

ISAS | Determination of Biomarkers in Human Breath by TDS/GC/MS

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Introduction

Nowadays, there is an increasing interest in new applications for analytical tools such as gas chromatography/mass spectrometry (GC/MS) in life sciences and medicine.

In the field of serious lung diseases there is a challenge for new techniques in addition to established ones such as bronchoscopy and x-ray. Lung cancer is often detected in a very late stadium. Most of the patients survive less than 5 years after diagnosis. Therefore, it is necessary to develop analytical methods which give early hints for anomalies.

Gas chromatography/mass spectrometry (GC/MS) has the advantage to be a non invasive method (easy sampling technique, easy to use even for children and weak persons), fast in analysis, ambulant, cheaper than invasive methods and without any side effects.

The aim of this work is to develop a suitable sampling technique and a GC/MS-method to analyse breath and to find selective, volatile organic compounds (VOCs); some of them are supposed to be biomarkers.



Fig. 2: Sampling while breathing clean, wet, synthetic air

Results

statistic control group of 20 persons:

- ◆ Analytes like ethanol, acetone, isopropanol, benzene, toluene and xylene are found in all samples.
- ◆ Sulphur compounds like 1-methylthiopropene and dimethyldisulfide are found in most samples.
- ◆ Terpenes like α -pinene, β -pinene and limonene are found in many samples.
- ◆ Small alkanes (C₆/C₇-body) are found in some samples.
- ◆ The concentration of the analytes is different in each sample.

general results:

- ◆ Ratio ethanol/acetone/isopropanol is in similar range for a group of healthy persons.
- ◆ Ratio ethanol/acetone/isopropanol is significantly depending on state of hunger.
- ◆ Smokers have numerous additional compounds and higher concentrations of normal markers such as 1-propanol, benzene, toluene and xylene in their breath. (fig. 4)

Difficulties in breath analysis

◆ Breath consists of many components

- surrounding air (including outdoor air)
- metabolites of the human body
- metabolites of bacteria
- analytes of pharmaceuticals
- analytes of food or living habits
- analytes of hygienic products

◆ GC/MS samples must almost be dry

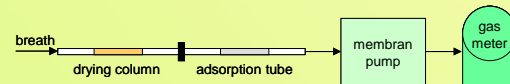
- 44 mg/L steam gas in human breath (37°C)
- loss of biomarkers with similar polarity like water is possible

◆ Quantitative analysis is very complex

- set up of a test gas source (filled with the biomarkers) based on diffusion principle
- similar conditions to the human body

Sampling technique

- ◆ drying step: adsorption of the water
- ◆ accumulation step: accumulation of volatile organic compounds (VOCs) from a defined breath gas volume (normally between 1L and 5L)



- ◆ reference samples: a) room air
b) sampling while inhaling clean, wet, synthetic air (fig. 2)
- ◆ storage of samples: sampling tubes are closed directly after sampling and measured either directly or stored at about 4°C

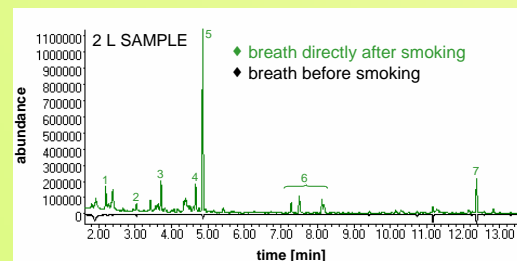


Fig. 4: Section of a chromatogram from a smoker before versus directly after smoking

outstanding analytes:

- 1) 1-propanol
- 2) benzene
- 3) 2,5-dimethylfuran
- 4) pyrrole
- 5) benzene
- 6) xylene
- 7) limonene



Fig. 1: TDS/GC/MS-system with Gerstel-autosampler (Mass Selective Detector, Agilent Technologies 6890N HP-SMS column 30 m x 250 μ m x 0.25 μ m)

Experimental

drying column: 7 cm molecular sieve (3 Å)

adsorption tube: 200 mg Tenax

desorption: 25°C → 30°C/min → 200°C (3.4 min)

cryo trap: -80°C → 25°C/s → 200°C (3 min)

GC: 37°C (2 min) → 5°C/min → 150°C → 10°C/min → 220°C (10 min)

MS: m/z range 35 – 300 amu, EI, full scan-mode

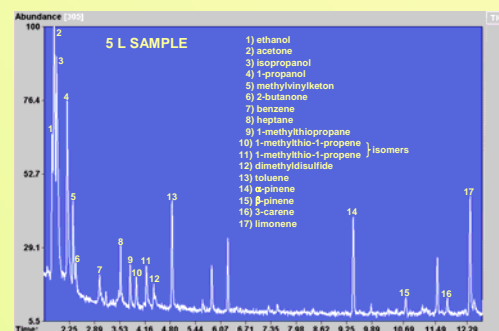


Fig. 3: Section of a typical chromatogram from a non-smoker (all analytes can be found in samples with synthetic air)

Conclusions

- ⇒ High intensity of expiration increases amount of biomarkers.
- ⇒ Many influences are determined by matrix effects (for example smoking, fig. 4).
- ⇒ Information about smoking, eating, drinking habits, etc. are often incomplete.
- ⇒ Ratio of biomarkers is relevant.
- ⇒ Concentration and polarity of biomarkers are very important for the distribution between gaseous and liquid phase.

⇒ liquid phase has to be analysed next

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