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Method for analysis of exhaled air by microwave energy desorption coupled with gas chromatography–flame ionization detection–mass spectrometry

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Abstract

A method for chemical analysis of volatile constituents in exhaled air of mechanically ventilated patients is described. Exhaled substances are adsorbed and concentrated onto activated charcoal, desorbed by microwave energy and transferred into a gas chromatograph for separation without prior cryofocusing. Substances are identified by flame ionisation detection and mass spectrometry. This method yields reproducible results and is well suited for clinical studies. © 1998 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Analysis of exhaled air may provide useful insights into metabolic processes of the body [1]. Acetone for instance is the main constituent in the exhaled breath of patients with uncontrolled diabetes mellitus and can clinically be detected by its characteristic odor [2]. Expired alkanes (ethane and pentane) are regarded as markers of lipid peroxidation [3]. The concentrations of ammonia and amines are increased in the expired air of patients with uraemia [4], dimethylsulfide and methylthiol in patients with liver cirrhosis [5–7].

The advantage of analysing expired air for characterization of metabolic processes and disease states is the non-invasive mode of obtaining material of sufficient quantity. Exhaled air, however, is rarely used for diagnostic purposes in clinical medicine due to analytical difficulties. The analysis is hampered (a) by the low concentration of the volatile constituents in the exhaled breath, (b) by condensation of volatile substances onto the wall of the breath collecting vessel and (c) by the high amount of water present in exhaled air.

Therefore a method was designed which overcomes the various obstacles. Expired gas is drawn through a ceramic sampling tube containing activated charcoal by a roller pump. The volume of gas passing through the sampling tube is measured by a volumeter. The constituents of the gas are adsorbed and concentrated onto activated charcoal. For analy-

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sis the volatile substances are desorbed by microwave energy and directly transferred into the gas chromatograph for separation. Substances are identified by flame ionisation detection (FID) and or mass spectrometry (MS). This method is tested in a clinical setting.

2. Experimental

2.1. Adsorption of volatile substances onto the adsorption material

Exhaled air is drawn by a pump (Model SV-367, Fa.Waters, Rochester, Minnesota, MN, USA) through ceramic tubes (9.5 cm×0.6 cm, Analyt, Muellheim, Germany) each filled with a material (80 mg) of different adsorption characteristics, depending on polarity, lipophilicity and the boiling point of the substances under investigation. The following adsorption materials were studied: Carboxen 1000 m^2/g, activated charcoal 1000–1500 m^2/g (Analyt, Muellheim, Germany) and graphitized activated carbon 120 m^2/g (Carbopack, Supelco, Deisenhofen, Germany). For assessment of the adsorption and desorption characteristics of the respective adsorption material a mixture of commercially available volatile compounds was prepared as follows: 5 μl of liquid ketones, hydrocarbons, aromatics and volatile anaesthetics listed in Table 1 were injected into an evacuated 100-ml glass vessel (Analyt, Muellheim, Germany) with a microsyringe (Hamilton, Bonaduz, Switzerland). The remaining vacuum was replaced by hydrocarbon-free air. For homogeneous distribution the gas mixture was continuously stirred with a polytetrafluoroethylene (PTFE)-coated magnet (Rüromag, IKA-Werk Staufen, Germany). Aliquots of 1 to 20 μl of the headspace gas mixture (calibration mixture) were withdrawn through a rubber fitting with a gastight microsyringe (Hamilton) and injected into the sampling tube containing the respective adsorption material. For completion of adsorption 50 ml of hydrocarbon-free air was drawn through the tube within 30 s. The quality of adsorption was studied by passing the calibration mixture through two sampling tubes placed in series.

Table 1
Constituents of the calibration mixture

Peak No.	Ret. time (min)	Substance
1	12.26	Ethanol
2	13.86	2-Methylbutane
3	15.09	Isoflurane
4	15.61	Acetone
5	16.42	Pentane
6	17.47	Isoprene
7	20.11	2,2-Dimethylbutane
8	22.68	2,3-Dimethylbutane
9	22.95	2-Methylpentane
10	23.94	3-Methylpentane
11	24.97	Hexane
12	26.28	2,2-Dimethylpentane
13	26.56	2,4-Dimethylpentane
14	27.92	3,3-Dimethylpentane
15	28.35	2-Methylhexane, benzene
16	28.72	3-Methylhexane
17	29.64	Heptane
18	30.51	Methoxyflurane
19	32.42	Toluene
20	32.90	Hexanal
21	35.31	Ethylbenzene

[a] Peak No.=numbers in chromatograms.
[b] Ret. time=retention time.

2.2. Desorption

Substances were desorbed from the adsorption material by means of a microwave thermoinjector (Model MW-1 Analyt) and directly transferred into the gas chromatograph. An intensive microwave pulse homogeneously heated the adsorption material to approximately 600–1000°C within 10 s. The microwave energy was predominantly dissipated to the adsorbent material, only a fraction of the energy was transmitted to the ceramic tube wall which resulted in a temperature increase of not more than 60–100°C. The microwave desorber was operated under the following conditions: backflush (time of purging the sampling tube before desorption) 10 s, desorption power (applied microwave energy) 400 Ah 10^{-6}, bypass delay (time of gas flow through the sampling tube after desorption) 30 s, desorption time (duration of the microwave pulse) 15 s, split ratio (ratio of gas flow through the GC column and total gas flow through the sampling tube) 10. The efficiency of desorption achieved by microwave energy was determined by a subsequent desorption step.

2.3. Internal standard

For detection of loss of volatile constituents during desorption and of differences in the adsorption characteristics of the adsorption material 2,2-dimethylbutane (65 ng) was added to the sampling tube as internal standard (I.S.). For this purpose 1 μl of 2,2-dimethylbutane was injected into an evacuated 100-ml glass vessel. The remaining vacuum was replaced by hydrocarbon-free air. The mixture was stable for at least 6 h. Ten μl of the headspace gas were transferred with a gastight microsyringe into the sampling tube which had been exposed to expired air before. For conclusion of the adsorption procedure 50 ml of hydrocarbon-free air was drawn through the tube within 30 s. The sampling tube was then sealed with inert caps of polyethylene. The mean peak area of the internal standard was derived from 223 measurements.

2.4. Gas chromatographic analysis

The desorbed substances were separated by gas chromatography (GC) (HP 5890 series A and HP 6890 Series, Hewlett-Packard, Bad Homburg, Germany). The columns used were CP Sil 8 CB capillary column (50 m×0.32 mm I.D.; 5 μm film, Chrompack, Frankfurt a. M., Germany) and Carboxen 1006 Plot column (30 m×0.32 mm I.D., Supelco). The temperature of the CP Sil column was held at 5°C for 2 min (cooled with dried ice) after injection, subsequently heated to 40°C by 2°C/min and thereafter to 280°C with a heating rate of 10°C/min. This final temperature was maintained for 5 min (run-time 48.5 min). The temperature of the Carboxen Plot column was held at 30°C for 2 min and heated to 200°C by 10°C/min. This final temperature was maintained for 15 min (run-time 39 min). The interface between microwave desorber and gas chromatograph was heated to 150°C and the injection port was kept at 250°C.

The separated substances were identified by MS, (HP 5988A and HP 6890 Series MSD, Hewlett-Packard) and by FID (Hewlett-Packard). The FID system was fed with hydrogen 40 ml/min, synthetic air 400 ml/min and helium 40 ml/min as make-up gas. Helium was used as carrier gas with a flow velocity of 40 cm/s. The column pressure was 0.6 bar. For measurements using the mass spectrometer the column pressure was maintained at 0.5 bar with a split ratio of 10. Reducing the split ratio resulted in higher signal intensity but lower reproducibility. In general the selected ion monitoring (SIM) technique was used. The following ions, representative for certain groups of substances were chosen for monitoring in the multiple selected ion mode: m/z 31 and 45 characteristic for alcohols, m/z 58 for ketones, m/z 43 and 57 for hydrocarbons, m/z 77 and 91 for aromates, and m/z 51 for volatile anaesthetics. The mass 18 m/z had to be faded out due to the presence of water vapor. Quantitative analysis was performed by plotting the peak area of the respective compound of the calibration mixture against its known concentration.

2.5. Direct injection versus microwave desorption technique

To compare the direct injection to the microwave desorption technique 10 μl of the gaseous calibration mixture were directly injected into the gas chromatograph under constant pressure. The resulting gas chromatogram was compared to that obtained by microwave desorption of a charcoal filled sampling tube loaded with an equal amount of the gaseous calibration mixture.

2.6. Regeneration of the sampling tube

Total removal of substances not desorbed (>5%) could be achieved by exposing the sampling tube to a desorption power of 1000 Ah 10^{-6} and a flow of carrier gas of 100 ml/min for 20 s. The tubes could be reused 50–100 times with no adverse effects on the gas chromatographic results.

2.7. Application of the method in a clinical setting

The method was tested in two different patient populations. Patients without lung pathology undergoing isoflurane anesthesia for elective surgery and patients suffering from acute lung injury. The injury

is characterized by an inflammatory response of the lungs to various internal and external noxious stimuli.

Approximately 1000 ml inspired and expired air were drawn through an activated charcoal containing sampling tube for 3 to 5 min by means of a roller pump (Cole-Parmer Instruments, Niles, IL, USA). Sample volume and gas flow through the sampling tube were measured by collecting the gas under water and recording the time of measurement (volumetric water trap). To avoid any contamination the gas volume was measured distal from the roller pump. The air collecting system (sampling tube, roller pump, volumetric water trap) was mounted in parallel to the respiratory circuit. The connections to the respiratory circuit consisted of t-shaped pieces equipped with Luerlock connectors made either of stainless steel or PTFE. Tubings made of PTFE were used to connect the sampling tube to the t-shaped pieces. Samples of inspired and expired air were taken at standardized sites of the respiratory circuit.

The amount of adsorbed water of 1000 ml of exhaled air was determined gravimetrically. No filters or traps were incorporated into the sampling system. For assessment of the adsorption stability different volumes of hydrocarbon-free air were drawn through the sampling tube exposed to exhaled air before. The thermal stability was studied by storing the sampling tube exposed to exhaled air at room temperature and at increased temperatures (60°C).

The unknown substances were identified by comparing their retention times and their sequence of elution to known test substances. The results obtained by MS were confirmed by submitting the respective commercially available test substance to the same analytical procedure. Calibration mixtures were prepared as described in Section 2.1. A calibration curve was constructed by plotting substance concentrations against corrected peak areas. For quantitative analysis GC peak areas were transformed into substance concentrations by means of the calibration curve. The ratio between the mean area and the area of the internal standard in the individual sample was calculated. If it differed from 1, the peak area of the substance to be determined was corrected by that factor.

2.8. Chemicals and materials

2-Methylbutane 59060, *n*-pentane 76870, 2,2-dimethylbutane 39730, 2,3-dimethylbutane 39760, 2-methylpentane 68310, 3-methylpentane 68320, *n*-hexane 52750, 2,2-dimethylpentane 41060, 2,4-dimethylpentane 41090, 3,3-dimethylpentane 41120, 2-methylhexane 67360, 3-methylhexane 67370, *n*-heptane 51730 were from Fluca (Neu Ulm, Germany). The chemicals were of gas chromatographic degree. Isoflurane, enflurane, methoxyflurane were from Abbott (Wiesbaden, Germany). Isoprene 98% (Riedel-de Haën, Seelze, Germany), methanol, ethanol, 2-propanol (all analytical-reagent grade) were from Merck (Darmstadt, Germany). Hydrogen 5.0, helium 5.0 and nitrogen 4.6 were from Sauerstoffwerk (Friedrichshafen, Germany). Synthetic air was from Messer Griesheim (Rheinfelden, Germany).

3. Results

3.1. Adsorption and desorption

The quality of adsorption depends on the adsorption material and the gas flow. Activated charcoal 1000–1500 m^2/g is very hydrophilic and adsorbs very volatile substances. At a gas flow of 200 ml/min activated charcoal adsorbs lipophilic hydrocarbons almost 100% and the hydrophilic substances ethanol and acetone up to 95% and 93%, respectively. Gas flows greater 500 ml/min reduce the amount of highly volatile substances. The adsorption stability of activated charcoal is satisfactory. Purging the tube with 2000 ml of hydrocarbon-free air reduced the peak areas only insignificantly. The activated charcoal loaded tubes exposed to exhaled air showed good thermal stability when tightly sealed. Even at 60°C there was no loss of the adsorbed substances. The sampling tubes can be stored several days at room temperature without loss of substances to be determined. Adsorption of 1000 ml exhaled air saturated with water vapor was associated with a weight gain of the sampling tube of nearly 10 mg. Retention times and peak areas were not affected by that amount of water.

Graphitized activated carbon 120 m^2/g is hydro-

phobic, adsorbs the smallest amount of water, and is suited for substances with a boiling point above 50°C. Tubes filled with graphitized carbon molecular sieve suffered from a loss of very volatile substances.

Carboxen 1000 m^2/g is very hydrophobic, acts as graphitized carbon molecular sieve and is suitable for highly volatile substances (hydrocarbons C_2–C_8, benzene, styrene).

The low boiling substances like isoprene were desorbed from activated charcoal at a range of 96±4% during the first desorption step. Heptane and methoxyflurane were liberated at a range of 90±4%. The desorption range was constant in the concentration range of interest (1 pmol–100 nmol). Tubes filled with graphitized activated carbon did not produce reproducible results in the analysis of exhaled air. Sampling tubes filled with Carboxen are difficult to clean. For these reasons we used activated charcoal 1000–1500 m^2/g as adsorption material for further studies.

A representative gas chromatogram (GC–FID) of the calibration mixture is shown in Fig. 1 and the retention times of the different compounds in Table 1.

A linear correlation between concentration and peak area of the volatile constituents of the calibration mixture was found over the whole range of interest. The correlation coefficients were always greater than 0.92 (Fig. 2). The variability of the peak area of the internal standard 2,2-dimethylbutane was 100±22 pA s (mean±standard deviation) resulting in a variation coefficient of 0.22.

The reproducibility of the measurements was improved by increasing the backflush (time) which affects the removal of ambient air and any substance not bound to the adsorption material prior to the microwave desorption. An increasing backflush reduced the amount of highly volatile substances only minimally. The energy of the microwave pulse (desorption power) determines the temperature dissipated to the tube. This parameter had only little effect on the chromatogram. The lowest available energy was sufficient for complete desorption of the volatile substances of interest.

Retention times of the substances did not differ between the direct injection technique with a split of 1:10 and the microwave desorption technique (Fig. 1). Relative peak areas normalized to the internal standard were identical for the microwave desorption and the direct injection with the exception of peak 6 (isoprene) and peak 20 (hexanal).

The peaks appearing in the very beginning of the chromatogram (Fig. 1A) are the result of the impact of the microwave pulse onto the activated charcoal. Microwave desorption of tubes containing only activated charcoal liberated substances of very low molecular mass. High desorption energy leads to an increased discharge of compounds produced by pyrolysis of the activated charcoal. The released substances were identified as acetylene, ethene, ethane, carbon monoxide, carbon dioxide and water (using a Carboxen 1006 Plot column for gas chromatographic analysis) (Fig. 3). Consecutive desorption processes resulted in the release of constant quantities (up to 30 pmol) of hydrocarbons depending only on the applied microwave energy and the humidity of the activated charcoal. Therefore, activated charcoal is not suited for studies where acetylene, ethylene or ethane are to be investigated.

Representative gas chromatograms of inspired and expired air of a mechanically ventilated patient suffering from acute lung injury are shown in Figs. 4 and 5. Constituents of inspired and exhaled air and their concentrations are listed in Table 2. Even several days after an isoflurane anaesthesia substantial concentrations of the volatile anaesthetic were found in patients' exhaled air (Fig. 6).

In contrast to FID, SIM chromatograms resulted in sharp peaks and very low background as shown in Fig. 7.

4. Discussion

The method described overcomes most of the obstacles associated with previously published procedures which rendered them cumbersome and less reliable for clinical studies. The method does not require cryofocusing because the sample is very quickly heated (300°C/s) [8]. Direct loading of the expired gas onto activated charcoal avoids the problems associated with condensation of volatile constituents onto the wall of the air collecting vessel.

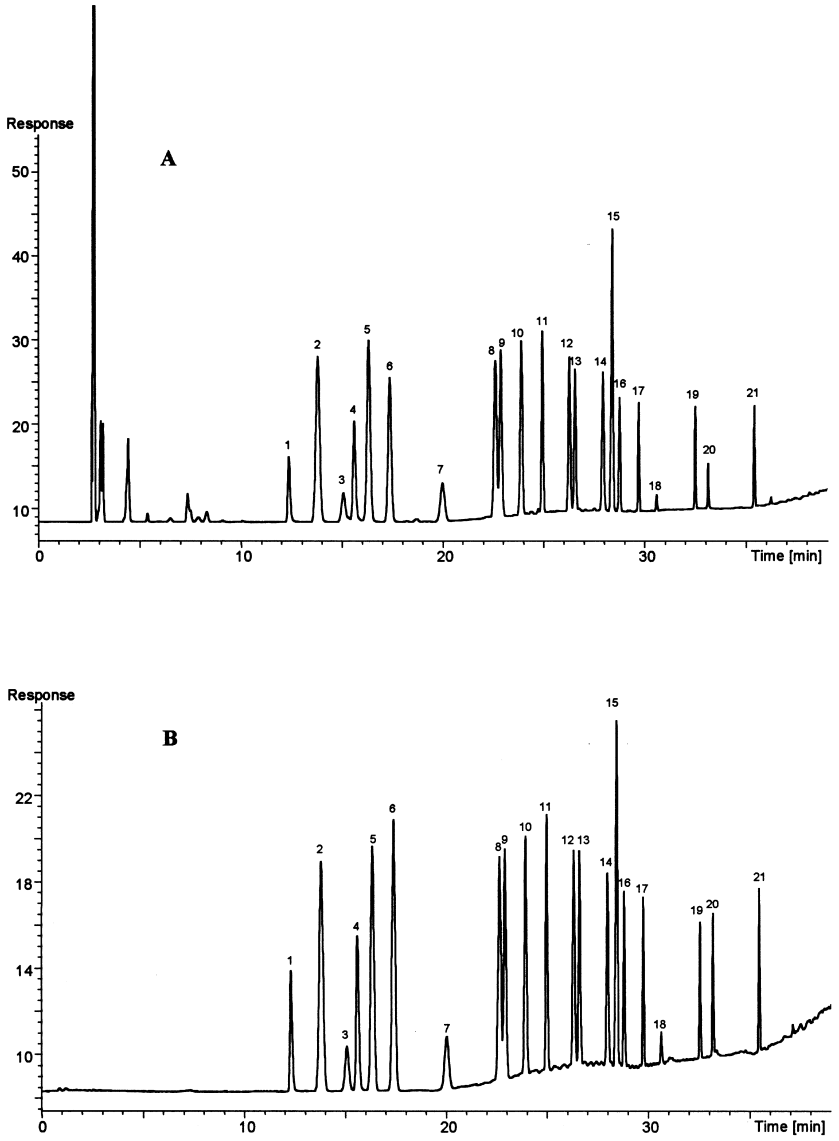

Fig. 1. (A) Gas chromatogram (GC–FID) of the gaseous calibration mixture using the microwave desorption technique. (B) Gas chromatogram (GC–FID) of the gaseous calibration mixture using the direct injection technique. Peak numbers as listed in Table 1.

Various methods have been published in the past for collecting gas in different containers [9,10]. The advantage of such collecting techniques is the possibility to sample large gas volumes within a short time which can serve numerous measurements. The disadvantage of collecting gas in a bag rather than loading it directly onto an appropriate adsorption medium is the loss of material due to the adsorption onto the wall of the collecting bag. Moreover, filled bags are voluminous and difficult to handle. The regeneration is complicated by the condensed water vapor on the wall, and they have to be purged with purified gas at least 10 times before they can be reused.

Constituents of exhaled air are best analysed by spectrophotometry and MS [11]. Most volatile

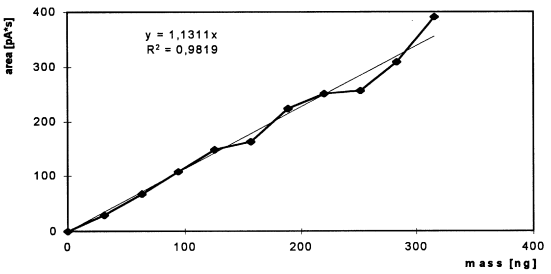

Fig. 2. Correlation between concentration and peak area of pentane analysed by microwave desorption technique (GC–FID).

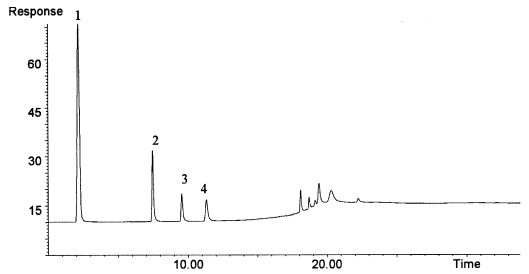

Fig. 3. Pyrolysis products after mircowave desorption of a blank sampling tube filled with activated charcoal, analyzed by a Carboxen 1006 Plot column (1=carbon monoxide, 2=acetylene, 3=ethylene, 4=ethane).

agents, however, cannot be directly analysed because of their very low concentrations present in the gas sample. Different processes like condensation in cryogenic traps or adsorption onto surface active materials (Tenax, activated charcoal) were used for enrichment in the past. All these procedures are hampered by problems of selectivity, discrimination of certain substances and completeness of adsorption and desorption.

Gas can be adsorbed onto silica gel, activated charcoal, molecular sieves and porous polymers [12]. It is difficult to find a material that allows complete adsorption as well as easy desorption of all volatile components present in exhaled air. Organic polymers do not have sufficient thermal stability for use in the microwave desorber. Only activated charcoal is stable enough and has suitable dielectric properties for use in the microwave desorber.

Problems associated with the analysis of ultra trace amounts of halocarbons in gas samples have been investigated by Bruner et al. [13]. The main problem is the fast and complete adsorption of all volatile constituents in a given gas volume and the transfer into the analyser without any loss.

Large amounts of water vapor in gas samples introduce a major problem into the chemical analysis of exhaled air. Silica gel looses its capacity of adsorbing organic substances in the presence of excess of water vapor [12]. The concentration of volatile substances in exhaled air is very low in relation to the quantity of condensed water (50 mg per liter of breath). Therefore Ghoos et al. [14] developed a system that should avoid water contami-

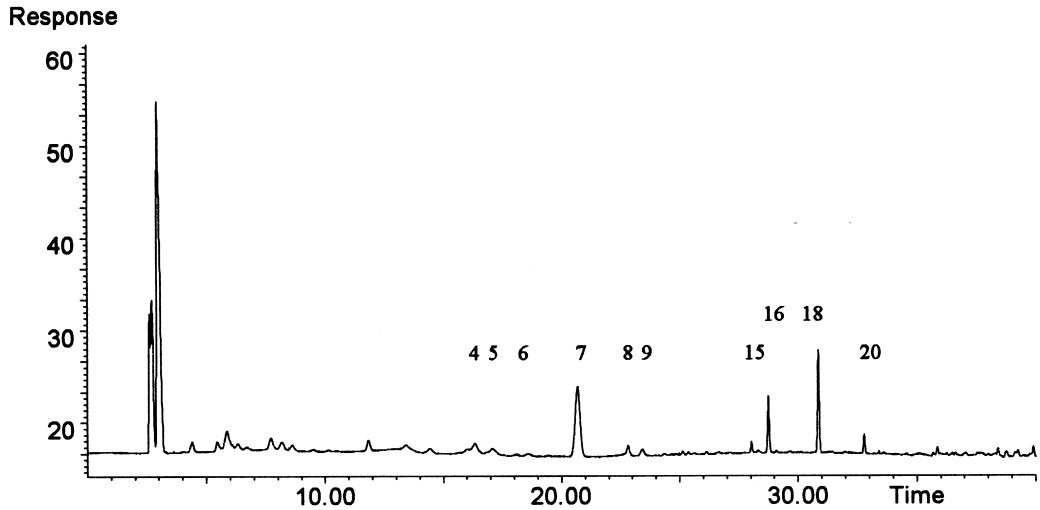

Fig. 4. Gas chromatogram of inspired air of a critically ill patient suffering from acute lung injury. Peak numbers as listed in Table 1.

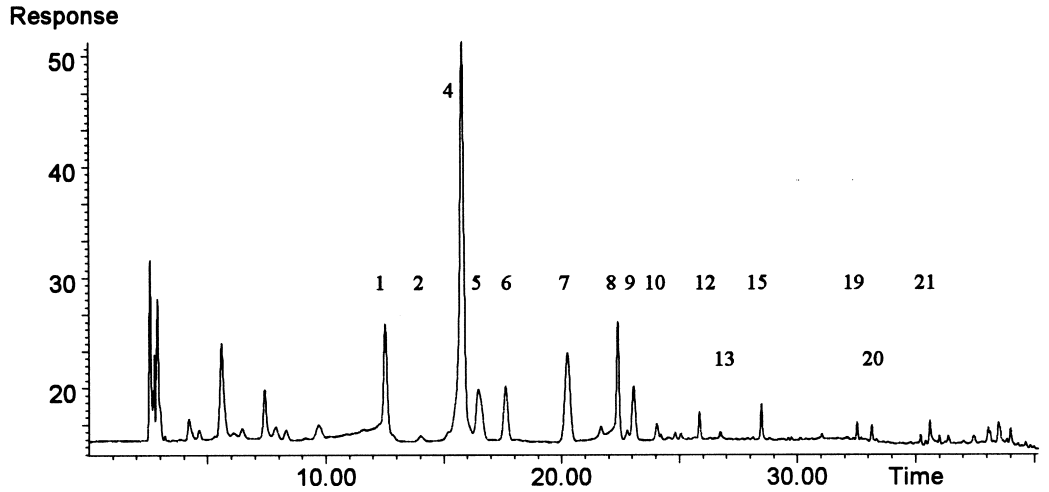

Fig. 5. Gas chromatogram of expired air of a critically ill patient suffering from acute lung injury. Peak numbers as listed in Table 1.

nation during the thermodesorption– cryofocusing procedure. In preliminary experiments we have tried to remove excess water vapor from the expired breath by using a series of filters, wash bottles, drying units or cryogenic traps. All the water removing devices proved to be of no value because they also removed hydrophilic and hydrophobic volatile substances in an unpredictable manner. Since the amount of water vapor had only little effect on the microwave desorption and the gas chromatographic analysis filters are not required for the method described.

In the past analysis of biological gas samples was performed using a thermodesorption–cryofocusing technique. Freezing of water in capillaries occurs frequently during cryofocusing if gas samples contain water vapor. This problem that can considerably complicate the analytical process is completely eliminated by the microwave desorption technique. Chemical reactions such as pyrolysis, isomerisation and other conversion reactions are favoured by high temperatures as occurring during microwave desorption. Rektorik [15] addressed this particular problem by studying the thermal conversion during microwave desorption. He reported that the concentration of the second isomer of γ-terpinene, a substance that is very sensitive to thermal conversion, showed only a small increase during microwave desorption. Com-

Table 2
Constituents found in inspired and expired air of patients suffering from acute lung injury with retention times (ret. time), representative ions (m/z) in case of mass spectrometrical detection and concentrations (conc.)

Substance	Ret. time GC (min)	Ion (m/z)	Conc. in expired air (nmol/l)	Conc. in inspired air (nmol/l)
Methanol	7.30	31	0.2–20	0–1.0
Ethanol	12.26	31	0.2–10	0–1.0
Isoflurane	15.09	51	0–1000	0–0.01
Acetone	15.61	43; 58	3–500	0–6.0
Pentane	16.42	43; 57	0.1–5.0	0.1–1.0
Isoprene	17.47	67	0.3–7.0	0–0.1
2,2-Dimethylbutane (I.S.)	20.11	43; 57	0.3	0.3
Benzene	28.35	78	0.01	0.01
Methoxyflurane	30.51	81	0.0–50	0.0
Toluene	32.42	77; 91	0.01	0.01
Hexanal	32.90	56	0.01	0.002

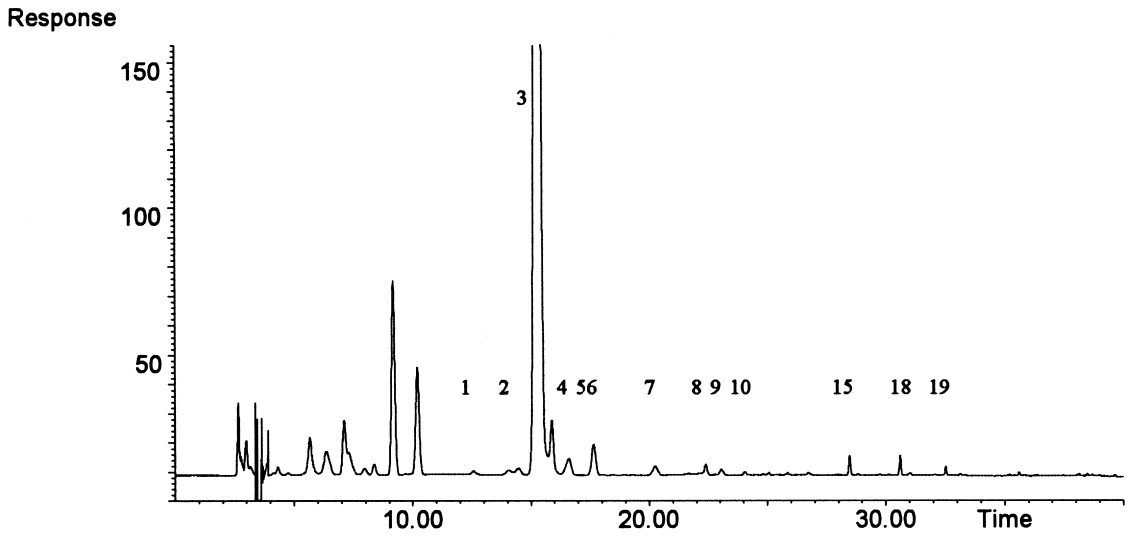

Fig. 6. Gas chromatogram of expired air three days after an isoflurane anaesthesia. Peak numbers as listed in Table 1.

paring microwave desorption to direct injection (split 1:10) we recognized that the very volatile substances ethane, ethylene and acetylene were generated by impact of the microwave pulse onto the activated charcoal in presence of water. Assessment of exhaled concentrations of the above mentioned substances, therefore, is not possible by means of charcoal adsorption followed by microwave desorption. Retention times for all substances of the calibration mixture did not differ between microwave desorption and direct injection, and relative peak intensities were identical except for isoprene [6] and hexanal [20]. The apparent loss of isoprene and hexanal during microwave desorption was most probably due to heat induced polymerisation of isoprene and reactions of the aldehyde body of hexanal, respectively. The extent of thermal conversion, however, was not sufficient to affect accuracy of quantitative analysis of isoprene and hexanal.

The regeneration of the activated charcoal in the sampling tube is of great advantage for routine use because it can easily be cleaned with the same microwave desorber, and the tube can be reused up to a hundred times. Unfortunately, an autosampling device is not yet available for the microwave desorber.

Increasing the desorption time resulted in an increase in peak intensity. The reason for that is not the prolongation of the desorption pulse since the peak intensity depends only on the time during which the sampling tube is in line with the mainstream of the carrier gas. The lowest energy is sufficient for complete desorption. Prolongation of the bypass delay which is the duration of gas flow through the sampling tube following the microwave pulse enhanced the peak intensity for the same reason. The effect on the sharpness of the peaks is only minimal. For analysis of highly volatile substances the best results were obtained with a low desorption power and a long desorption time.

For the transfer of released substances onto the GC column a minimum flow of 10 ml/min of carrier gas has to pass through the sampling tube during desorption. Since the flow through the GC column is limited by its diameter only a fraction of the total gas flow can be used for analysis. Therefore the microwave desorber in combination with a capillary column has always to operate in a split mode. The split (quotient of total gas flow through the sampling tube to gas flow through the GC column) leads to a loss of intensity but not to a discrimination of the individual peaks.

FID is a sensitive system for analysing hydrocarbons. One advantage of this system is the low sensitivity of the detector towards water vapor, oxygen, nitrogen and carbon dioxide. Otherwise

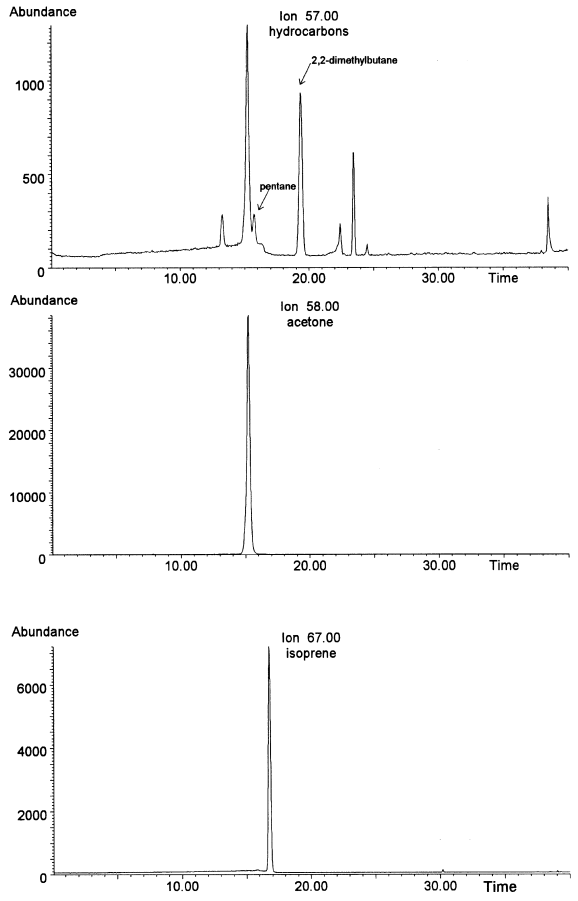

Fig. 7. SIM chromatograms of expired air of a critically ill patient suffering from acute lung injury. Extracted ion chromatograms of three different ions (m/z 57, 58 and 67) representative for alkanes, acetone and isoprene are shown.

these substances occurring in high quantities in exhaled air would interfere with the detection of the organic substances.

Detection of carbon disulfide or sulfur hexafluoride is not possible with FID but detectable with an electron-capture detector or a mass spectrometer. The mass spectrometer is the only suitable device for identification of unknown substances in the expired gas. With the SIM technique the sensitivity was about 10-times higher than it was with FID. However, even after 10 consecutive desorption steps a marked signal of water could be detected by the mass spectrometer. Therefore the mass 18 m/z had to be faded out for this kind of measurements. Best results were obtained by monitoring multiple selected ions. Eight different ions (Table 2) were chosen representative for certain groups of substances such as alcohols, aldehydes, hydrocarbons, aromates, volatile anaesthetics. Plotting chromatograms of single ions resulted in sharp peaks and very low background (Fig. 7).

A more efficient method of quantification can be achieved by adding an internal standard to the exhaled gas sample or by administering to the patient. That can be accomplished by a steady state infusion of a very volatile substance having a small distribution volume like nitrous oxide. Another possibility is the application of a lipophilic substance being very slowly eliminated from the body. Patients who received isoflurane or another volatile anaesthetic for an operation exhibited nearly stable concentrations of the respective agent in the exhaled air for a few hours.

The method has been tested in mechanically ventilated patients suffering from acute lung injury. The substances found in the inhaled and exhaled air are summarized in Table 2.

Pentane is synthesized in the body and metabolized in liver microsomes by cytochrome-P450 [16]. Moodie and Labadarios [17] found pentane levels in healthy female subjects varying from 0.62 to 3.16 nmol/l and in healthy males from 3.20 to 8.76 nmol/l. Sexton and Westberg [18] reported pentane concentrations in the air of urban centers ranging from 110–340 pmol/l.

Our findings are in agreement with Cailleux and co-workers [19–22]. The high levels of pentane found in healthy humans by other investigators [23] are probably the result of inappropriate chromatographic techniques being unable to separate pentane from isoprene, a volatile hydrocarbon always present in human breath [24]. The concentrations of pentane and isoprene we found differ significantly from those reported by Kohlmueller and Kochen [9]. That difference can be explained by patient population. They studied spontaneously breathing healthy subjects, while our patients were critically ill and mechanically ventilated. Guilbaud et al. [25] believe that pentane is a reliable index of lipid peroxidation in vivo. Their lowest detectable level of pentane was 8 pmol [25]. They observed a significant increase of

exhaled pentane in patients with rheumatoid arthritis and in rats with CCl_4 intoxication.

The exact origin of isoprene is unknown. Stone et al. [26] consider isoprene a normal constituent of human breath originating from the cholesterol synthetic pathway, while Stein and Mead [27] believe that isoprene is formed by peroxidation of squalene. The findings of Foster et al. [28] suggest that exhaled isoprene might serve as a marker of a physiological response to an oxidant injury to epithelial membranes and fluid linings of the lower respiratory tract.

Cailleux et al. [20] observed a decrease of the isoprene concentration in blood during general anaesthesia. The isoprene concentrations in human blood are quite different from those in rabbit, dog, ewe and cow blood [21]. In all animal species the isoprene concentration is always lower than 1 nmol/l whereas in humans it ranges from 15 to 70 nmol/l.

There was no difference between the inspiratory and expiratory concentrations of hexanal in our patients suggesting that hexanal does not originate from within the body. Cecchini et al. [29] found several compounds with carbonyl functions among them hexanal and 4-hydroxy-nonenal using high-performance liquid chromatography. They suggested that these substances represent peroxidative breakdown products of lipid membranes. Frankel et al. [30] looked for the presence of hexanal in rat liver using a capillary gas chromatographic method, since it is an important volatile metabolite of hydroperoxides formed from polyunsaturated fatty acids.

No organic sulfide could be detected in our patients. Phillips and co-workers [31,32] found carbon disulfide (CS_2) in exhaled air (mean concentration of 5.25 pmol/l) as well as in ambient air (mean concentration 8.26 pmol/l indoor and 3.92 pmol/l outdoor). The same authors observed significantly higher alveolar gradients of pentane and carbon disulfide in patients with schizophrenia than in the control group [33]. They concluded that schizophrenia may be accompanied by accelerated lipid peroxidation in cell membranes as well as by increased production of CS_2. Patients with liver cirrhosis have a fivefold increase in dimethylsulfide concentration in the expired air (113.4 ± 31.9 ng/l; normal 21.1 ± 1.7 ng/l) [5].

All gas samples contained ethanol and methanol. Their concentrations varied widely. After ingestion of 5 g ethanol the blood concentration blood increases up to 200-fold [34]. After disinfection with alcohol containing solutions laboratory results may considerably be affected.

Acetone levels can be detected in human breath in a wide range of variation. The highest values are found in patients suffering from uncontrolled diabetes mellitus.

Following anaesthesia with isoflurane that anaesthetic could be detected in the exhaled air for several weeks. Anaesthesia machines and delivery systems exposed to a volatile anaesthetic may liberate it in trace amounts even after thorough decontamination.

5. Conclusions

The method described overcomes methodological problems that rendered previous techniques cumbersome and unreliable. It allows quantitative analysis of gas samples with low substance concentrations. The method is well suited for clinical studies because the microwave desorption technique eliminates the problems associated with the gas collection and the high water content in the expired breath. Correlation of the concentration of volatile substances in exhaled air and in blood may allow better insights into metabolic processes of the body and the diffusion of substances across the alveolar membrane.

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