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VOLATILE ORGANIC COMPOUNDS IN THE EXHALED BREATH OF YOUNG PATIENTS WITH CYSTIC FIBROSIS

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ABSTRACT

Inflammatory mediators in the exhaled breath are receiving growing medical interest as non-invasive disease markers. Volatile organic compounds have been investigated in this context, but clinical information and methodological standards are limited.

We measured the levels of ethane, propane, n-pentane, methanol, ethanol, 2-propanol, acetone, isoprene, benzene, toluene, dimethylsulfide and limonene in repeated breath samples from 20 cystic fibrosis patients and 20 healthy controls (age 8-29 years). Three end-exhaled and one ambient air sample were collected per person and analysed on a customised gas chromatography system.

Intra-subject coefficients of variation ranged between 9-34%, and hydrocarbon breath levels were influenced by their inspired concentrations. The alveolar gradient for pentane was higher in cystic fibrosis patients than in healthy controls (0.36 vs. 0.21 ppb, *p=0.04*) and inversely proportional to FEV_1 (r=-0.62, *p=0.004*), highest values were observed in patients with pulmonary exacerbations (0.73 vs. 0.24 ppb, *p=0.006)*. Cystic fibrosis patients also exhibited a lower output of dimethylsulfide (3.9 vs. 7.6 ppb, *p=0.003*). Group differences were not significant for ethane and the remaining substances.

We conclude that chemical breath analysis for volatile organic compounds is feasible and may hold potential for the non-invasive diagnosis and follow-up of inflammatory processes in cystic fibrosis lung disease.

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INTRODUCTION

More than 30 years after Pauling et al. described the abundance of volatile organic compounds (VOC) in human breath [1], regular automated measurement has only been realised for ethanol in the context of traffic-related toxicology. Other exhaled compounds have attracted attention in a medical context: Ethane and n-pentane were linked to the *in vivo* level of lipid peroxidation and oxidative stress [2-4]; breath acetone was shown to correlate with the metabolic state of diabetic patients [5] or mice on a ketogenic diet [6]; and a decrease in exhaled isoprene was reported shortly after ozone exposure [7] and in acute pulmonary exacerbations of cystic fibrosis [8]. Yet despite a growing scientific focus on nitric oxide and other exhaled biomarkers of pulmonary disease [9, 10], data on breath VOC remain scarce and important methodological questions unanswered such as the standardised collection, handling and analysis of human breath samples. Gas chromatography as the most appropriate method can be affected by the high water and carbon dioxide content of breath samples, and no standard has been established regarding the choice of preconcentration procedures, temperature programs, column and detector [11]. This has impeded the development of biochemical breath analysis as a clinical tool.

Lung disease in cystic fibrosis (CF) is characterised by chronic airway inflammation, retention of viscous secretions, bronchiectasis, and often bacterial infection [12, 13]. These factors contribute to a variable clinical course with progressive bronchial obstruction and hyperinflation. Scoring systems based on the level of symptoms and findings from X-ray, microbiology and pulmonary function tests may be helpful in a research setting and for defining a pulmonary exacerbation. Bronchoscopic lavage or biopsy can be applied for the objective assessment of inflammatory processes, but the procedural risks are often not justified. Hence, most treatment decisions remain based on clinical judgement and secondary

parameters derived from pulmonary function testing, chest radiography or blood analysis [14].

We hypothesised that exhaled VOC can be accurately and reproducibly determined in breath samples from CF patients and matched healthy controls, and that the pulmonary exchange rate of these potential biochemical markers is related to other descriptors of CF lung disease.

METHODS

Subjects

Fifteen patients were recruited in a stable state during scheduled outpatient visits to Aachen CF center, and five hospitalised patients were included during intravenous antibiotic treatment for pulmonary exacerbations. Details on their medical history, current status and environmental tobacco smoke exposure were gathered from chart records and a structured interview. Long-term medication was unchanged over the previous month and included inhaled antibiotics in 5, DNase in 4 and inhaled steroids in 7 patients; six subjects received insulin due to CF-related diabetes.

Twenty healthy subjects free from chronic lung disease or respiratory tract infection in the preceding month served as control group. All subjects were non-smokers; distribution of age (8-29 years), gender (11 females each), and height were comparable between groups. However, CF patients had significantly lower body mass index Z-scores (-0.8 vs. +0.3, reference data from [15]). Spirometry was normal in all control subjects (median FEV_1 108% predicted, range 85-138), but revealed a varying degree of bronchial obstruction in the CF group (median FEV_1 65%, range 21-105).

All test persons (and legal guardians if applicable) gave their written informed consent, and the study protocol had been approved by the local ethics committee.

Breath sampling

Prior to the investigation, subjects were asked to refrain from eating and strenuous physical activity for at least 3 hours. After 10 minutes of rest in the study room, they were instructed to deliver their exhaled breath through a steel mouthpiece into 6-Liter canisters of electropolished stainless steel with a fused-silica inner lining (SilcoSteel®, Supelco Co., Bellefonte PA, USA). These had been pre-conditioned by fourfold evacuation to <5 hPa and

subsequent pressurisation with pure synthetic air to 3,000 hPa; followed by a final evacuation. Residual VOC content was assessed by gas chromatography, canisters were only released if the integrated peak areas of all remaining signals corresponded to a total VOC volume mixing ratio <20 ppt.

The target breathing maneuvre consisted of a deep inspiration and a 5-second breathhold followed by slow and complete exhalation over 10 seconds. Breath air was discarded during the first two seconds of exhalation and then directed into the canister by a magnetic valve. This was repeated over 1-3 breaths, typically producing final pressures of 300–400 hPa in the canister. Per session, three breath samples were collected at 15-minute intervals together with one sample of room air. All measurements were performed in a window-less conference facility inside Aachen University Medical Center, controlled by a central ventilation system and without disinfectant dispensers or frequent person traffic. Gas samples were transported to Research Center Jülich within 24 hours of collection for the determination of VOC content.

Analytical procedure

After measuring their initial gas pressure, canisters were pressurized to 3,000 hPa with pure synthetic air (quality 5.0 = 99.999 %) in order to further reduce the relative humidity of the sample. Gas specimens were then analysed using a gas chromatograph (HP 6890, Hewlett Packard Co., Palo Alto CA, USA) and a specially designed sampling manifold as previously described [17] and illustrated in *Figure 1*. Trace species from a sample volume of 800 cm^3 were pre-concentrated at a flow rate of 80 ml/min on a sample loop (20 cm length, ID 2 mm) packed with glass beads of 0.25 mm diameter at liquid nitrogen temperature. Subsequently, the sample was thermally desorbed at 120°C and injected on a capillary column (DB-1, 120 m x 0.32 mm ID, 3 µm film thickness). After injection, the column was kept isothermal at -60°C for 5 min, then heated up to 200°C at a rate of 5°C per minute and finally maintained at

200°C for 15 minutes. Signals were gathered from a flame ionisation detector which received 98% of the column output through a split valve. The remaining flow was directed to an ion trap mass spectrometer (Saturn 9000, Varian Inc., Palo Alto CA, U.S.A) used for the identification of specific peaks. In the order of elution from the column, signals for ethane, propane, methanol, ethanol, n-pentane, acetone, 2-propanol, isoprene, dimethylsulfide (DMS), benzene, toluene and limonene were identified and the corresponding peak areas were determined by semi-automated integration. All readings were quality-controlled by the same experienced investigator (BM), *Figure 2* shows a typical breath chromatogram. Analysis of one sample lasted for about 90 minutes, and sets of ten canisters could be analysed on the system in unattended operation.

VOC volume mixing ratios were finally calculated from the product of their peak area and the respective mass response factor. These factors were derived from analyses of a 74 compound high-precision mixture containing alkanes, aromatics, terpenes, aldehydes and ketones at known mixing ratios between 1 and 5 ppb (Cylinder CC169190, Apel-Riemer Environmental Inc., Denver CO, USA). Calibration measurements were performed every 1-2 weeks and showed extremely stable retention times and mass response factors with negligible variation and no drift across the study period.

Experimental validation

Before engaging in the clinical protocol, we studied the potential impact of humidity in the exhaled breath on our system's analytical performance. To this end, we added 50 µL of ultrapure water to 500 hPa of a calibration gas sample, stored pairs of humidified and dry standard mixtures for one day and measured them on the gas chromatography system as described above. The resulting chromatograms (illustrated in *Figure 3*) showed identical retention times; it should be noted that the few peaks with apparent reduction in the

humidified mixture belong to substances that were not analysed in the exhaled samples. Furthermore, only minimal variation in peak amplitude was observed for the compounds under investigation (as shown in *Figure 4*). Slightly higher relative errors for methanol may be attributed to its low relative content in the standard mixture (5 ppb or approximately 3% of exhaled concentrations).

Error estimation

Experimental uncertainties can be introduced by a number of factors and are an important consideration. We assessed the impact of canister transport and storage by analysing the VOC content in parallel samples of gas standards, ambient or exhaled air after 0, 1, 2 and 3 days. Results from a total of 30 measurements consistently showed that the mixing ratios of nonmethane hydrocarbons, aromatic compounds, DMS and terpenes were stable within 5% over 2 days compared to an instantaneously analysed sample. Alcohol concentrations differed by up to 10% in breath samples.

In addition, measurement accuracy depends on the quality of the calibration standard (≤5% between true and declared gas concentrations, Apel-Riemer Environmental Inc.) and of the mass flow controller used for diluting the calibration gas (≤2% deviation, MKS Instruments, Wilmington MA, USA). Analytical accuracy is affected by uncertainties of the calibration function and the graphical analysis. The linear regression calculated across a number of dilution steps of the standard mixture carries a relative uncertainty of <5% for the individual species. Uncertainties of the peak areas are caused by their reproducibility (known to lie within ≤4%), and by an integration uncertainty estimated at <6%. The integration uncertainty is highest at low concentration levels. Geometric addition of all these factors yielded an overall experimental uncertainty of 12%.

Statistics

Reproducibility of breath measurements was assessed by calculating intra-subject coefficients of variation across the three successive samples. Group comparison was performed by Mann-Whitney-U-test, associations between parameters were investigated by linear regression analysis. $P<0.05$ was considered statistically significant, calculations were performed using SPSS 11.0 for Windows software (SPSS Inc., Chicago IL, USA).

RESULTS

Breath sampling proved to be feasible in all subjects without discomfort or adverse events. Due to chromatographic interference, exhaled concentrations were unavailable for 2-propanol in 4 samples and limonene in one sample. DMS could not be detected in all but one ambient probe. On all other occasions, discrete volume mixing ratios were determined for the compounds specified above. Their short-term reproducibility was generally good, average intra-subject coefficients of variance across the three consecutive measurements ranged from 9% for acetone up to 34% for ethanol (cf. *Table 1*). A significant and linear influence of ambient conditions on exhaled concentrations was demonstrated for ethane (r=0.63), propane (r=0.72), pentane (r=0.6), and methanol (r=0.59, all with $p<0.001$). Therefore, subsequent analyses were based on the alveolar gradient of a specific compound: $\Delta[VOC_i] = [VOC_i]_{breath} - [VOC_i]_{room}$. These differences are equivalent to the endogenous rate of production or absorption of a specific compound per unit ventilation rate; assuming a steady state equilibrium between body tissues, lungs and expired gas. Values for exhaled, ambient and $\Delta[VOC_i]$ are given in *Table 2*.

Group comparison showed a significantly higher pentane output ($p=0.04$), lower DMS production ($p=0.001$) and higher 2-propanol uptake ($p=0.003$) in CF patients versus healthy controls. No significant differences were observed for ethane, propane, methanol, ethanol, acetone, isoprene, benzene, toluene or limonene (cf. *Table 2*). Among cystic fibrosis patients only, correlation with FEV_1 (expressed as % predicted) was demonstrated for breath toluene (r=0.72, $p<0.005$) and Δ[pentane] (r=-0.62 and $p<0.005$, illustrated in *Figure 5*). Comparison between CF subjects on i.v. antibiotics and stable outpatients revealed significantly higher values for Δ[pentane] in the former group (0.73 *vs.* 0.26 ppb, $p=0.007$). Furthermore, Δ[pentane] was higher in patients with malnutrition (r=-0.46, $p=0.04$ for linear regression to BMI Z-score) or chronic *Pseudomonas* infection (n=16, $p<0.001$). The breath of diabetic

patients contained more isoprene (141 vs. 89 ppb, $p=0.003$) and less DMS (1.43 vs. 4.95 ppb, $p=0.04$) or methanol (109 vs. 229 ppb, $p=0.04$) than in CF subjects with normal glucose tolerance. Inhaled antibiotic treatment was associated with lower Δ[ethane] (-0.49 vs. 0.68 ppb, $p=0.001$), and patients with domestic tobacco smoke exposure (n=9) exhibited higher values for Δ[methanol] (281 vs. 120 ppb, $p<0.01$) and exhaled or Δ[DMS] (6.1 vs. 2.1 ppb, $p<0.01$). Exhaled VOC were not significantly associated with atopic status, treatment with inhaled steroids and DNase, or with CF genotye (stratified according to the number of delF508 alleles).

DISCUSSION

In this study, we measured ambient and exhaled breath concentrations of 12 volatile trace gases in children, adolescents and young adults who were either healthy or affected by chronic airway inflammation due to cystic fibrosis. Based on the assumption that different breathing patterns might affect exhaled parameters, as recently demonstrated by Cope et al. [16], we developed a standardised breathing manoeuvre to define the degree of in- and expiratory gas mixing and ensure an air sample of alveolar origin.

Exhaled gas was delivered into chemically inert containers through a custom-built device. Sampling and analytical technology with a single column-two detector gas chromatography system were adapted from atmospheric chemistry [17] and had not been applied to human breath measurements before. Partial canister filling and subsequent dilution contributed to a lower water content of the breath sample that was finally transferred to the gas chromatograph. Since this is a critical point in the analysis of exhaled air, the effects of humidity had been thoroughly assessed beforehand. Interferences might be expected at three different levels: Condensation on the canister surface with adsorption of compounds, blockage of the pre-concentration loop by ice, and alteration of the chromatographic signal. Our validation experiments gave no evidence for any of these problems, but demonstrated high storage stability and no relevant water effect on retention times and peak amplitudes. Therefore we are confident that the employed method of gas collection, separation and VOC detection is robust and equally applicable to dry gas standards, partly humid ambient air and water-saturated human breath samples.

Analytical performance for exhaled VOC then proved to be very good, with lower limits of detection in the range of 10-20 ppt. Ambient levels were not negligible for a number of compounds, therefore we chose to determine the pulmonary exchange rate of a specific gas from the difference between in- and expired concentration. The results showed substantial

inter-subject variability within both groups with significant differences for pentane, DMS and 2-propanol. Pentane and toluene were linearly correlated to lung function in CF patients only, ethane levels did not discriminate CF patients from healthy controls.

Inflammatory processes have been recognised as central in the pathophysiology of many chronic lung diseases, and numerous research groups are committed to the development of sensitive markers and methods. Apparent advantages of biochemical breath analysis are its intrinsically non-invasive nature as well as the close physiological proximity between target tissue and analytical substrate. In the context of cystic fibrosis, non-invasive markers of pulmonary inflammation could contribute to an individual tailoring of treatment through monitoring of oxidative stress and disease progression [14, 18]. Exhaled pentane has been proposed as such a marker for airway pathology [10]; and available evidence suggests that peroxidation of polyunsaturated fatty acids in the liver is its major source. Increased pentane concentrations were reported in the breath of patients with acute asthma [19], pulmonary infections [20], and after acute cigarette smoke exposure [21]. A single observation in adult CF patients has previously been reported in abstract form [22] and as letter to the editor [23], apparently without a subsequent original contribution. The authors collected tidal air over 2 minutes after a 10 minute "washout" and found substantially higher breath pentane output in CF patients compared to healthy controls, but details on patient characteristics, analytical methods and results are not available. Our investigation demonstrated that Δ[pentane] was significantly higher in patients than in healthy controls and inversely correlated with lung function and nutritional status. Moreover, even higher pentane gradients were detected during pulmonary exacerbations and in the presence of chronic *Pseudomonas* infection, which is a well-known stimulant of neutrophilic airway inflammation. All of these findings support the hypothesis that exhaled pentane reflects *in vivo* oxidative processes in our CF patients. For individual subjects however there was wide scatter (cf. *Figure 5*) with substantial overlap

between results from CF patients and controls. So far, the long-term variability and discriminative power of pentane in specific clinical situations (such as the diagnosis of a pulmonary exacerbation) have not yet been assessed. As proposed by Springfield & Levitt [24], chromatographic analysis should be performed with diligence and ambient sources taken into account.

Ethane is another major product of lipid peroxidation, and increased breath levels have been reported in patients with asthma or CF [25, 26]. We were unable to reproduce this finding in our study; this may be due to different methodology and to the correction for ambient ethane which is not mentioned by Paredi et al. in their CF paper [26]. However, inhaled antibiotic treatment was associated with lower ethane output in our patients, raising the possibility of an additional source from bacterial production. Similarly, propane was given off at comparable levels in patients and controls. This C_3-hydrocarbon is probably derived mainly from protein degradation and fecal flora and of doubtful use as a marker for lipid peroxidation [10].

Decreased pulmonary output of isoprene has been described in acute exacerbations of CF, with a return to normal values after treatment [9]. Again, this finding was not reproducible in our cross-sectional study: Exhaled isoprene differed neither between stable and exacerbated CF patients nor between CF patients and healthy controls. The implication of higher isoprene levels in diabetic patients remains unclear, a previous investigation failed to show a difference between schoolchildren with type I diabetes and healthy peers [27].

Volatile sulfur compounds in the breath have previously been assessed in the context of oral malodor [28], occupational exposure [29], congenital enzyme deficiency [30] and liver cirrhosis [31]. While elevation of DMS was found in all of these conditions, we measured a markedly decreased breath output in our CF population. Although progressive cholestasis and hepatic fibrosis occur regularly in the course of CF, none of our study patients had severe liver involvement. Furthermore, exhaled DMS levels were significantly higher in patients

with environmental tobacco smoke exposure and lower in those on insulin or inhaled antibiotics. Due to the complexity of biochemical pathways and the potential number of unknown confounding factors, these interesting results remain descriptive at the moment.

Limonene has been described as elevated in the breath of patients with liver disease [32]. Monoterpenes have been identified as potential antioxidants with a primarily nutritional source, their exact role in the human metabolism is unclear. We detected limonene at mixing ratios between 0.1 and 15 ppb in the exhaled breath of our CF patients and healthy controls without a systematic group difference.

Among the remaining compounds, 2-propanol was actually taken up from the ambient air in significantly higher quantities by CF patients than controls. Ethanol release exhibited the highest variability, while methanol output was higher in the presence of tobacco smoke exposure and lower in chronically *Pseudomonas* infected patients. However, no differences between CF patients and healthy controls were observed for these alcohols. Likewise, a net uptake was described for the aromatic compounds benzene and toluene without a significant group difference. Acetone proved to be the most abundant VOC in human breath (cf. *Figure 2*) with high repeatability, but not associated with diabetes and again not discriminating between CF patients and healthy controls.

In conclusion, young patients with CF showed a higher pentane and lower DMS output in their breath compared to normal controls. Their alveolar gradient of n-pentane was related to pulmonary function, nutritional status, *Pseudomonas* infection and the presence of a pulmonary exacerbation. Additional evidence on the prognostic value of exhaled pentane as an inflammatory marker might be gathered by serial measurements in a patient cohort before and after antibiotic treatment of exacerbations. This was beyond the scope of the present

study, but should be addressed in the near future. Group comparison yielded no differences for ethane, isoprene, limonene and several other VOC.

We admit that some of these observations result in more new questions than answers: Long-term variability of breath VOC has neither been assessed in healthy people nor in patients with respiratory disease. Today's equipment and procedures for sampling and analysis are fairly complex and unstandardised, not to mention the lack of established reference values. Therefore with our current knowledge, it is still of ambiguous practical value to determine the concentration of a specific VOC in the exhaled breath of a patient. Nevertheless, our investigation may add strength to the evolving concept that chemical breath analysis has the potential to open a new diagnostic window on important aspects of pulmonary physiology and pathology in CF and other chronic inflammatory conditions.

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FIGURE LEGENDS:

Figure 1: Experimental setup of the gas chromatography system with canister manifold, pneumatic valves (V 1-6), mass flow controller (MFC), pre-concentration loop, capillary column, oven, flame ionisation detector (FID) and mass spectrometer (MS). A set of 10 individual 6-liter canisters can be analysed in unattended operation.

Figure 2: Original chromatogram of a breath sample; investigated compounds are labeled with names and retention times above the respective peaks. The left insertion shows the complete chromatogram demonstrating that acetone and isoprene are the dominating compounds in exhaled breath air, the right insertion shows a blow-up of the region adjacent to the isoprene peak.

Figure 3: Chromatograms obtained from dry (left panel) and humidified (right panel) samples of calibration gas.

Figure 4: Difference in peak areas for specific VOC (normalized to the peak area of propane, which is not affected by water) between dry (light bars) and humidified standard gas mixtures (grey bars).

Figure 5: Relation between exhaled pentane output and FEV_1 in healthy controls (light triangles), stable CF patients (grey circles) and CF patients during exacerbation (full circles), including regression line for the combined CF group ($r=-0.62$, $p=0.004$).

TABLES

Table 1: Short-term variability of exhaled VOC in cystic fibrosis patients (CF), healthy controls and both groups as expressed by mean, minimal and maximal intra-subject coefficients of variation.

	CF	Controls	All	Range
Ethane	16%	8%	12%	0-38%
Propane	11%	13%	12%	1-50%
Pentane	22%	19%	20%	1-66%
Methanol	14%	11%	13%	1-77%
Ethanol	38%	29%	34%	4-94%
2-Propanol	25%	22%	23%	3-64%
Acetone	9%	8%	9%	1-41%
Isoprene	21%	22%	21%	3-77%
Benzene	28%	12%	20%	3-68%
Toluene	23%	12%	18%	2-66%
DMS	14%	14%	14%	0-60%
Limonene	34%	27%	30%	2-116%

Table 2: Mean exhaled and ambient VOC concentrations [in ppb] with their mean difference (Δ) and 95% confidence intervals (CI) in cystic fibrosis patients (CF) and healthy controls.

	CF				Controls				
	Breath	**Room**	**Δ**	**CI**	**Breath**	**Room**	**Δ**	**CI**	
Ethane	2.76	2.38	0.39	(-0.04 - 0.82)	2.47	2.37	0.10	(-0.25 - 0.44)	
Propane	1.95	1.42	0.53	(0.31 - 0.75)	1.95	1.38	0.58	(0.08 - 1.08)	
Pentane	0.55	0.19	0.36	(0.24 - 0.48)	0.43	0.22	0.21	(0.13 - 0.29)	*
Methanol	200	7.30	193	(125 - 261)	272	7.88	265	(198 - 331)	
Ethanol	157	13.3	144	(-15 - 302)	195	30.0	165	(87 - 243)	
2-Propanol	6.99	21.9	-14.9	(-22.8 - -6.94)	9.96	15.7	-5.76	(-11.7 - 0.16)	#
Acetone	402	2.39	400	(322 - 478)	469	1.74	467	(383 - 551)	
Isoprene	106	0.80	105	(83 - 127)	115	0.45	114	(88 - 140)	
Benzene	0.16	0.19	-0.04	(-0.13 - 0.05)	0.13	0.21	-0.08	(-0.15 - -0.01)	
Toluene	0.43	0.68	-0.25	(-0.53 - 0.03)	0.29	0.80	-0.51	(-0.82 - -0.20)	
DMS	3.89	0.00	3.89	(2.24 - 5.54)	7.58	0.00	7.58	(5.73 - 9.43)	#
Limonene	2.42	0.14	2.28	(0.53 - 4.02)	2.30	0.09	2.21	(0.83 - 3.59)	

* - $p<0.05$ for comparison of Δ between groups

- $p<0.005$ for comparison of Δ between groups

FIGURES

Figure 1:

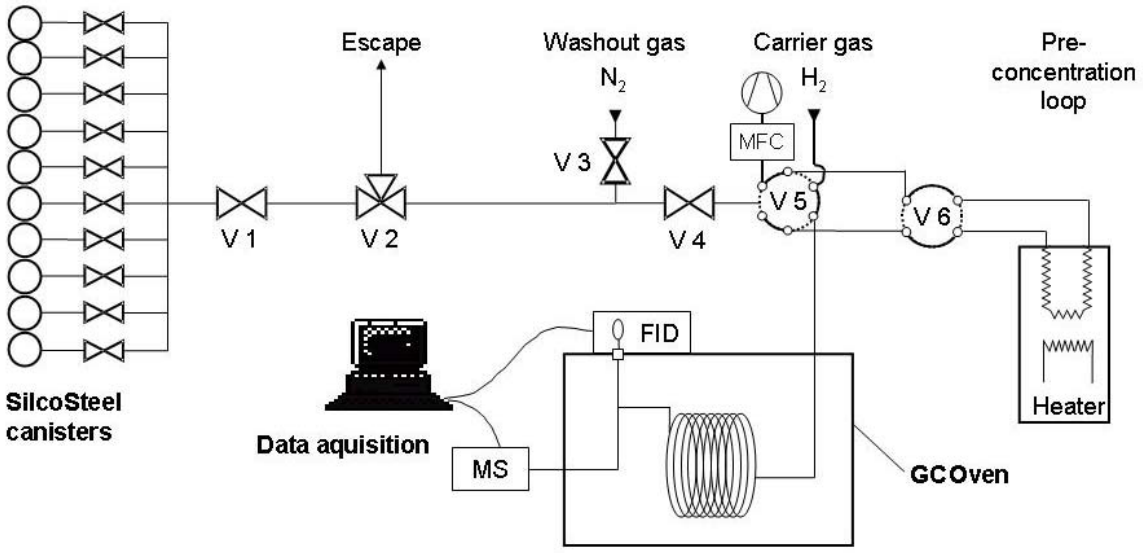


Figure 2:

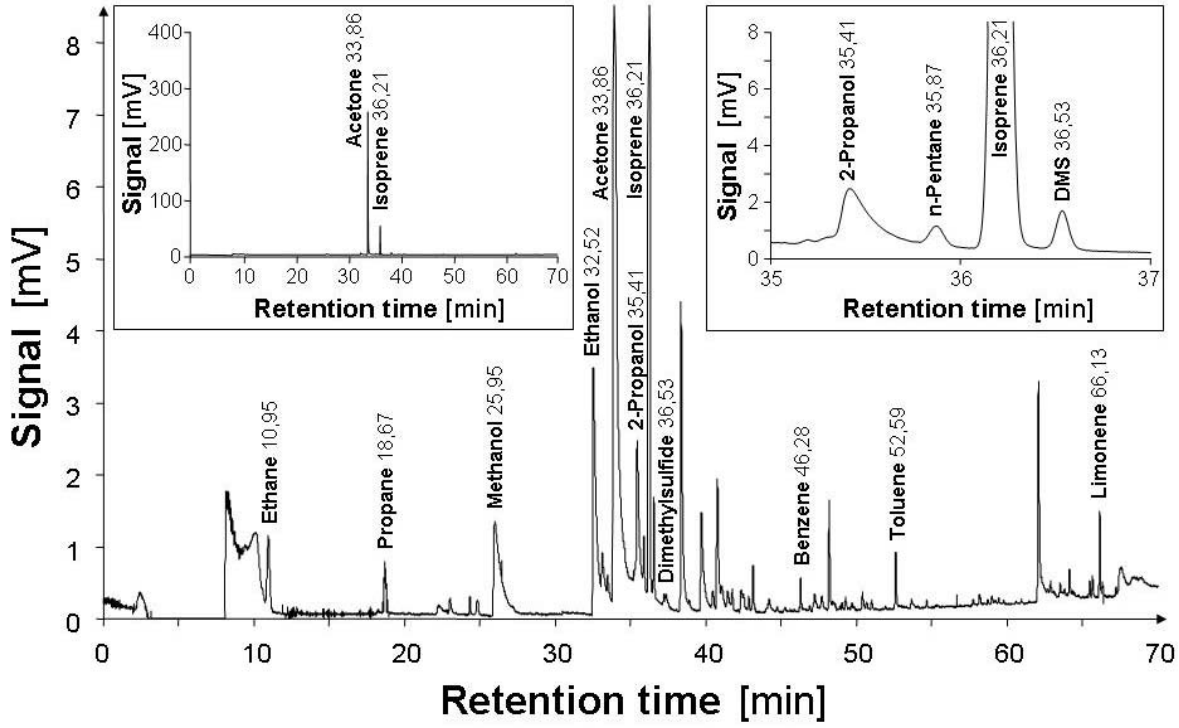

Signal [mV]
Retention time [min]
Ethane 10,95
Propane 18,67
Methanol 25,95
Ethanol 32,52
Acetone 33,86
2-Propanol 35,41
Isoprene 36,21
Dimethylsulfide 36,53
Benzene 46,28
Toluene 52,59
Limonene 66,13
Acetone 33,86
Isoprene 36,21
2-Propanol 35,41
n-Pentane 35,87
Isoprene 36,21
DMS 36,53

Figure 3:

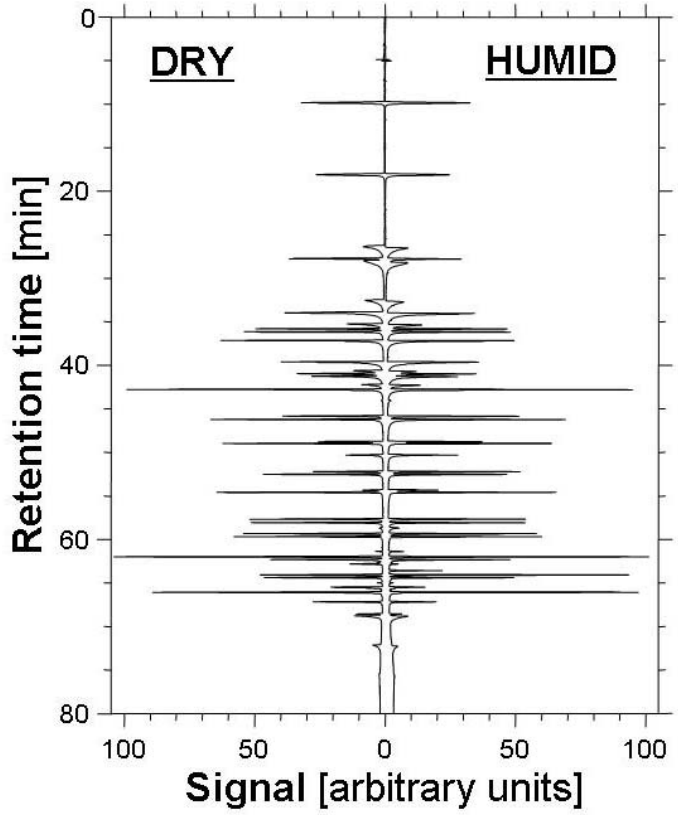

DRY
HUMID
Retention time [min]
0
20
40
60
80
100
50
0
50
100
Signal [arbitrary units]

Figure 4:

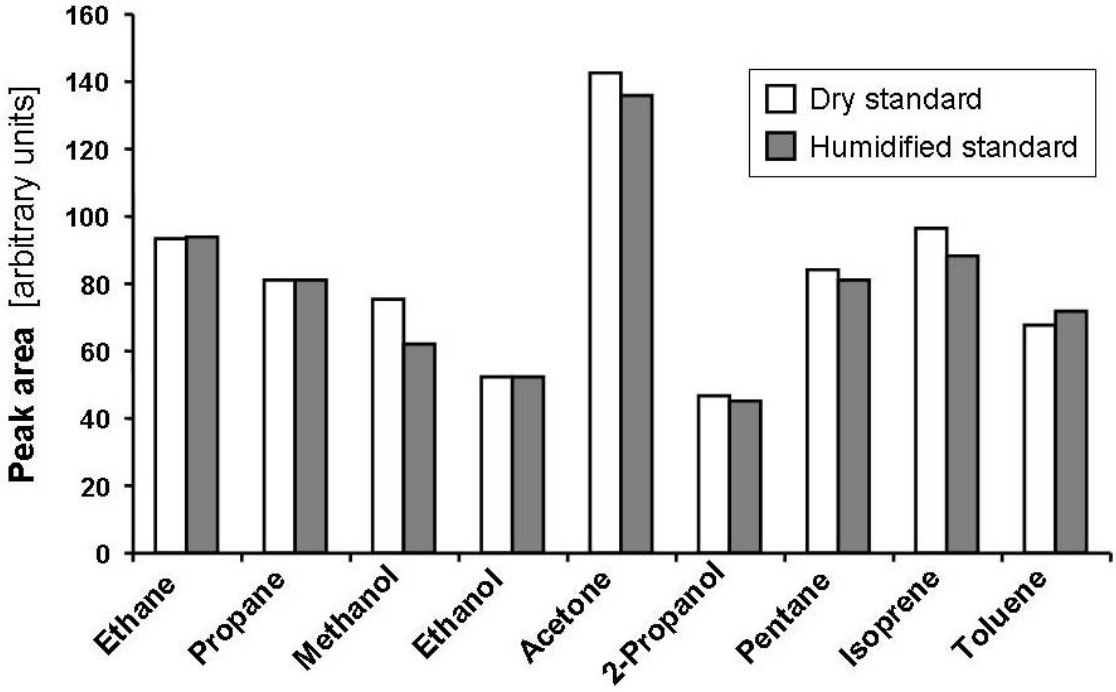
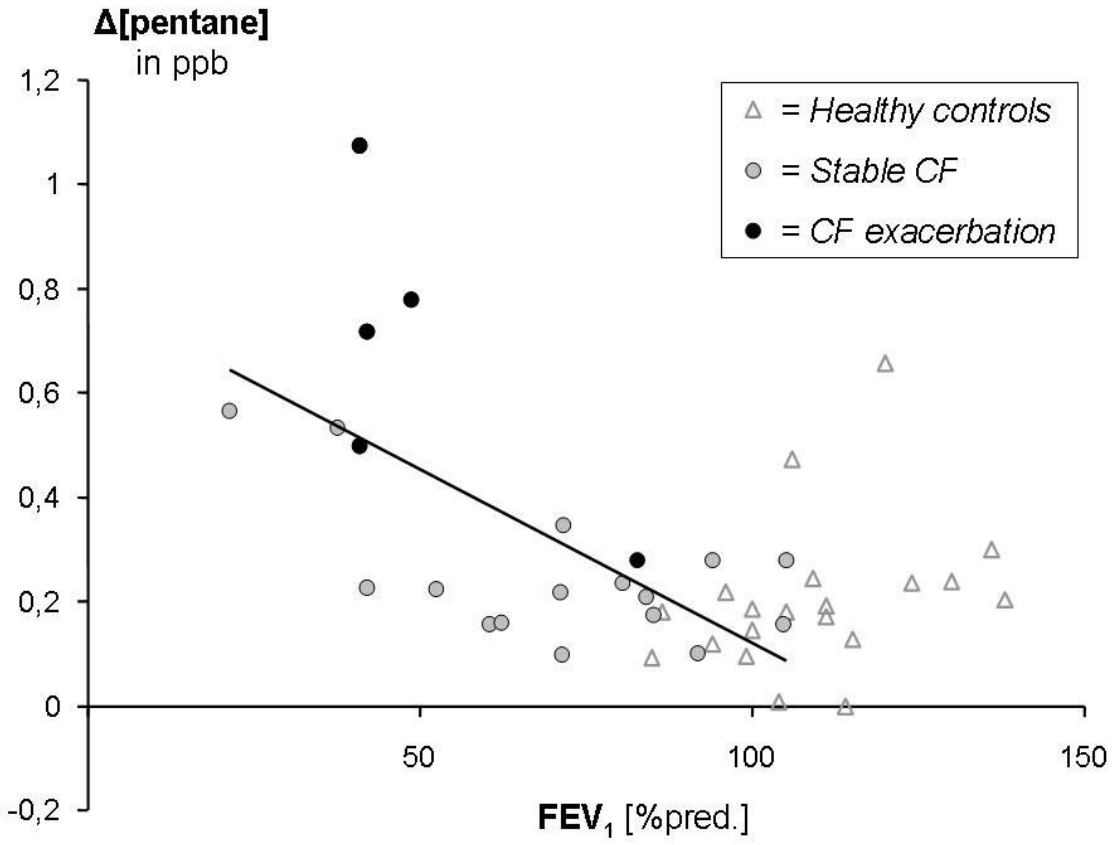

Peak area [arbitrary units]
160
140
120
100
80
60
40
20
0
Dry standard
Humidified standard
Ethane
Propane
Methanol
Ethanol
Acetone
2-Propanol
Pentane
Isoprene
Toluene

Figure 5:


Δ[pentane]
in ppb
1,2
1
0,8
0,6
0,4
0,2
0
-0,2
50
100
150
FEV1 [%pred.]
= Healthy controls
= Stable CF
= CF exacerbation