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RESEARCH PAPER

Analysis of volatile organic compounds from deep airway in the lung through intubation sampling

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Abstract

Exhaled volatile organic compounds (VOCs) have been widely applied for the study of disease biomarkers. Oral exhalation and nasal exhalation are two of the most common sampling methods. However, VOCs released from food residues and bacteria in the mouth or upper respiratory tract were also sampled and usually mistaken as that produced from body metabolism. In this study, exhalation from deep airway was first directly collected through intubation sampling and analyzed. The exhalation samples of 35 subjects were collected through a catheter, which was inserted into the trachea or bronchus through the mouth and upper respiratory tract. Then, the VOCs in these samples were detected by proton transfer reaction mass spectrometry (PTR-MS). In addition, fast gas chromatography proton transfer reaction mass spectrometry (FGC-PTR-MS) was used to further determine the VOCs with the same mass-to-charge ratios. The results showed that there was methanol, acetonitrile, ethanol, methyl mercaptan, acetone, isoprene, and phenol in the deep airway. Compared with that in oral exhalation, ethanol, methyl mercaptan, and phenol had lower concentrations. In detail, the median concentrations of ethanol, methyl mercaptan, and phenol were 7.3, 0.6, and 23.9 ppbv, while those in the oral exhalation were 80.0, 5.1, and 71.3 ppbv, respectively, which meant the three VOCs mainly originated from the food residues and bacteria in the mouth or upper respiratory tract, rather than body metabolism. The research results in our study can provide references for expiratory VOC research based on oral and nasal exhalation samplings, which are more feasible in clinical practice.

Keywords VOCs · Exhaled breath · Intubation · Deep airway · PTR-MS

Introduction

Volatile organic compounds (VOCs) can be produced in significant quantities by human metabolism [1]. After entering the blood circulation system from cells, tissues, and organs, these VOCs can be discharged from the body through the bronchi, trachea, pharynx, larynx, and oral or nasal cavity after a blood-gas exchange in the alveoli [2]. They can also reflect the metabolic level and physical condition of the human body [3]. Presently, more than 1000 kinds of VOCs were detected in human exhalation, primarily including aldehydes, ketones, alcohols, and benzene homologues [4]. Oral exhalation and nasal exhalation are two of the most commonly used methods of breath sampling. However, previous studies have found that VOCs produced from food residues [5], saliva [6], and bacteria [7] in the oral [8] and nasal cavity [9] would interfere with the detection of human metabolic VOCs.

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Breath test has been widely used in the diagnosis of gastrointestinal diseases [10], liver diseases [11], respiratory diseases [12], diabetes [13], and cancer [14]. However, up to now, the study results could not be consistent and verified, and there were even contradictory phenomena. For example, the study of expiratory biomarkers in patients with lung cancer has lasted for 37 years [15]. Phillips et al. [16] found that the concentration of pentane in the exhalation of lung cancer patients increased. While Rudnicka et al. [17] found that the concentration of pentane decreased. One of the reasons for this phenomenon might be the interference of VOCs released by food residues, saliva, and bacteria during oral/nasal exhalation collection. Only by distinguishing such interfering VOCs from metabolic VOCs can we find biomarkers that can stand verification. However, with the current two mainstream sampling methods, it is temporarily impossible to obtain undisturbed exhalation samples in the lung and trachea.

This study is the first to directly collect the exhalation from deep airway by intubation sampling. Endotracheal intubation is the most common respiratory management measure for critically ill patients with respiratory dysfunction [18]. Usually, a catheter passing through the oral or nasal cavity is inserted into the trachea or bronchus through the glottis. It is an important method to implement mechanical ventilation for patients. Then, the VOCs in these samples were detected by a proton transfer reaction mass spectrometry (PTR-MS). In addition, a fast-gas-chromatography proton-transfer-reaction mass spectrometry (FGC-PTR-MS) was used to further determine the VOCs with the same mass-to-charge ratios. Then, we compared the exhalation from deep airway with the oral exhalation to distinguish interfering VOCs from metabolic VOCs. The research results in our study can provide references for expiratory VOCs research based on the oral and nasal exhalation samplings, which are more feasible in clinical practice.

Experimental method

Subject selection

From January 2020 to May 2021, this study was carried out at the Second Affiliated Hospital of Anhui Medical University. The volunteers included 35 hospitalized patients who were receiving endotracheal intubation. They were critically ill patients in the intensive care unit (ICU). In addition, 35 healthy people were recruited to obtain the concentration distribution of VOCs in the oral exhalation. The healthy people were scientific researchers or medical personnel. Table 1 shows the basic information of the two groups participating in the breath test.

PTR-MS and FGC-PTR-MS

This study was conducted on our self-developed PTR-MS instrument as shown in Fig. 1B. PTR-MS is a real-time and online analysis technology [19]. The principal diagram of PTR is shown in Fig. 1D. A high concentration of H_3O^+ was generated in the hollow cathode discharge and entered the drift tube under the force of the electric field. VOCs were sampled to the drift tube. If its proton affinity (PA) was higher than 691 kJ/mol (PA of H_2O), it could undergo a proton transfer reaction with H_3O^+. Then, the produced ions $VOCsH^+$ and H_3O^+ entered the quadrupole mass filter for detection [19]. PTR-MS has been widely used to detect trace VOCs exhaled from the human body [20]. Compared with conventional mass spectrometry detection technology, PTR-MS has the advantages of high detection speed, high sensitivity, and the ability to measure absolute concentration through calculation, without a complex sample pretreatment process [21]. It has great potential for development and application prospect in clinical breath tests. To prevent uncontrollable changes in the exhaled samples during long-distance transportation, the PTR-MS instrument was placed in a room in the ICU and the environmental temperature was maintained at 25 ± 1℃.

Table 1 Basic information of the volunteers participating in the breath test

	Sampling through intubation	Oral exhalation
Number of subjects	35	35
Age (mean ± SD*, min ~ max, years)	66.9 ± 16.3, 30 ~ 89	35.2 ± 12.3, 20 ~ 65
Sex (female/male)	15/20	12/23
Prevalence of respiratory diseases (%)	34.3%	0
Prevalence of neurological diseases (%)	37.1%	0
Prevalence of cardiovascular diseases (%)	14.3%	0
Prevalence of gastrointestinal diseases (%)	14.3%	0

SD standard deviation

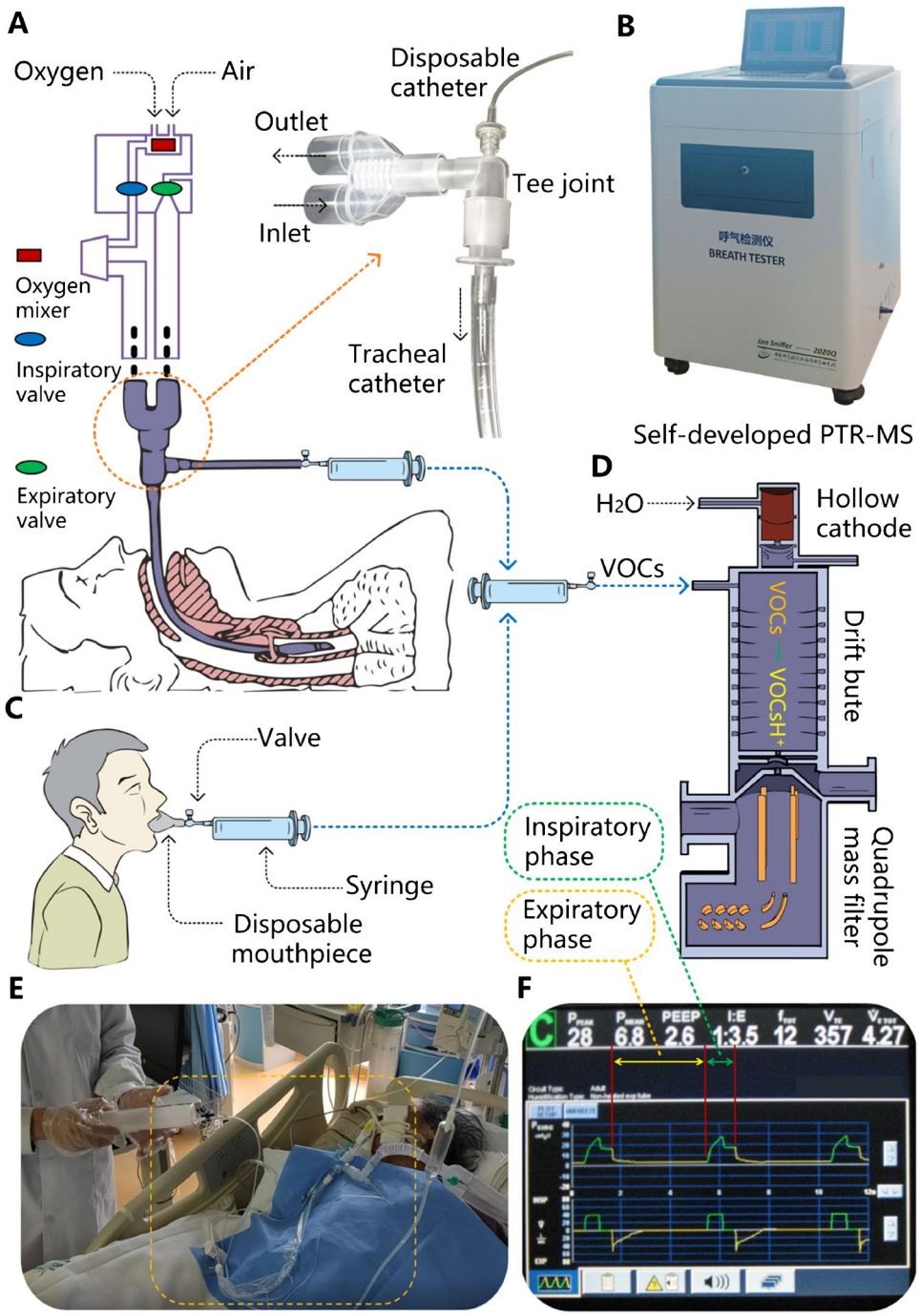
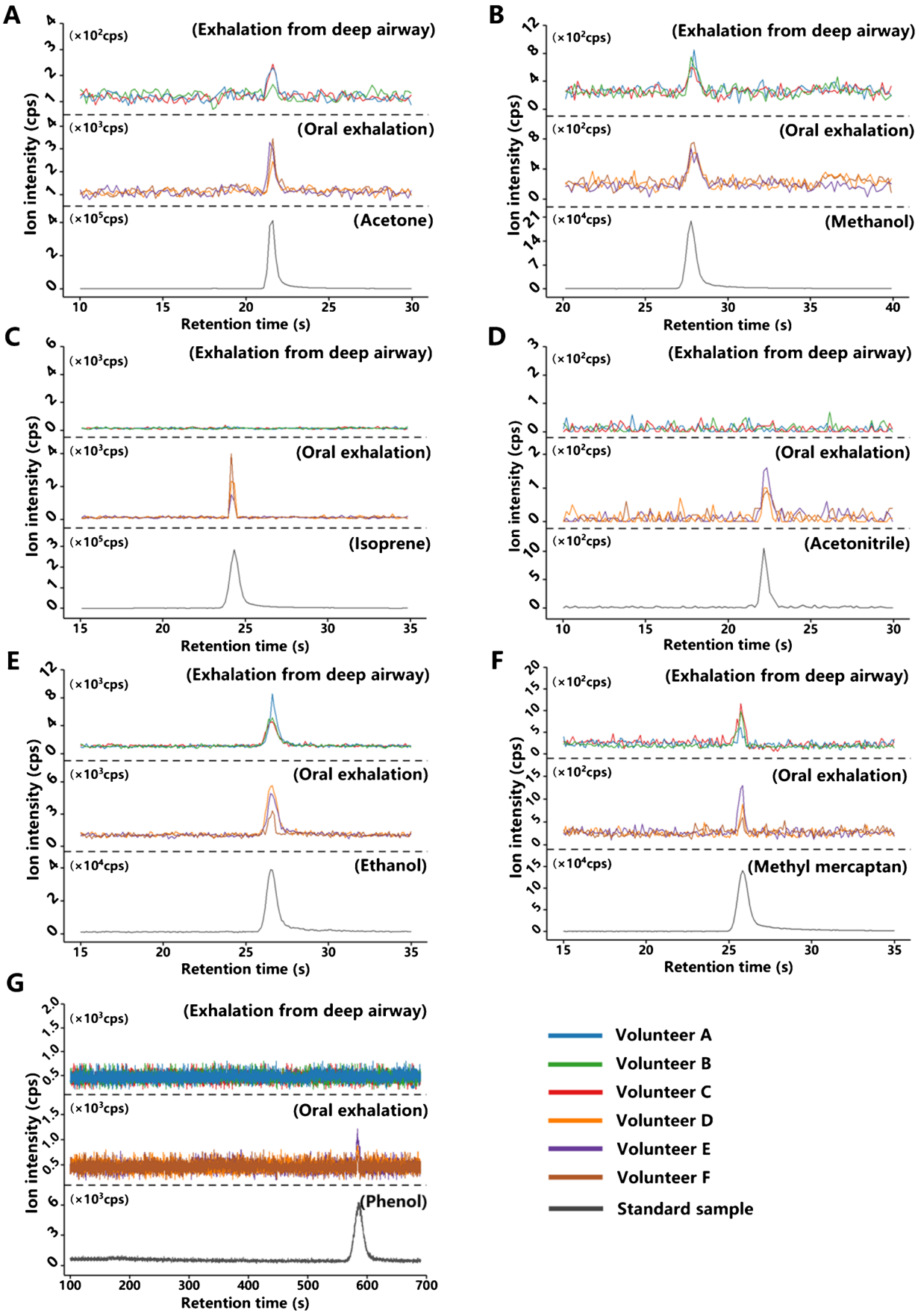


Fig. 1 Schematic diagram of exhalation samples collection and detection. **A** The schematic diagram of sampling through intubation. **B** The physical drawing of the self-developed PTR-MS instrument. **C** The collection method of oral exhalation. **D** The principal diagram of PTR-MS. **E** The scene photo of sampling through intubation in ICU. **F** A specific demonstration of the respiratory waveform in the inspiratory and expiratory phase

Nevertheless, the PTR-MS can only determine the mass charge ratio (*m/z*), and there are fragment ions for some VOCs, which reduces its qualitative ability [22]. Therefore, an FGC-PTR-MS was used in this study to improve the accuracy of qualitative analysis. The chromatographic column (TG-624SILMS, 30 m × 0.53 mm × 3 μm) was purchased from Thermo Scientific, the temperature of the injection port and transfer line were set at 120 and 70℃, the column temperature was set at 90 ℃, and the flow rate of carrier gas (nitrogen) was 10 mL/min.

Exhalation sampling from deep airway

Tracheal catheters serve as a connection between patients and ventilators. When the patient was treated with mechanical ventilation, the tracheal tube was inserted into the deep trachea or bronchus through the oral cavity and glottis. The ventilator was connected to the outside of the catheter to maintain the patency of the patient's airway and improve the patient's respiratory function and oxygenation index. The ventilator was mainly composed of an air supply device,


A
(Exhalation from deep airway)
(Oral exhalation)
(Acetone)
B
(Exhalation from deep airway)
(Oral exhalation)
(Methanol)
C
(Exhalation from deep airway)
(Oral exhalation)
(Isoprene)
D
(Exhalation from deep airway)
(Oral exhalation)
(Acetonitrile)
E
(Exhalation from deep airway)
(Oral exhalation)
(Ethanol)
F
(Exhalation from deep airway)
(Oral exhalation)
(Methyl mercaptan)
G
(Exhalation from deep airway)
(Oral exhalation)
(Phenol)
Ion intensity (cps)
Retention time (s)
Volunteer A
Volunteer B
Volunteer C
Volunteer D
Volunteer E
Volunteer F
Standard sample

◂**Fig. 2** Chromatogram of 7 kinds of ion signals in the exhalation from deep airway and the oral exhalation detected by FGC-PTR-MS. **A** Acetone ($m/z=59$). **B** Methanol ($m/z=33$). **C** Isoprene ($m/z=69$). **D** Acetonitrile ($m/z=42$). **E** Ethanol ($m/z=47$). **F** Methyl mercaptan ($m/z=49$). **G** Phenol ($m/z=95$)

control device, and patient circuit. The air supply device mixed the quantitative oxygen and air through the oxygen mixer, to adjust the oxygen supply concentration of the ventilator. The patient circuit was a complex external air circuit to realize specific functions. The control device was composed of sensors, an expiratory valve, and an inspiratory valve. These two valves cooperated with each other to close and open alternately, which could realize the process that the ventilator injected oxygen into the patient's lungs and then slowly discharged it out of the body. As shown in Fig. 1A, to obtain a stable and continuous patient exhalation sample with high purity, we added an L-shaped tee joint at the connection between the tracheal catheter and the ventilator pipeline to connect a disposable catheter (1 m length, 2 mm inner diameter), and the end of the catheter was connected to a glass syringe with a valve with a capacity of 100 mL. In this way, the exhalation of the patient could be extracted from the catheter intubated by the patient, and the airflow velocity in the catheter (≈50 mL/s) was far lower than the peak respiratory velocity of the ventilator (670 ~ 1670 mL/s), which would not affect the normal operation of the ventilator.

Breath samples were collected during the expiratory phase according to the patient's respiratory curve. When the inspiratory valve of the ventilator was opened and the expiratory valve was closed, it was the inspiratory phase. Conversely, it was the expiratory phase. The respiratory curve displayed by the ventilator can reflect the change in the patient's respiratory state in real time. Taking the volume-time curve as an example, the inspiratory and expiratory phases were distinguished by different colors, as shown in Fig. 1F. During the patient's stable expiratory phase (about 2 ~ 3 s), we used a syringe to extract 50 mL of exhaled gas each time. A total of 100 mL was extracted from one volunteer. A valve on the glass syringe was turned off after sampling. The collected samples were detected within 10 min after sampling. Figure 1E shows the photo of the sampling process. All the above-used pipeline parts were medical disposable accessories, and the glass syringe was reused after high-pressure sterilization.

The exhalation samples were pushed into the PTR-MS instrument for detection using a syringe pump. The valve on the glass syringe was turned on before the detection. The injection flow rate was 10 mL/min. A full scan mode was adopted for the PTR-MS detection. The scan range was set as m/z 20 ~ 150. The ion intensity of m/z 37 ($H_2O{\cdot}H_3O^+$) was too high, which will cause irreversible damage to the electron multiplier in the PTR-MS. So it was eliminated from the scan. The dwell time and settle time were set as 1 and 0.1 s, respectively.

Chemicals

The standard samples of methanol, acetonitrile, ethanol, acetone, isoprene, and phenol used in the experiment were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Methanol, acetonitrile, acetone, and methyl mercaptan standard gases were purchased from Shanghai Haizhou Special Gas Co., Ltd.

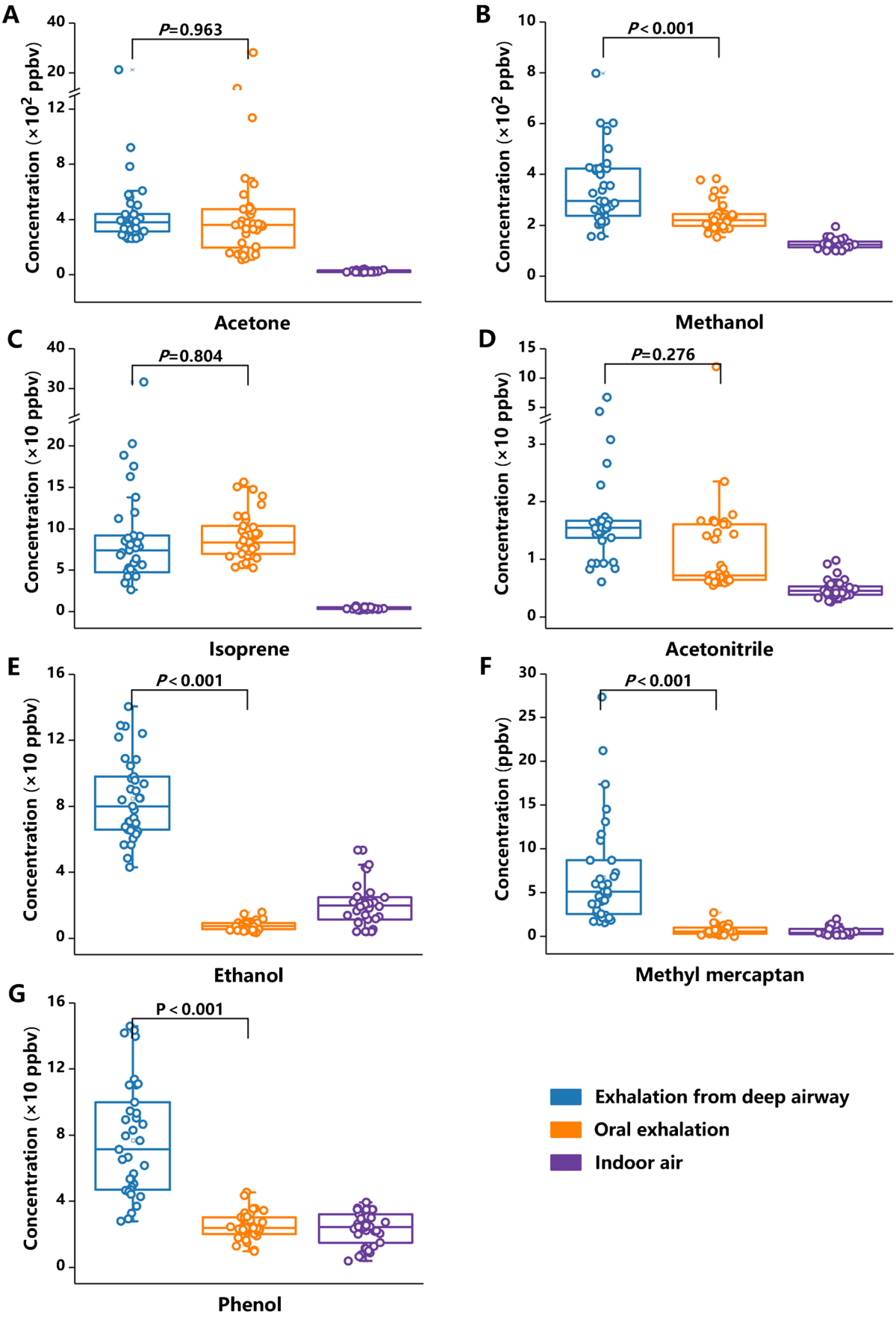
Results and discussion

VOCs in deep airway

By using the aforementioned way, we collected the exhalation samples from deep airway of 35 subjects with stable respiratory status. Then, we used the same glass syringe with a disposable mouthpiece (Fig. 1C) to collect the oral exhalation samples of 35 healthy people as a control reference. These exhalation samples were immediately detected by the PTR-MS instrument placed in the ICU. Ions with average signal intensity greater than twice the air background were selected for analysis. An FGC-PTR-MS was used to determine these ions. The exhalation samples used in the qualitative analysis included the exhalation from deep airway of 3 intubated subjects and the oral exhalation of 3 healthy subjects. The basic information of the 6 subjects is shown in Table S1.

Acetone

The chromatographic retention time of ion at m/z 59 in the air from deep airway was 26.5 s, resembling the acetone standard gas, as illustrated in Fig. 2A. So the ion at m/z 59 was determined as acetone. Acetone was the highest amount of endogenous VOCs in human breath and was one of three ketone bodies produced by the liver and other organs [23]. Its generation pathways in the human body included spontaneous decarboxylation of acetoacetic acid [24] and dehydrogenation of isopropyl alcohol [25]. Fasting, vigorous exercise, and ketogenic diets all led to the concentration elevation of exhaled acetone [25, 26]. Current studies suggested that exhaled acetone can be used as a biomarker for diagnosing type I diabetes [27]. The concentration of acetone in the exhalation of normal adults was 100 ~ 1500 ppbv [23]. As shown in Fig. 3A, the median concentration of acetone in the deep airway and oral exhalation were 361.3 and 378.1 ppbv, respectively. The difference between the two groups was tiny, which indicates that the exhaled acetone was primarily originated from human metabolism.


A
P=0.963
Concentration (×10² ppbv)
Acetone
B
P< 0.001
Concentration (×10² ppbv)
Methanol
C
P=0.804
Concentration (×10 ppbv)
Isoprene
D
P=0.276
Concentration (×10 ppbv)
Acetonitrile
E
P< 0.001
Concentration (×10 ppbv)
Ethanol
F
P< 0.001
Concentration (ppbv)
Methyl mercaptan
G
P < 0.001
Concentration (×10 ppbv)
Phenol
Exhalation from deep airway
Oral exhalation
Indoor air

◂**Fig. 3** Concentration distribution of 7 kinds of VOCs in the exhalation from deep airway and the oral exhalation. **A** Acetone. **B** Methanol. **C** Isoprene. **D** Acetonitrile. **E** Ethanol. **F** Methyl mercaptan. **G** Phenol

Methanol

The retention time of ion at *m/z* 33 in the air from deep airway was 21.6 s, the same as that of methanol standard gas, as shown in Fig. 2B. Methanol was a metabolite widely present in human breath, blood, and urine [28], and it was also a main component of the atmosphere [29]. Particularly, the intake of fruits, vegetables, alcohol, and aspartame (a sweetener) beverages could increase the amount of exhaled methanol. Previous animal studies had demonstrated that it was produced from microbial metabolism in the cecum [30, 31]. In addition, the biochemical reactions of hydroxymethylated protein and S-adenosine could also produce a small amount of methanol [30]. In our study, the median concentration of methanol in the deep airway was 219.4 ppbv, and that in oral exhalation was 295.4 ppbv. As shown in Fig. 3B, the methanol in deep airway exhalation was slightly lower. The most likely reason was that the intubated group depended on intravenous fluids way of nutrition for a long time, and lack of dietary fiber. This caused lower exhaled methanol, which was consistent with the detection results of Lee et al. in patients with end-stage renal disease [32].

Isoprene

According to Fig. 2C, the retention time of ion at *m/z* 69 was 26.5 s in the air from deep airway and was determined as isoprene. Isoprene was considered a by-product of the mevalonate pathway in cholesterol synthesis [33]. It was the second-largest amount of endogenous VOC in human exhalation, and the concentration in the adult exhalation was 100 ~ 300 ppbv [34]. The median concentration of isoprene in the deep airway was 83.6 ppbv, and that in oral exhalation was 74.1 ppbv, as shown in Fig. 3C. No obvious differences were there. So the isoprene would be an endogenous metabolite like acetone.

Acetonitrile

The retention time of ion at *m/z* 42 in the air from deep airway was 27.7 s, determined as acetonitrile, as shown in Fig. 2D. Exhaled acetonitrile was considered partly originated from an exogenous source and was regarded as a biomarker of recent smoking [35]. The concentration of exhaled acetonitrile increased significantly when exposed to acetonitrile in the working places [36]. The concentration of exhaled acetonitrile in healthy adults was 5 ~ 124 ppbv [36, 37]. The median concentration of acetonitrile in the air from deep airway was 7.2 ppbv, lower than 15.4 ppbv in oral exhalation, as shown in Fig. 3D. The elevated part in oral exhalation may be originated from external sources, such as smoking, exposure to second-hand smoke, and bacteria in the mouth.

Ethanol

Ethanol was one of the most frequently detected VOCs in exhalation [38]. The retention time of ion at *m/z* 47 in the air from deep airway was 24.0 s, determined as ethanol, as shown in Fig. 2E. In a previous study, the concentration of exhaled ethanol ranged from 13 to 1000 ppbv [39]. We found the median concentration of ethanol in oral exhalation was 80.0ppbv, but only 7.3ppbv in the exhalation from deep airway, as shown in Fig. 3E. Wang et al. [40] used selective ion flow mass spectrometry (SIFT-MS) for comparing the oral exhalation with nasal exhalation of 3 subjects for a month. They found the average concentration of ethanol in oral exhalation to be higher than that in nasal exhalation (151 ppbv vs 27 ppbv), which indirectly proved that the oral cavity was the main source of ethanol in oral exhalation. Our results directly indicated that the contribution of the oral cavity was much higher than that of the blood-gas exchange in the alveoli. Ethanol in the oral cavity was produced by the decomposition of glucose by oral bacteria through anaerobic respiration [40]. Our previous study also found that the ion signal of *m/z* 47 significantly decreased after gargling [41], which was consistent with the conclusion in this study.

Methyl mercaptan

Methyl mercaptan was a major volatile sulfur compound (VSC) with a foul odor and can cause halitosis. The retention time of *m/z* 49 ion in the oral exhalation was 22.1 s, so it was determined as methyl mercaptan, as shown in Fig. 2F. Methyl mercaptan was produced from bacterial degradation of sulfur-containing amino acids (methionine, cystine, and cysteine) in the oral cavity [42, 43]. The people without halitosis also had lower concentrations of exhaled methyl mercaptan. When it reached 6.3 ppbv, it produced a slight but noticeable odor [44]. In our study, the median concentration of methyl mercaptan in oral exhalation was 5.1 ppbv, but that of air from deep airway was only 0.6 ppbv, as shown in Fig. 3F. The level of methyl mercaptan in the air from deep airway was similar to that in indoor air. This phenomenon suggested that exhaled methyl mercaptan was almost completely originated from the oral cavity rather than alveoli in healthy individuals. In our previous study, we also found that the intensity of ion at *m/z* 49 in exhalation decreased significantly after gargling [41], which was consistent with the result here.

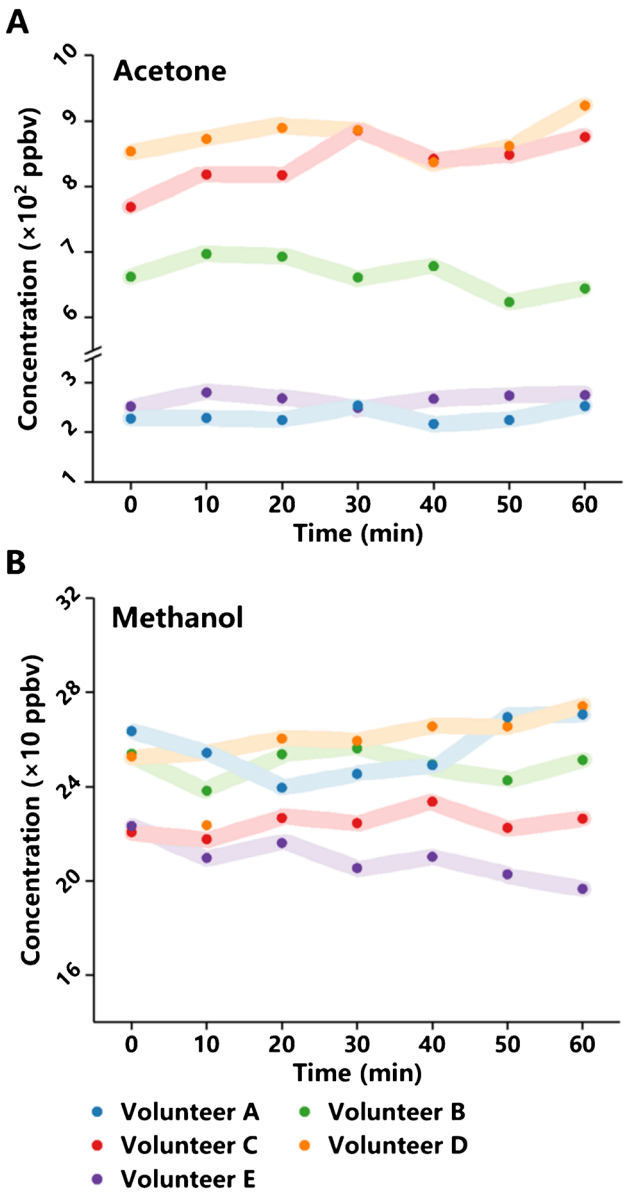


Fig. 4 The changing curve of exhaled VOCs was monitored at multiple points by sampling through intubation. **A** Acetone, **B** Methanol

Phenol was a VOC with a sweet odor existing in human expiratory and saliva [45]. The retention time of ion at *m/z* 95 in the air from deep airway was 585.8 s, determined as phenol, as shown in Fig. 2G. The phenol in human expiratory has been found to be related to the metabolism of tyrosine by bacteria in the oral cavity and intestinal tract [46]. In this study, the detection results of phenol were like that of ethanol and methyl mercaptan. As shown in Fig. 3G, the median concentration of phenol in the air from deep airway was 23.9 ppbv, closing to 24.4 ppbv in the indoor air, far lower than 71.3 ppbv in oral exhalation. This result directly verified that phenol in exhalation mainly originated from the oral cavity or upper respiratory tract. In our recent study, it was found that after gargling, the exhaled phenol was reduced by 68% and 69% in patients with esophageal cancer and healthy people [47], which indirectly indicated that at least half of phenol originated from the oral cavity, which was consistent with the conclusion of this study.

Repeatability evaluation of the sampling method

The repeatability of the intubation sampling method was evaluated in this study. We conducted continuous intubation sampling within 1 h with an interval of 10 min. The duration of a single expiratory phase of these subjects was larger than 1.5 s. Seven samples were collected from each subject. Acetone and ethanol were selected for the evaluations. Acetone was a small molecule product produced from fat oxidation in human metabolism, and it was one of the VOCs with the highest concentration in exhalation [48]. Methanol was a metabolite widely found in human exhalation, blood, and urine [28]. Five subjects were recruited for this study. The basic information of these subjects was shown in the supplementary materials (Table S2). As shown in Fig. 4, the concentrations of acetone and methanol changed slightly. As shown in Table 2, the average RSDs of exhaled acetone and methanol for 5 subjects were 4.5% and 4.0%, respectively, indicating good repeatability.

Phenol

Table 2 Monitoring fluctuations of acetone and methanol in the exhalation from deep airway of 5 volunteers

Volunteer	Acetone			Methanol		
	Average concentration (ppbv)	SD* (ppbv)	RSD* (%)	Average concentration (ppbv)	SD* (ppbv)	RSD* (%)
A	232.6	14.5	6.2	256.0	12.2	4.8
B	665.7	26.4	4.0	249.4	6.6	2.6
C	836.43	39.5	4.7	224.6	5.1	2.2
D	874.7	28.2	3.2	257.4	16.3	6.3
E	266.4	11.7	4.4	209.1	8.8	4.2

**SD* standard deviation, *RSD* relative standard deviation

Table 3 Three different VOCs had been reported as biomarkers in exhalation

VOCs	Disease group (number)	Concentration (AVE* or MED*)	Control group (number)	Concentration (AVE or MED)	Change*	Detection technology	Reference
Ethanol	Cystic fibrosis patients (20)	AVE 157.0 ppbv	Healthy people (20)	AVE 195.0 ppbv	↓	GC–MS	[42]
	Crohn's disease patients (24)	MED 123.2 ppbv	Healthy people (53)	MED 89.1 ppbv	↑	SIFT-MS	[43]
	Type 1 diabetes mellitus children (53)	MED 107.0 ppbv	Healthy children (60)	MED 85.0 ppbv	↑	PTR-TOF–MS	[49]
	Cirrhotic patients (80)	MED 129.0 ppbv	Healthy people (43)	MED 44.8 ppbv	↑	SIFT-MS	[50]
	Chronic kidney disease children (48)	MED 146.4 ppbv	Healthy children (60)	MED 82.4 ppbv	↑	PTR-TOF–MS	[51]
	Colorectal cancer patients (65)	AVE 95.9 ppbv	Healthy people (122)	AVE 464.0 ppbv	↓	GC–MS	[52]
	Lung cancer patients (108)	MED 193.0 ppbv	Healthy people (121); other lung diseases patients (24)	MED 1203.0 ppbv	↑	GC–MS	[53]
Methyl mercaptan	Chronic renal failure patients before hemodialysis (50)	AVE 1.0 ppbv	Chronic renal failure patients after hemodialysis (50)	AVE 0.5 ppbv	↑	GC–MS	[54]
Phenol	Esophagogastric cancer patients (18)	MED 17.0 ppbv	Healthy people (17)	MED 6.0 ppbv	↑	SIFT-MS	[55]
	Esophageal cancer patients (48)	MED 9.0 ppbv	Non-cancer controls (129)	MED 4.0 ppbv	↑	SIFT-MS	[56]
	Gastric cancer patients(96)	N/A*	Healthy people (78)	N/A	↑	SPI-MS	[57]
	Lung cancer patients (79)	AVE 1.1×10^6 PA*	Healthy people (38)	AVE 1.0×10^6 PA	↑	GC–MS	[58]
	Lung cancer patients (16)	AVE 0.0 ppbv	Healthy people (20)	AVE 75.02 ppbv	↑	GC–MS	[59]
	Esophageal cancer patients (29)	MED 31 cps	Healthy people (57)	MED 47 cps	↓	PTR-MS	[3]
	Maligant pleural mesothelioma Patients (14)	N/A	Non-cancer controls (19)	N/A	N/A	GC–MS	[60]
	Thyroid cancer patients (39)	N/A	Healthy people (32)	N/A	↑	GC–MS	[61]
	Breast cancer (71)	N/A	Healthy people (78)	N/A	N/A	GC–MS	[62]

**AVE* average value, *MED* median value, *Change* the change in the disease group compared to the control group, *N/A* the concentration information of this biomarker is not mentioned in the literature, *PA* peak area

Potential impact of oral breath sampling on breath research

Analysis of the air from deep airway is an indication that some VOCs were originated from the oral cavity and upper respiratory tract (nose, pharynx, and larynx) rather than the body metabolism. In our study, we found that ethanol, methyl mercaptan, and phenol were mainly originated from the oral cavity. Nevertheless, these three VOCs had been previously reported as expiratory biomarkers of cancer and other diseases. Exhaled ethanol had been reported as a biomarker of cystic fibrosis, Crohn's disease, pediatric diabetes, cirrhosis, pediatric chronic kidney disease, colorectal cancer, and lung cancer [42, 43, 49–53]. Methyl mercaptan had been reported as an exhaled biomarker of chronic renal failure [54]. Phenol was generally elevated in the breath of patients with esophageal

cancer, gastric cancer, lung cancer, thyroid carcinoma, and other cancers [3, 55–62]. The detailed information has been shown in Table 3. The conclusions in this study should be reconsidered.

Conclusion

In this study, through intubation sampling, the air in deep airway was first directly collected and analyzed. According to our findings, it contained methanol, acetonitrile, ethanol, methyl mercaptan, acetone, isoprene, and phenol. Among them, the median concentrations of ethanol, methyl mercaptan, and phenol in the deep airway were much lower than that in oral exhalation. This phenomenon indicated that some VOCs in exhaled breath may originate from the food residues and bacteria in the mouth or upper respiratory tract, rather than the body's metabolism. They should not be considered biomarkers of diseases. Nevertheless, ethanol, methyl mercaptan, and phenol have been reported as expiratory biomarkers for cancers as well as other diseases in numerous previous papers. This may be the reason why most previous study results could not be consistent. We suggest researchers should consider the contributions of food residues and bacteria to exhaled VOCs in future breath studies. The research results in our study can provide references for expiratory VOC research based on the oral and nasal exhalation samplings, which are more feasible in clinical practice.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-022-04295-x.

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Declarations

Ethics approval This breath test project passed the check by the Ethics Committee of the Second Affiliated Hospital of Anhui Medical University (approval number: YX 2020–003). All the exhalation sampling and breath tests were carried out with the informed consent of the subjects or their accompanying family members.

Conflict of interest The authors declare no competing interests.

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