A^{+\cdot}$), as shown in Eq. 1. Compounds with high proton affinity (PA) may then form protonated molecules by self-protonation (Eq. 2), or react with water clusters [17]. Additionally, the acetone contained in human exhalation acts as a dopant (D) to assist ionization of analytes, as shown in Eqs. 3, 4 and 5.

$$A + h\nu \rightarrow A^{+\cdot} + e^- \tag{1}$$

$$A^+ + A \rightarrow [A + H]^+ + [A\text{-}H] \tag{2}$$

$$D + h\nu \rightarrow D^{+\cdot} + e^- \tag{3}$$

$$D^{+\cdot} + H_2O \rightarrow [H_2O + H]^+ + [D-H] \tag{4}$$

$$[H_2O + H]^+ + A \rightarrow [A + H]^+ + H_2O \tag{5}$$

Subjects Five healthy volunteers in our research group, including three male and two female participants, were enrolled to test the performance of the modified APPI-QTOFMS by testing their exhaled breath. Subjects were asked to exhale directly through a disposable mouthpiece after gargling, which may eliminate the influence of contaminants such as food debris in the oral cavity.

Data processing All raw spectra were acquired by micrOTOF control software, and analysis was processed in DataAnalysis 4.0 software (Bruker Daltonics).

Results and discussion

Optimization of the APPI source Three parameters including the capillary voltage, the sampling flow and the curtain gas flow were found to significantly affect the ion intensity. Accordingly, each parameter was individually optimized by keeping the other parameters at constant values. Since the conditions of exhalation like humidity may influence ionization efficiency of breath components, protonated acetone ion (m/z 59.049) was selected for optimization. The whole process was accomplished within 1 h to minimize the influence of variable acetone concentration.

Capillary voltage significantly influenced the ion transfer efficiency, and high voltage may lead to in-source collision-induced dissociation. For the capillary voltage, five different voltages, viz. 600, 700, 800, 900 and 1000 V, were evaluated for improving the ion signal. As exhibited in Electronic Supplementary Material (ESM) Fig. S1a, voltage at 700 V led to the highest signal.

A high sampling flow rate results in high sample throughput. However, too high of a sampling flow rate could influence the efficiency of ionization and ion transport. The sampling flow rate was set in the range of 1–5 L/min with an interval of 1 L/min. The effect of sampling flow rate on the signal intensity level of protonated acetone is presented in ESM Fig. S1b. The intensity of acetone decreased when the flow rate was over 2 L/min and. Therefore, 2 L/min was set as the optimal value for the sampling flow rate.

Curtain gas flow aids in evaporation of a sample and cleaning of the inlet of the instrument to avoid blocking. However, if the gas flow is too high, it blows away analytes in the ionization zone and dilutes the sample. After optimization of the curtain gas flow rate, 3.0 L/min was selected as the optimal value, as shown in ESM Fig. S1c.

Effect of humidity It is well known that human breath contains a high level of moisture, which may influence the breath spectra. Studies based on APCI-MS have shown the impact of humidity on ion intensity [18]. Martínez-Lozano et al. also revealed that breath humidity increases some background ion signals in ESI-MS [19]. In APPI-MS, the effect of humidity in exhaled breath should be taken into account.

To simulate the humidity of breath, humidification of ambient air was performed upstream of the ion source chamber by passing air through the headspace of a flask containing pure water at 37 ± 2 °C. And the humidified air showed ~95% relative humidity, which is comparable to breath humidity. The relative humidity was measured by a hygrometer. As is shown in ESM Fig. S2, after sampling of humidified air, the air background was changed. This phenomenon indicates that some ion signals in breath spectra may be attributed to changes in humidity rather than ionization of analytes. The direct subtraction of ambient air background is not able to eliminate these ion signals. To minimize the influence of breath humidity, subtraction of humidified air background was carried out for signal correction.

Validation of APPI-QTOFMS The performance of this method was evaluated using acetone, a typical metabolite in human breath. Saturated acetone vapor was stored in an airtight syringe and then injected into the inlet tube using a microsyringe pump. By controlling the injection flow rate, the acetone can be diluted in carrier gas air. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated with Eqs. (6) and (7) [20], where S is the mean value of ion signals, n is the measurement times (here n is 8), σ is the standard deviation of the measurement and C is the concentration of acetone (here 500 ppbv).

$$LOD = \frac{C \times 3\sigma}{S} \tag{6}$$

$$LOQ = \frac{C \times 10\sigma}{S} \tag{7}$$

The LOD was calculated to be 1.32 ppbv, and the LOQ was 4.41 ppbv. The repeatability was expressed as relative

standard deviation (RSD) of 3.4%, indicating that the developed method has good repeatability.

Online breath analysis Online breath analysis was performed to test the performance of the modified APPI-QTOFMS. The typical spectrum of exhaled breath is shown in Fig. 2a. Within a mass range from m/z 20 to 1000, many signals were detected in each breath sample. The high resolution of the TOF analyzer provides accurate measurement of the m/z value to differentiate those chemical compounds with the same nominal mass. As is shown in the inset of Fig. 2a, the ion signal of m/z 114.023, 114.063 and 114.091 can be clearly distinguished.

A drawback of APPI is the complex ionization mechanisms, which would produce many fragment ions [21]. As for unknown mixture samples, these fragment ions disturb the identification of analytes. To better understand the fragmentation of compounds in the APPI source, CID analysis was carried out with different collision energy. According to our results, the amounts of fragments generated by the CID with collision energy of 8~9 eV and their relative abundance
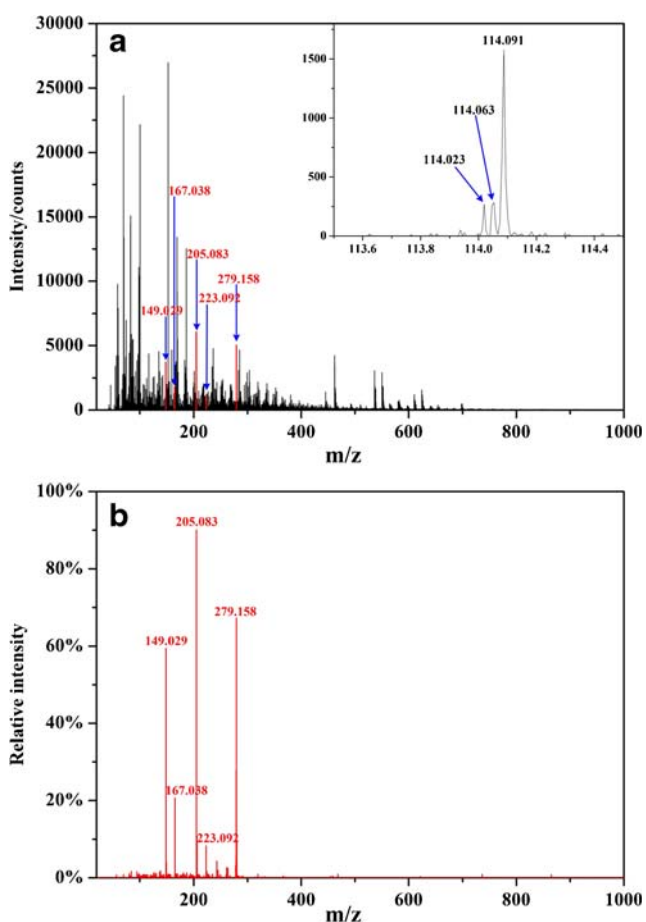

Fig. 2 (**a**) Typical spectrum of exhaled breath from a male subject, and partial enlargement of the spectrum. (**b**) Online CID spectrum of m/z 279.158. By comparing with online CID spectra, fragment ions m/z 223.092, 205.083, 167.038 and 148.029 in full-scan mass spectra can be excluded

are similar to those compounds detected in the full mass scan mode. In this case, the fragments in the APPI experiments can be identified by comparing CID spectra with full-scan mass spectra. For instance, the ion signal at m/z 279.158 was detected in the full mass scan mode, as shown in Fig. 2a, and was recognized as a protonated ion with no precursor ions. This ion was then selected to carry out the CID experiment under collision energy of 8~9 eV. It was found that this ion yields fragments of m/z 223.092, m/z 205.083, m/z 167.038, m/z 149.029 and m/z 121.033, with a relative intensity ratio of 13%:133%:32%:87% as shown in Fig. 2b. It is noted that the m/z 279.158 was set as 100%. Returning to the full-scan mass spectra, as marked with red lines in Fig. 2a, ion signals at m/z 223.092, m/z 205.083, m/z 167.038, m/z 149.029 and m/z 121.033 with a similar relative intensity ratio of 24%:122%:36%:75% were also observed. Therefore, ion signals at m/z 223.092, m/z 205.083, m/z 167.038, m/z 149.029 and m/z 121.033 in the exhaled breath spectra were recognized as fragments produced from the ion of m/z 279.158.

Using this method, 21 molecular ions were differentiated, as shown in Table 1, along with their fragment ions. However, another 39 ion signals with lower intensity could not be unambiguously identified because their fragment ions were difficult to observe in CID mode.

Tentative identification of components in exhalation The assignment of these ions was established based on their exact formula, ion fragments from CID analysis, a search of open-access databases (METLIN Metabolomics Database, Human Metabolome Database, MassBank database, NIST database) and also referring to previous studies. Twenty-one molecular ions were tentatively identified, as listed in Table 1. Possible assignments of 39 ions are also presented in ESM Table S1.

Among these compounds, acetone, isoprene and urea are known as endogenous metabolites from blood. Acetone can be converted from acetoacetate via decarboxylation of excess acetyl–CoA [2, 40], and the breath acetone is related to the blood glucose level [41]. Breath isoprene is from the mevalonic pathway of cholesterol synthesis in the human body [2]. 3-Buten-2-one and 2,3-butanedione are also from blood, despite their unclear metabolic pathway [34]. As a catabolite of protein, urea is formed from ammonia generated by the deamination of amino acids. This compound may also be released via the airway and mouth [13]. For healthy people, exhaled propionic acid, pyrroline and trimethylamine may be derived from the mouth and pharynx. Propionic acid is considered as the metabolite of oral bacteria [28, 35]. However, compounds like dihydropyran, pentenoic acid and caprolactam are still unknown with regard to both origin and metabolic pathway.

Besides the abovementioned compounds, some new components were observed in exhaled breath for the first time. As for the signal at m/z 100.075, it was assigned as the formula of $[C_5H_{10}NO]^+$ which can yield fragments of $[C_5H_8N]^+$,

Table 1 Tentative identification of 21 ions and their corresponding fragments

m/z	Ion formula	CID fragments	Tentative assignments	Reference reported in breath
59.049	$[C_3H_6O+H]^+$	$43.018[C_2H_3O]^+$	Acetone	GC-MS [22], PTR-MS [23], SIFT-MS [24], SESI-MS [25],
60.080	$[C_3H_9N+H]^+$	$44.053[C_2H_6N]^+$	Trimethylamine	SIFT-MS [26] GC-MS [27] SESI-MS [28]
61.040 44.013*	$[CH_4N_2O+H]^+$ $[CH_2NO]^+$	*44.013 $[CH_2NO]^+$	Urea	EESI-MS [13], LC-MS [29]
69.068 53.038*	$[C_5H_8+H]^+$ $[C_4H_5]^+$	$53.038[C_4H_5]^+$	Isoprene	PTR-MS [30], SIFT-MS [31], GC-MS [32]
70.065 53.039*	$[C_4H_7N+H]^+$	$53.039[C_4H_5]^+$	Pyrroline	SESI-MS [33]
71.048 53.038*	$[C_4H_6O+H]^+$ $[C_4H_5]^+$,	$53.038[C_4H_5]^+$, $43.019[C_2H_3O]^+$	3-Buten-2-one	GC-MS [34]
75.043 57.033*	$[C_3H_6O_2+H]^+$ $[C_3H_5O]^+$	$57.033[C_3H_5O]^+$	Propionic acid	SESI-MS [35]
85.061 67.053*	$[C_5H_8O+H]^+$ $[C_5H_7]^+$	$67.053[C_5H_7]^+$	Dihydropyran	GC-MS [36]
87.041 69.034*	$[C_4H_7NO+H]^+$ $[C_4H_5O]^+$	$69.034[C_4H_5O]^+$	2,3-Butanedione	GC-MS [34]
90.086	$[C_4H_{11}NO+H]^+$	$72.079 [C_4H_{10}N]^+$, $57.057[C_3H_7N]^+$	N,N-Dimethylethanolamine	EESI-MS [37] SESI-MS [28]
100.075	$[C_5H_9NO+H]^+$	$82.064[C_5H_8N]^+$ $72.081[C_4H_{10}N]^+$, $56.051[C_3H_6N]^+$, $55.054[C_4H_7]^+$,	δ-Valerolactam(2-piperidinone)	No
101.057, 83.047* 55.054*	$[C_5H_8O_2+H]^+$, $[C_5H_7O]^+$, $[C_4H_7]^+$	$83.047[C_5H_7O]^+$, $55.054[C_4H_7]^+$	Pentenoic acid	[3]
114.091	$[C_6H_{11}NO+H]^+$,	$97.062[C_6H_9O]^+$, $96.076[C_6H_{10}N]^+$, $79.056[C_6H_7]^+$, $69.069 [C_5H_9]^+$	Caprolactam	GC-MS [38]
126.965	$[C_2H_6SeO+H]^+$	$111.939[CH_4SeO]^+$, $109.958[C_2H_6Se]^+$	Dimethyl selenoxide	No
184.061 166.050*	$[C_8H_9NO_4+H]^+$ $[C_8H_8NO_3]^+$	$166.050[C_8H_8NO_3]^+$ $148.039[C_8H_6NO_2]^+$	4-Pyridoxic acid	No
256.259 239.233*	$[C_{16}H_{33}NO+H]^+$ $[C_{16}H_{30}O]^+$	$239.233[C_{16}H_{30}O]^+$, $221.224[C_{16}H_{28}]^+$,	Palmitic amide (palmitamide)	No
279.158 223.092* 205.083* 167.038* 149.029*	$[C_{16}H_{22}O_4+H]^+$ $[C_{12}H_{15}O_4]^+$ $[C_{12}H_{13}O_3]^+$ $[C_8H_7O_4]^+$ $[C_8H_5O_3]^+$	$223.092[C_{12}H_{15}O_4]^+$ $205.083[C_{12}H_{13}O_3]^+$, $167.038[C_8H_7O_4]^+$, $149.029[C_8H_5O_3]^+$ $121.033[C_7H_5O_2]^+$ $93.034[C_6H_5O_5]^+$	Dibutyl phthalate	No
462.147 445.122*	$[C_{12}H_{36}Si_6O_6+NH_4]^+$ $[C_{12}H_{36}Si_6O_6+H]^+$	$445.122[C_{12}H_{37}Si_6O_6]^+$ $429.081[C_{11}H_{33}Si_6O_6]^+$ $355.063[C_9H_{27}O_5Si_5]^+$ $324.982[C_6H_{17}O_6Si_5]^+$	Dodecamethylcyclohexasiloxane(D6)	GC-MS [39]
536.168 519.132*	$[C_{14}H_{42}Si_7O_7+NH_4]^+$ $[C_{14}H_{42}Si_7O_7+H]^+$	$519.132[C_{14}H_{43}Si_7O_7]^+$ $503.101[C_{13}H_{39}Si_7O_7]^+$ $415.069[C_{10}H_{31}Si_6O_6]^+$	Tetradecamethylcycloheptasiloxane(D7)	No
610.187 593.149*	$[C_{16}H_{48}Si_8O_8+NH_4]^+$ $[C_{16}H_{48}Si_8O_8+H]^+$	$593.149[C_{16}H_{49}Si_8O_8]^+$ $577.118[C_{15}H_{45}Si_8O_8]^+$ $489.085[C_{12}H_{37}Si_7O_7]^+$ $373.108[C_{10}H_{33}Si_5O_5]^+$ $371.097[C_{10}H_{31}Si_5O_5]^+$ $355.062[C_9H_{27}O_5Si_5]^+$	Hexadecamethylcyclooctasiloxane(D8)	No
684.198 667.169*	$[C_{18}H_{54}Si_9O_9+NH_4]^+$ $[C_{18}H_{54}Si_9O_9+H]^+$	$667.169[C_{18}H_{55}Si_9O_9]^+$ $429.082[C_{11}H_{33}Si_6O_6]^+$ $355.061[C_9H_{27}O_5Si_5]^+$	Octadecamethylcyclononasiloxane(D9)	No

*Fragments observed in full-scan mass spectra

$[C_4H_{10}N]^+$, $[C_3H_6N]^+$ and $[C_4H_7]^+$. By comparing its CID spectra with an online spectral database reference (metlin. scripps.edu), δ-valerolactam was the best-matched candidate, as shown in Fig. 3. Moreover, the CID spectrum of standard of δ-valerolactam also agrees well with our result [42], which further confirmed this assignment. This compound has been observed in saliva as a metabolite in previous studies [43, 44].

It is noted that two selenium-containing compounds were observed in the exhalation. The ion signal at m/z 126.965, which presents a specific isotope ratio (Fig. 4a), was assigned to $[C_2H_7SeO]^+$, which was further confirmed by the comparison between breath spectra and theoretical standard spectra (Fig. 4b). In CID analysis, with collision energy of 10 eV, only one fragment (m/z 111.939) was observed with a formula of $[CH_4SeO]^+$, with the loss of methyl from precursor ion $[C_2H_7SeO]^+$. Two fragments, $[CH_4SeO]^+$ and $[C_2H_6Se]^+$, appeared when the collision energy was set at 20 eV (Fig. 4c). Therefore, $[C_2H_7SeO]^+$ was identified as having been generated from the ionization of dimethyl selenoxide [45]. The signal at m/z 110.958 with a formula

of $[C_2H_7Se]^+$ was assigned to dimethyl selenide. Although with low ion intensity, the specific isotope ratio of this compound confirmed assignment. As demonstrated in ESM Fig. S3, dimethyl selenoxide and dimethyl selenide showed almost no signals in laboratory air background and presented different content in each breath subject, which indicate endogenous compounds with individual differences in metabolism. Dimethyl selenide has been reported in exhaled
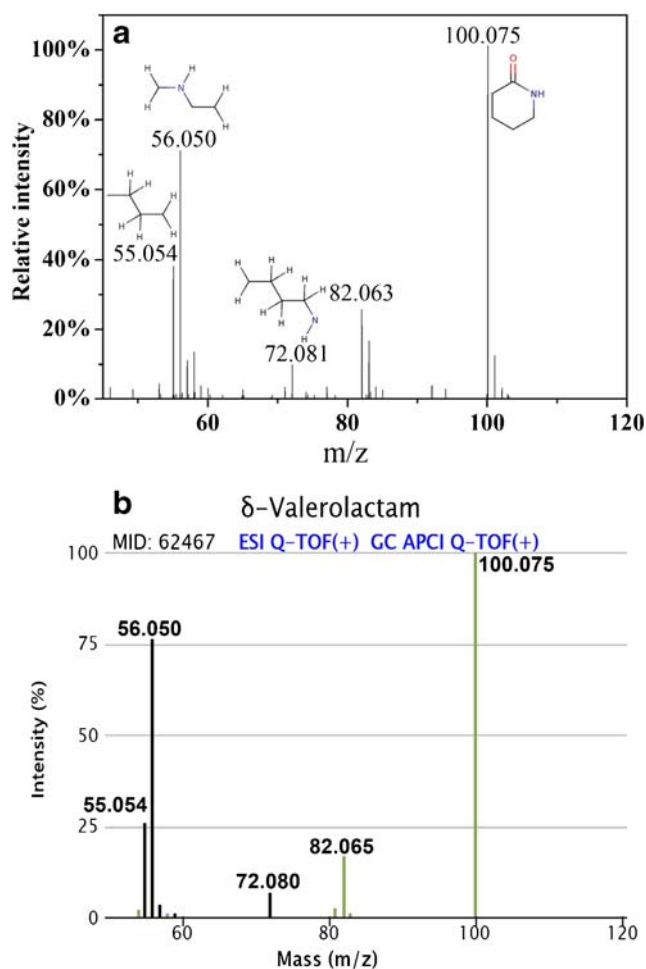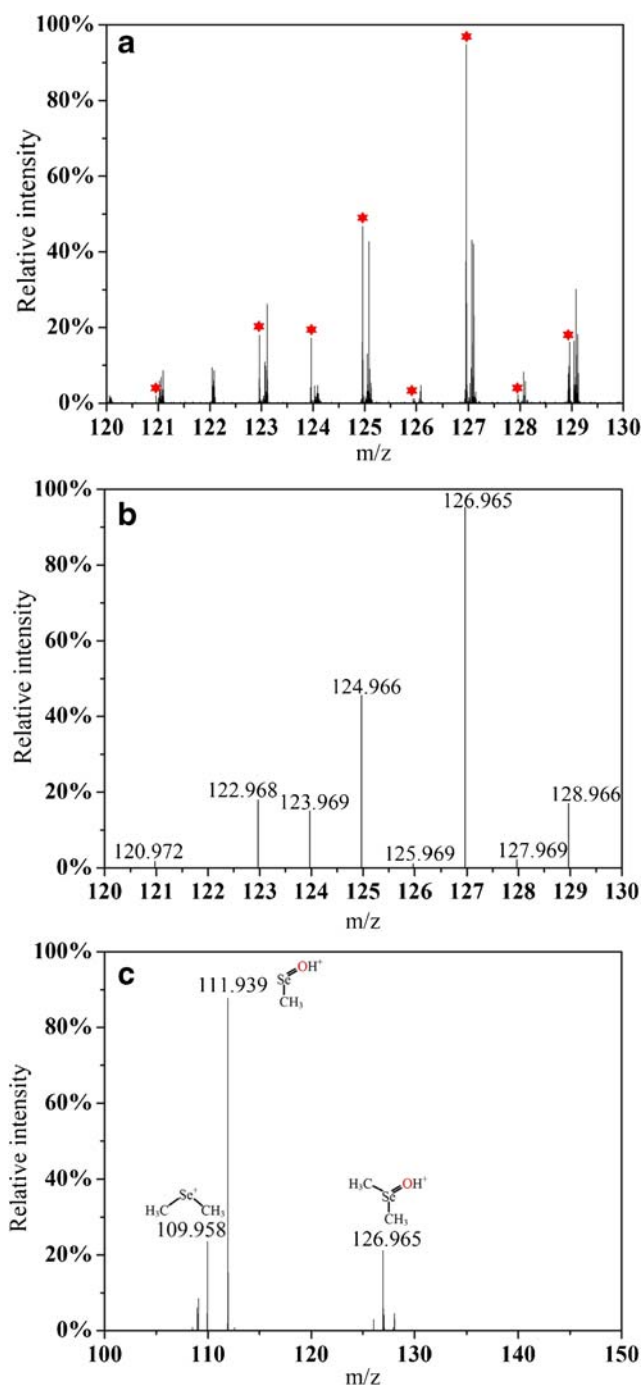

Fig. 3 (a) CID spectrum of m/z 100.075 in exhaled breath with collision energy of 20 eV, (b) standard CID spectrum of δ-valerolactam with collision energy of 20 eV from the METLIN database

Fig. 4 (a) Online breath spectra of m/z 126.965, (b) simulated standard spectra of [C2H7SeO]+, (c) CID spectrum of m/z 126.965

breath [46]. It is known as an intermediate metabolite from the reduction of selenates combined with its methylation in the human body. The hydrolysis of selenoamino acids and methylselenoamino acids also contributes to the generation of this compound [47, 48]. Dimethyl selenoxide was detected in exhaled breath for the first time. Studies have demonstrated the oxidation of dimethyl selenide to dimethyl selenoxide by microsome and flavin-containing monooxygenase from the lungs and liver of some mammals [49]. Observation of dimethyl selenoxide may indicate a similar oxidation process occurring in the human body. Both dimethyl selenoxide and dimethyl selenide reflect the metabolism of selenium in exhaled breath.

The signal at m/z 184.061 with the ion formula of $[C_8H_{10}NO_4]^+$ yields the fragments of $[C_8H_8NO_3]^+$ and $[C_8H_6NO_2]^+$. This compound was identified as 4-pyridoxic acid, which has been detected in human saliva and blood [50, 51]. It is the catabolic product of vitamin B6 that can be formed from the action of aldehyde oxidase I and microbial enzymes [52].

The signal at m/z 256.259 yields the fragments of $[C_{16}H_{30}O]^+$ and $[C_{16}H_{28}]^+$. It was identified as palmitic amide and has been found in human blood. This component could be from palmitic acid, and palmitic acid is one of the most common saturated fatty acids observed in human breath and saliva [53, 54]. Palmitic amide is a long-chain fatty amide (C16:0) with relatively low polarity; therefore, it can be ionized more efficiently in APPI. Tian et al. also showed that long-chain fatty amides are more likely to be detected using an APPI source compared with ESI and APCI sources [55].

The fragmentation of m/z 279.158 has been mentioned above. This compound was identified as dibutyl phthalate (DBP). It is a plasticizer commonly used in PVC products and also can be found in room air [56]. Signals in the mass range of m/z 400–800 were assigned to a set of cyclic volatile methylsiloxanes (cVMS) and their adducts from D6 to D10. These compounds are well known as exogenous contaminants and exist ubiquitously in breath samples and ambient air [57, 58]. In this work, siloxanes may derive from tubes, pipes and seals on the experimental device. Both DBP and cVMS are considered as exogenous inhalations that reflect exposure to these substances.

It is interesting to note that some breath components like urea, palmitic amide and dibutyl phthalate are nonvolatile compounds according to the tentative identifications. This phenomenon indicates this method is also capable of detecting some nonvolatile components in breath. This merit may be attributed to the high sampling flow rate of the modified APPI, which allows direct sampling of both VOCs and aerosols in exhalation. Nonvolatile compounds might be dissolved in aerosol and then introduced into the APPI source for ionization.

Conclusion

In this study, APPI-QTOFMS was modified for online analysis of exhaled breath. No chemicals were required during the sampling and ionizing, which minimizes contamination of analytes. The capillary voltage, sampling flow rate and curtain gas flow rate of the APPI source were optimized, and humidified air was employed for background subtraction to eliminate the impact of breath humidity on signal changes. To exclude fragment ions from APPI ionization, online CID analysis with collision energy of 8~9 eV was performed to assist recognition of molecular ions by comparing CID spectra with full-scan mass spectra. Twenty-one compounds were tentatively identified, and four of them, including dimethyl selenoxide, δ-valerolactam, 4-pyridoxic acid and palmitic amide, were observed for the first time as breath metabolites. The results indicate that this method is suitable for online breath analysis, and potential for expanding metabolite coverage in exhalation.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethics approval and consent to participate This study was approved by the Ethical Committee of the Hefei Institutes of Physical Science, Chinese Academy of Science. All participants understood the breath test very well and gave their informed consent.

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