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Breath Analysis from Patients with Metabolic Disorders: GC-MS Analysis with a Combined Thermodesorption - Cooled Injection System

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ABSTRACT

The combination of a new thermodesorption module with a cooled injection system (TDS-2,CIS-3, Gerstel, Mülheim, Germany) now provides a powerful thermodesorption system for direct analysis of volatile trace compounds in gaseous, liquid and solid samples. As a cooled injection system is used for the cryofocusing of the desorbed volatiles the GC-MS system still can be used for the regular analysis of liquid samples. Breath samples were collected by a 1 liter-tedlar bag and transferred onto a freshly conditioned thermodesorption tube filled with Tenax. Breath analysis were performed from patients with various metabolic disorders, smoking and non smoking healthy volunteers. A MS-library was used to identify 72 components.

INTRODUCTION

Analysis of trace components in air often requires the sampling of large volumes. Direct loading of such amounts is not possible and usually the air sample is passed through a trap containing an adsorbent for enrichment or into various sampling containers such as evacuated electropolished canisters [1]. After desorption the components are transferred to the column in separate steps [2, 3]. Breath analysis could become an important diagnostic tool in medicine for many reasons: volatile sulfur compounds in hepatic diseases and oral malodor [4, 5], ethane, pentane and isoprene as potential markers of lipid peroxidation [2, 6] and different volatiles due to environmental pollution [3]. 2,5-Dimethyl furan is supposed to have high discriminatory power in GC-MS profiles to allow differentiation between smokers and non-smokers [7]. The method described here allows simple breath collecting at the patients site and sufficient sensitivity to identifiy major metabolites used for diagnosis.

Experimental

Breath collecting. Different gas sampling bags were tested and the final analysis were done with a 1 liter valve tedlar bag from Supelco (Supelco, Bellefonte, PA, USA). The closed bag was connected directly to the adsorption tube and flow controlled sucked onto it by a pump. A blank glass tube (4.0 mm i.d.) filled with Tenax (60mm, 35-60 mesh) was used for adsorbing the major metabolites.

Instrumentation. The system consists of a thermodesorption system (TDS 2, Gerstel GmbH, Mülheim an der Ruhr, Germany, **Figure 1**), a temperature programmable cooled injection system (CIS 3, Gerstel GmbH, Mülheim an der Ruhr, Germany, **Figure 1**), a gas chromatograph HP 6890, and a mass selective detector HP 5972 (both Hewlett-Packard, Waldbronn, Germany).

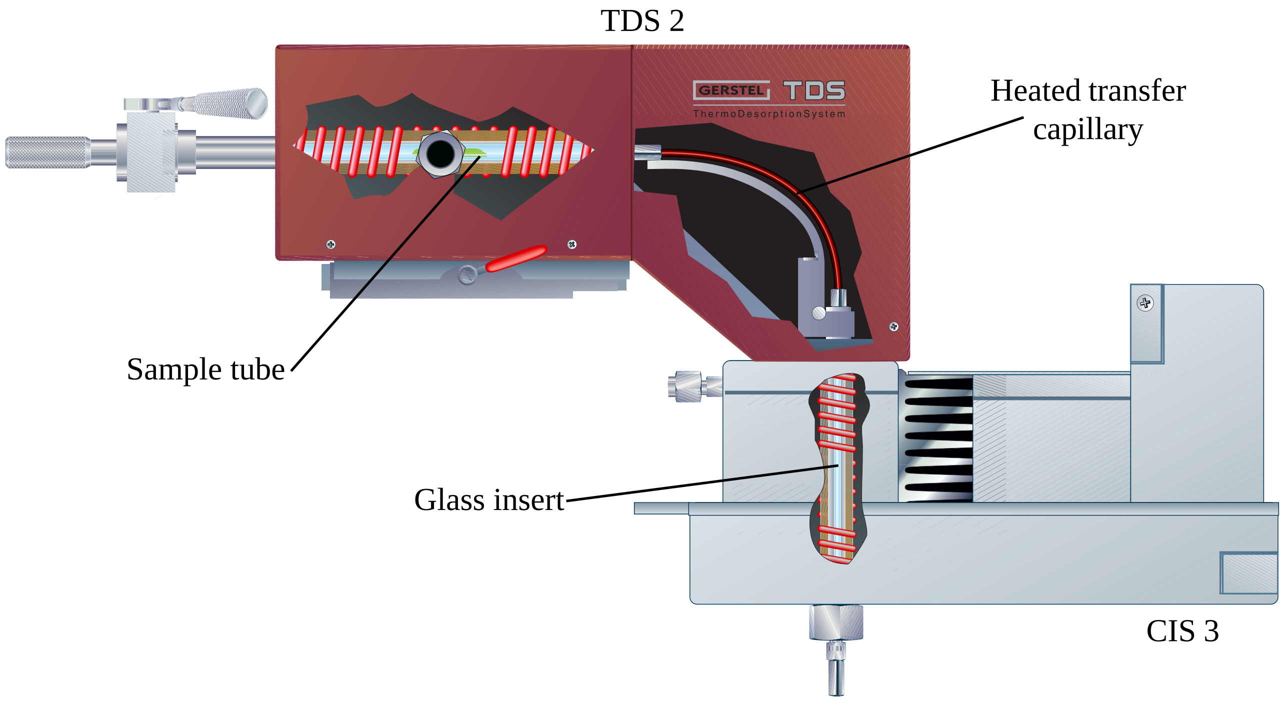

Figure 1. *Thermodesorption system TDS 2 attached to CIS 3.*

Operation. The Tenax tube is inserted into the TDS desorption chamber which is cooled down to ambient temperatures in order to prevent premature desorption. After purging the air out of the system, the tube is then heated to 240°C , while the carrier gas flowing through the tube transfers the volatiles in splitless-mode (**Figure 2**) into the pre-cooled CIS, where they are cryofocused and concentrated.

After the desorption has finished the CIS is heated to 280°C to allow split- or splitless transfer of the trapped compounds to the analytical column and further mass spectrometric detection.

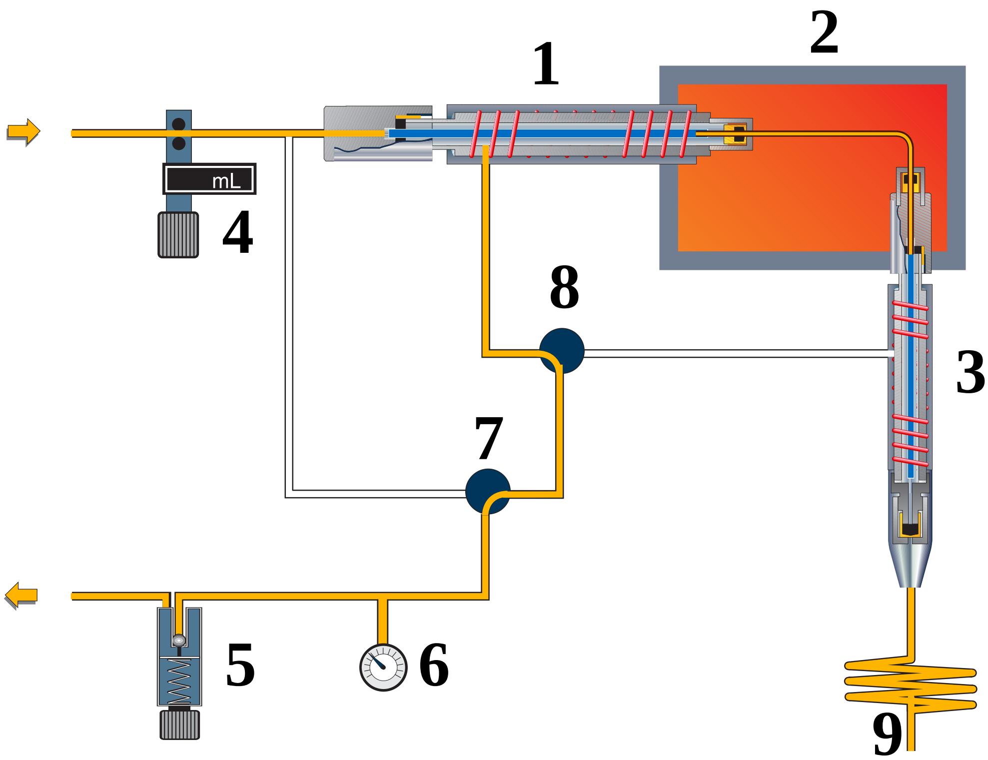

Figure 2. *Schematic of the applied system which consists of a thermodesorption system (1), a temperature controlled transfer capillary (2), a cooled injection system (3), standard backpressure pneumatics with mass-flow controller (4), backpressure regulator (5), pressure gauge (6) and split/splitless valve (7), including an additional 3/2-way solenoid (8) to switch the splitflow between TDS and CIS. The analytical column (9) is directly connected to a mass selective detector.*

Analysis conditions.

TDS-tube:	60 mm Tenax TA,	d_i = 4.0 mm,	35 - 60 mesh
Column:	60 m DB 1 (J&W),	d_i = 0.32 mm,	d_f = 5.0 µm
Pneumatics:	Carriergas He,	constant flow mode (1 ml/min), split 1:50	
		1 min splitless	
Temperatures:	TDS	20°C (2 min); with 60°C/minto 240°C (5 min)	
	CIS	-150°C; with 12°C/s to 280°C (3 min)	
	Oven	50°C (1 min); with 5°C/min to 260°C (20 min)	
	MSD	280°C	
MSD:	Scan, 20 - 300 amu		

Results and Discussion

Figure 3 shows a typical chromatogram from a single breath sample. Components identified in different samples are listed in **Table I**.

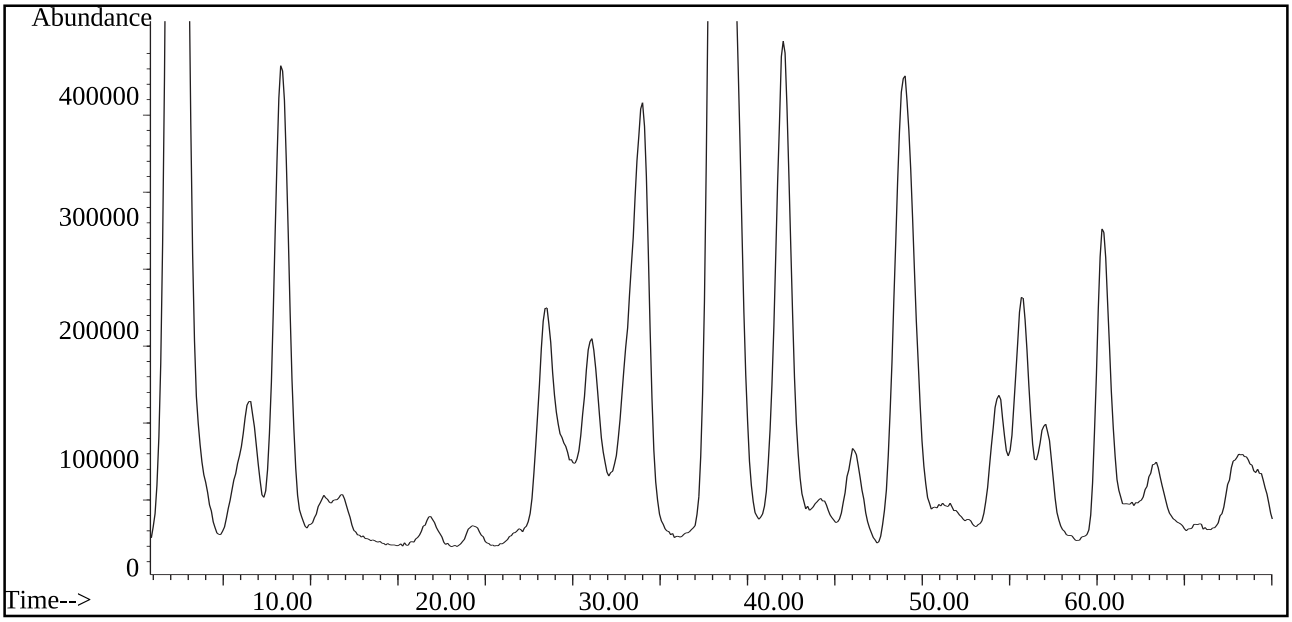

Figure 3. *Total ion chromatogram of a single breath sample from a healthy volunteer.*

Volatiles	RT (min)	Volatiles	RT (min)
Acetaldehyde	7.24	3-Methyl hexane	17.62
Methanethiol	8.09	3-Hydroxy-2-butanone	17.91
Trimethyl amine	8.39	2,5-Dimethyl furan	18.45
Ethanol	8.58	Acetamide	19.03
Acetonitrile	9.17	Pyridine	19.88
Acetone	9.63	Toluene	21.52
Isopropanol	9.86	Hexanal	22.03
Pentane	10.55	2-Pentanol	22.18
2-Methyl-1,3-butadiene	10.74	2-Cyclopenten-1-one	23.28
Acetic acid methyl ester	10.93	N,N-Dimethyl acetamide	24.42
1-Propanol	11.93	Ethyl benzene	25.55
Acetic acid	12.69	o-Xylene	25.85
2-Methyl pentane	13.13	Heptanal	26.27
3-Methyl pentane	13.76	Glycerine	27.72
Ethyl acetate	14.05	2-Hydroxy propanamide	28.19
2-Methyl furan	14.13	3-Methyl-2(5H-) furanone	28.82
Hexane	14.36	Phenol	28.77
2-Methyl-1-propanol	14.69	Benzaldehyde	29.04
1-Hydroxy-2-propanone	15.85	Aniline	29.46
3-Methyl butanal	15.91	1,2,3-Trimethyl benzene	30.85
1-Butanol	16.13	2-Ethyl-1-hexanol	31.24
1,1,1-Trichloro ethane	16.25	D-Limonene	32.32
Propanoic acid	16.37	Cyclohexene	32.30
1-Methoxy-2-propanol	16.87	Acetophenone	32.98
Benzene	16.93	2-Ethyl hexanoic acid	33.53
2,3-Pentanedione	17.22	1-Heptene	33.85
2-Methyl-1-pentene	17.45	Nicotine	44.14

Table I. *Peak identification.*

Both pentane and isoprene are excreted in human breath. Although pentane is considered to be an index of lipid peroxidation, the significance of isoprene is unknown. Most GC-columns do not separate these components, which therefore might be calculated wrong. Baseline separation was achieved with a Poraplot Q and Poraplot U column [2], but these columns are not of practical use for the rest of the volatiles of interest in human breath. With the method described here we were able to separate them fairly good (**Figure 4**): pentane elutes at 10.55 min and isoprene at 10.74 min.

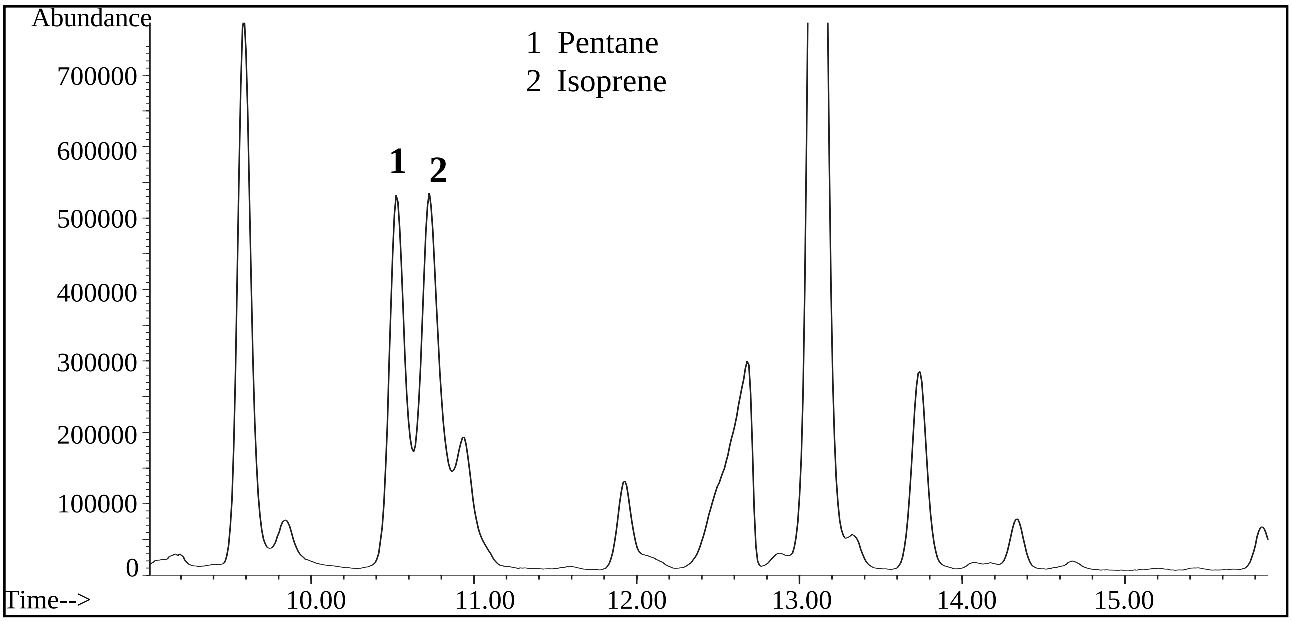

Figure 4. *Separation of pentane and isoprene.*

Many of the volatiles found in human urine and plasma [8-12] could be identified in breath. **Figure 5** shows the chromatogram of a diabetic patient undergoing hemodialysis. Acetaldehyde, n-butanol and isobutanol are clearly elevated compared to the concentration of a healthy volunteers breath (**Figure 6**). Also raised were the concentrations of phenol and ethyl hexanol, which arises from the plastizicer DEHP in dialysis tubings.

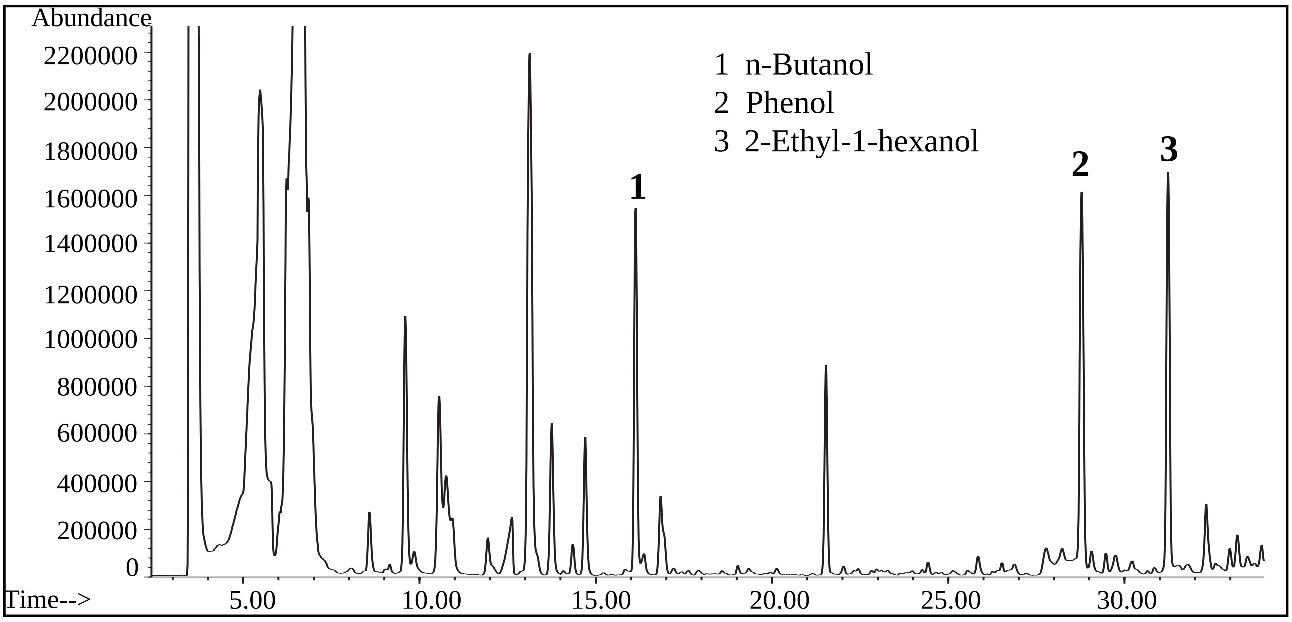

Figure 5. *Breath analysis of diabetic patient on hemodialysis.*

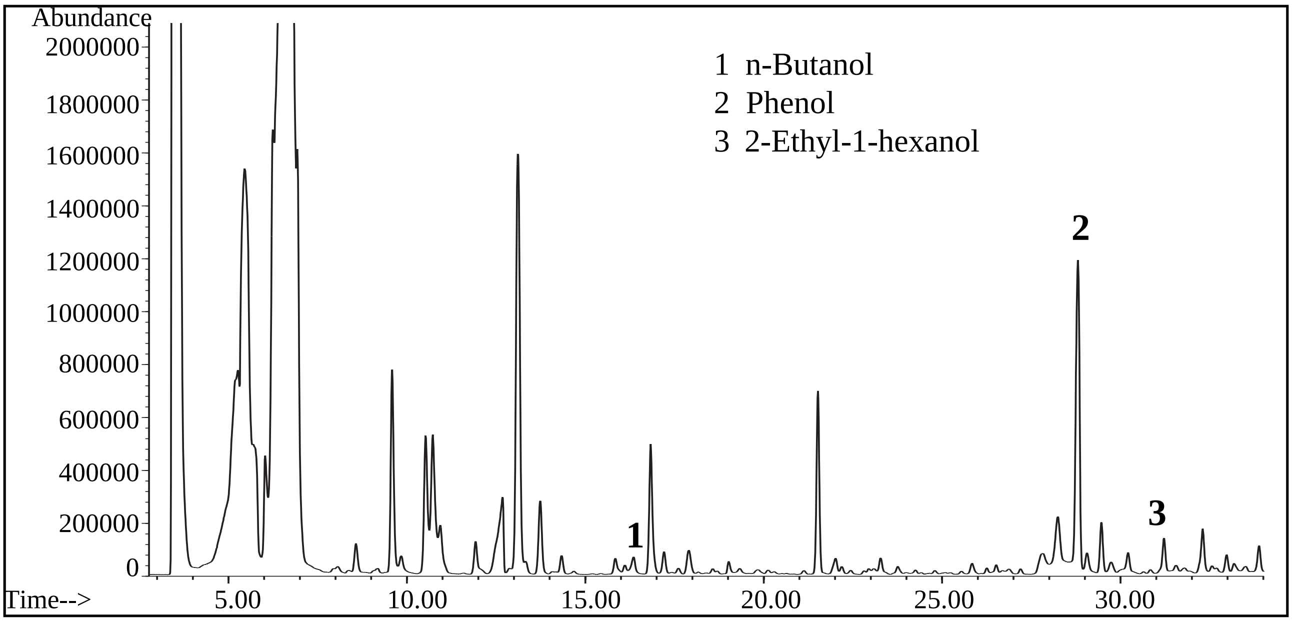

Figure 6. *Breath analysis of healthy volunteer.*

Breath samples taken before and after 3 hour hemodialysis showed an significant increase between 1.5- and 2.5-fold in 2-ethyl hexanol concentration. Further metabolites of DEHP were also present in small amounts: 2-ethyl hexanoic acid and 4-heptanone. Breath samples taken from a volunteer before and after a 3 hour infusion of 500 ml NaCl showed a 2-fold increase in 2-ethyl hexanol concentration.

2,5-Dimethyl furan is supposed to have high discriminatory power in GC-MS profiles to allow differentiation between smokers and non-smokers [7]. We could detect it in the breath of our smoking volunteer (**Figure 8**), but not in any of the non smoking persons (**Figure 7**). **Figure 8** shows the total ion chromatogram and **Figure 9** the extracted ion chromatogram of 2,5-dimethyl furan (RT 18.45) in a smokers breath.

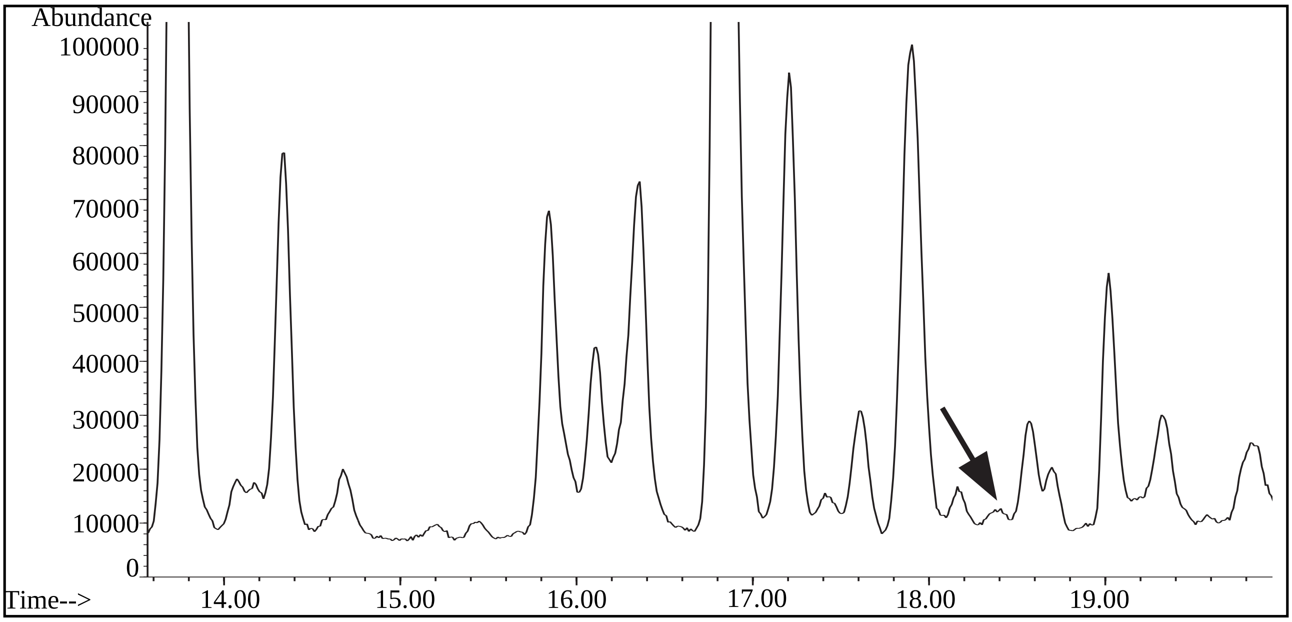

Figure 7. *Total ion chromatogram of a non-smokers breath: no 2,5-dimethyl furan.*

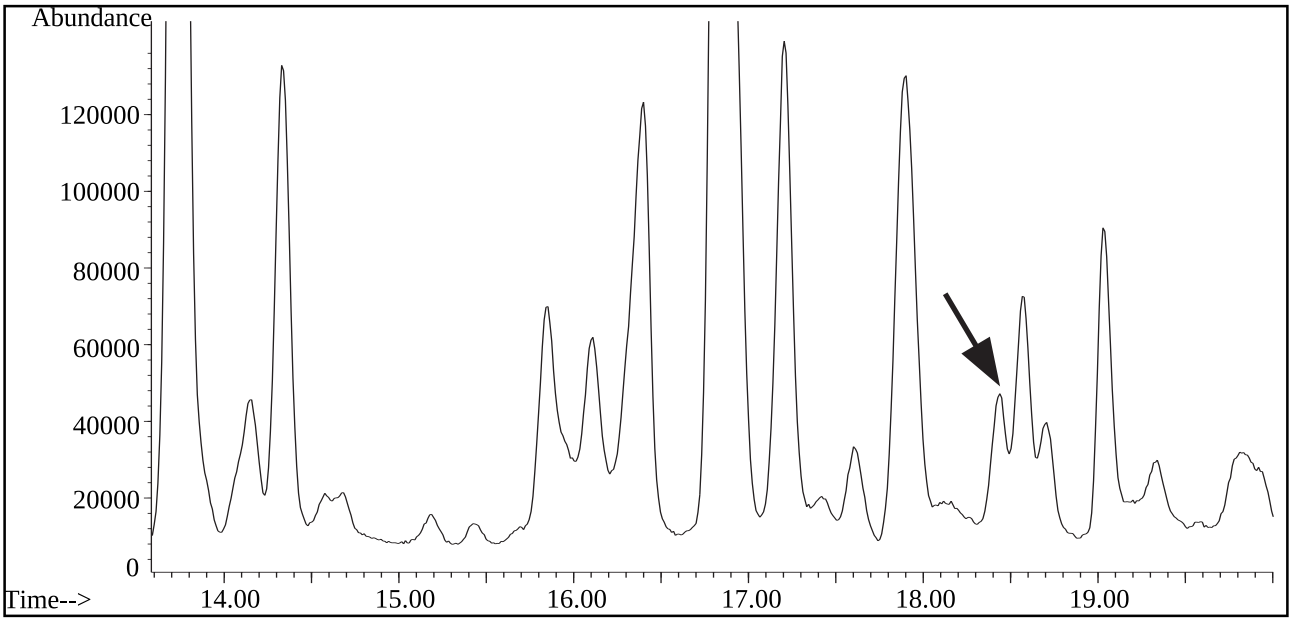

Figure 8. *Total ion chromatogram of a smokers breath: 2,5-dimethyl furan.*

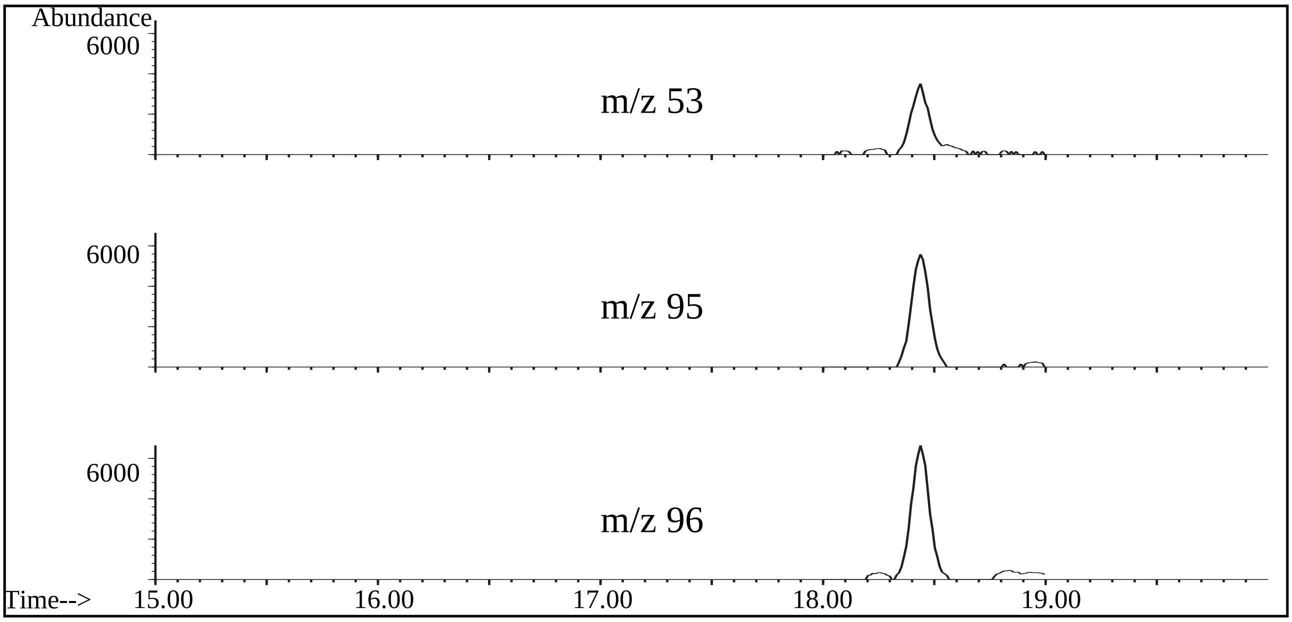

Figure 9. *Extracted ion chromatogram of a smokers breath: 2,5-dimethyl furan traces.*

One difficulty all breath analysis have to cope with are the volatile organic compounds from the air (**Figure 10**). Attempts have been made to apply purified air to the patients, but this will inevitable lead to a more complicated system to set up and difficulties in breath tests implementation in clinical routine.

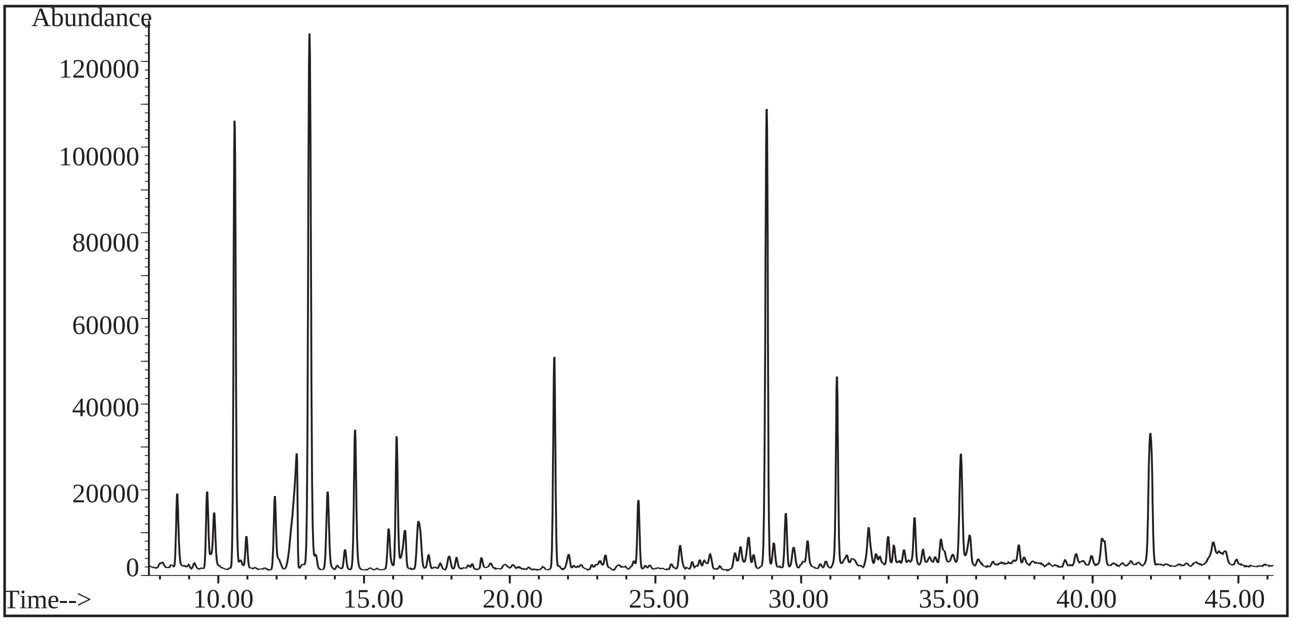

Figure 10. *Volatile organic compounds from air.*

Collecting breath samples still is another problem: electropolished canisters are expensive and can not easily be used for many patients. Bags are also sources for contamination: depending on the plastic and valves used alkanes and plasticizers will be found. In addition the adsorbent traps have to be carefully conditioned before usage.

Conclusion

Breath analysis could become an important diagnostic tool in medicine for many reasons: volatile sulfur compounds in hepatic diseases, ethane, pentane and isoprene as potential markers of lipid peroxidation and different volatiles due to metabolic disorders or environmental pollution. The method described here allows simple breath collecting at the patients site and shows sufficient sensitivity to identifiy major metabolites used for diagnosis. However further improvement of the materials used for breath collecting and adsorption has to be achieved.

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