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Comparison of Proton Transfer Reaction-Mass Spectrometry and Gas Chromatography–Mass Spectrometry in Analysis of Breath Samples

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Proton transfer reaction–mass spectrometry (PTR–MS) and gas chromatography–mass spectrometry (GC–MS) methods were compared and used for determination of volatile organic compounds in breath of patients suffering from COPD (chronic obstructive pulmonary disease). The working ranges available in PTR–MS and GC–MS were 0.3–5.8 nmol dm^{-3} and 0.9–1.8 nmol dm^{-3}, respectively. Principal component analysis was used for comparison of sensitivity of PTR–MS and GC–MS towards particular compounds (alkanes, ketones and aldehydes).

W pracy porównano chromatografię gazową (GC) sprzężoną ze spektrometrią mas (MS) oraz spektrometrię mas z jonizacją przez przeniesienie protonu (PTR). Obie techniki zostały użyte do oznaczeń lotnych związków organicznych w wydychanym powietrzu. Technika PTR zapewnia wykonanie oznaczeń w zakresie roboczym 0.3–5.8 nmol dm^{-3} a GC–MS w zakresie 0.9–1.8 nmol dm^{-3}. Do oceny przydatności obu technik do oznaczeń poszczególnych związków (alkanów, ketonów i aldehydów) zastosowano analizę głównej składowej.

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Breath analysis has been proposed as convenient and safe complementary method to blood and urine analysis. It offers a number of advantages compared to traditional diagnostic techniques [1]. It is a non-invasive and painless procedure, and it does not require skilled medical staff for sampling [2–4]. In addition, the exhaled breath analysis is attractive due to relatively simple sample matrix compared to other biological samples, such as blood.

Modern breath analyses began in 1970s when Pauling detected around 200 different volatile organic compounds (VOCs) in exhaled air by gas chromatography. In the last 30 years it has turned out that exhaled breath may contain small inorganic molecules like nitric oxide, carbon monoxide, carbonyl sulfide, and traces of many volatile organic compounds like acetone, organosulfur compounds [5], amines [6], isoprene, and other hydrocarbons [7, 8]. Concentration of VOCs in breath is very low (nmol dm^{-3}) and can be detected only using the most sensitive techniques. Separation is mostly done by gas chromatography. Hydrocarbons in the nmol dm^{-3}–pmol dm^{-3} (ppbv–pptv) range, such as ethane, pentane, or isoprene are usually determined using gas chromatography with flame ionisation detector. However, mass spectrometers are the most important detectors for identification of the exhaled breath constituents. MS is often capable of differentiation between co-eluting compounds. Chromatographic methods in turn require preconcentration step – usually adsorption on solid sorbents followed by thermal desorption. Solid sorbents for preconcentration of VOCs include polymers, graphitized carbon blacks, and carbon molecular sieves. However, they suffer from particular problems *i.e.* excess of water present in breath and carryover effects.

Preconcentration procedure has been already simplified by introduction of solid phase microextraction (SPME). SPME was developed in late 1980s by Arthur and Pawliszyn [9]. The PDMS–Carboxen coating is the most sensitive for many analytes occurring in exhaled breath and most of the work in this area has been done using this fiber.

Recently, proton transfer reaction mass spectrometry (PTR–MS) has emerged as a new technique for detection of human breath gases. This technique allows for rapid and online determination of VOCs. PTR–MS instruments consist of four parts: an ion source where H_3O^+ ions are produced, a drift tube, a transition chamber, and an ion detection section containing a quadrupole mass filter and an electron multiplier (Fig 1). In the drift tube, trace gases from the sample are ionized in proton transfer reactions with primary H_3O^+ ions. This reaction takes place when proton affinity of the trace compound is higher than that of water (E = 166.5 kcal mol^{-1} = 7.16 eV). The major advantage of using H_3O^+ as the primary reactant ion is that proton affinity of typical air constituents present at high concentrations, like NO, O_2, CO, CO_2, N_2, is lower than that of H_2O molecules. These compounds do not interfere with others because they do not react with H_3O^+ ions [10, 11].

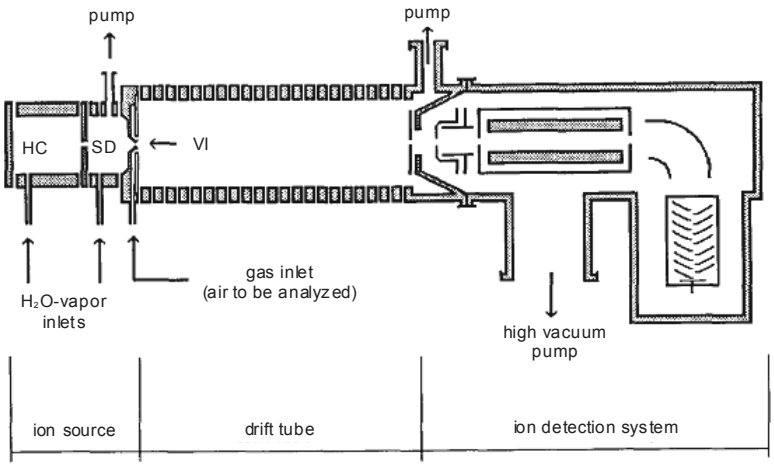

Figure 1. Scheme of PTR–MS system

PTR–MS characterizes the substances according to their mass-to-charge ratio. Accordingly, their chemical identification is not possible and must be performed by other techniques *i.e.* GC–MS. VOCs of identical masses can not be analysed separately using PTR–MS. The GC–MS is still the most valuable technique because it provides an advantage of separation and retention time data. PT–MS enables monitoring of metabolic processes for several hours without preconcentration. The main advantage of proton transfer chemical ionisation is that in most of cases it does not induce any significant fragmentation, and when it does only few fragments are produced. Soft-ionization results in only one or two characteristic ions, and the matrix of signals is much less complex than in other mass spectrometric techniques [12].

In this paper PTR–MS was applied for determination of volatile organic compounds in exhaled breath of patients with chronic obstructive pulmonary disease. The comparison of results obtained by PTR–MS and GC–MS has been performed.

EXPERIMENTAL

Materials

All chromatographic standards: butane, pentane, hexane, butanal, pentanal, hexanal, heptanal, 2-butanone, acetone, 2-methylbutane, 2,3-dimethylbutane, 2-methylpentane, 3-methylpentane, 2,4-dimethylpentane, toluene, benzene, isopropanol and acetonitryle were purchased from Sigma–Aldrich (Steinheim, Germany). Pure nitrogen and helium (5.0 grade) were purchased from Air Products (Warszawa, Poland).

Apparatus

Proton transfer reaction mass spectrometer was purchased from Ionicon (Innsbruck, Austria). GC–MS analysis was performed on an Agilent 5975 Inert XL MSD apparatus coupled with a 6890 gas chromatograph (Hewlett–Packard, Waldbronn, Germany) with the split-splitless injector. The temperature of the split-splitless injector was 200°C. The splitless time was 1 min, while the split ratio was 1:40. Helium served as a carrier gas. Linear velocity of 40 cm s^{-1} was applied. The MS analyses were carried out in a full scan mode, with scan range 15–220 amu. The following conditions were applied: scan rate 3.5 scans s^{-1}, ionization EI 70 eV, ion source and quad temperatures 190°C and 150°C, respectively. Chromatographic data acquisition was performed using ChemStation software (Agilent). A CP–Porabond–Q 25 m × 0.25 mm × 3 μm capillary column (Varian Inc., Middelburg, the Netherlands) was used. Oven temperature program was as follows: 40°C held for 2 min, ramped at 5°C min^{-1} to 140°C, then ramped at 10°C min^{-1} to 270°C and held for 5 min. 1 L-in-volume Tedlar bags were supplied by SKC (West Fullerton, CA, USA). Glass round-bottom flasks (750 mL) with a screw cap and a silicon septum were supplied by Alltech. The SPME fiber (Carboxen–PDMS 65 mm), a 20 mL crimp top HS glass vial, and PTFE-coated butyl septa were supplied by Sigma–Aldrich (Steinheim, Germany). Gas-tight syringes were purchased from Hamilton (Hamilton, Reno, NV, USA). A side-stream capnometer, model NPB–75, was obtained from Nellcor (Pleasanton CA, USA).

Exhaled breath sampling

Exhaled air is a mixture of alveolar air and ambient retained in the respiratory dead space. Alveolar air is a part of exhaled air, which has been in contact with blood inside alveoli. The real-time measurement of the carbon dioxide profile in the collected alveolar breath was performed (Fig. 2).

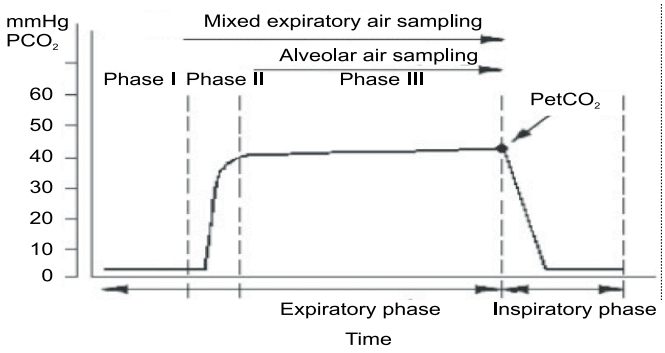

Figure 2. Scheme of typical capnogram. Concentration of CO_2 reaches plateau at phase III

During sample collection dead-space air was placed in the sampling system and diluted the alveolar air. Expiratory phase time could be divided into three phases. In the first phase partial pressure of CO_2 was in the range 0–10 mmHg. Afterwards, partial pressure increased to 40 mmHg (II phase). Sample was collected during III phase, when the pressure of carbon dioxide was the highest. The concentrations of endogenous molecules increased at the end of expiration when end tidal pressure of expired CO_2 reached *plateau*. Therefore, collection of breath samples should be done at the end of expiration. Ambient air samples were also collected for background correction. The exhaled breath from every patient was collected into two bags – the first one was used for GC–MS and the second one for PTR–MS.

Sample enrichment

There was no preparation step needed for PTR–MS system. Gas sample from the bag was directly inserted to the mass spectrometer. In case of GC–MS analysis, solid phase microextraction was used to enrich the samples with VOCs. SPME fiber was introduced into a glass vial containing either chromatographic standards or breath sample and exposed for 15 min at the temp. 30°C. After extraction, the fiber was injected into the GC and desorption was performed at 200°C for 1 min.

Calibration

Standards were prepared in round-bottom glass flasks. First, 2 μL of each standard were placed in the flask and after evaporation a certain amount of the mixture was transferred to the Tedlar bag containing 1000 mL of nitrogen. Afterwards, 15 mL of this mixture were injected to the evacuated HS vial. Prior to SPME sampling, each vial was equilibrated with nitrogen. In case of PTR–MS measurements, dilution of standards was similar except for gas transfer to vials.

RESULTS AND DISCUSSION

Eighteen compounds, including alkanes and aldehydes, were selected for experiments. Table 1 shows respective limits of detection (LODs) and limits of quantitation (LOQs). PTR–MS assured lower LODs and LOQs in determination of aldehydes, ketones, alcohols and esters, due to ionization processes.

Table 1. Comparison of sensitivity and linearity of established GC–MS and PTR MS procedures

Name	PTR–MS			GC–MS		
	LOD nmol dm^{-3}	LOQ nmol dm^{-3}	R^2	LOD nmol dm^{-3}	LOQ nmol dm^{-3}	R^2
Butane	–	–	–	1.8	5.4	0.955
Pentane	5.8	17.4	0.899	0.4	1.2	0.986
Hexane	4.5	1.35	0.905	0.3	0.9	0.967
Butyrylaldehyde	0.3	0.9	0.950	0.5	1.5	0.974
Pentanal	0.4	1.2	0.968	0.5	1.5	0.960

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Table 1. (Continuation)

Name	PTR–MS			GC–MS		
	LOD nmol dm^{-3}	LOQ nmol dm^{-3}	R^2	LOD nmol dm^{-3}	LOQ nmol dm^{-3}	R^2
Heksanal	0.6	1.8	0.880	0.6	1.8	0.926
Heptanal	0.5	1.5	0.316	1.0	3.0	0.751
2-Butanone	0.2	0.6	0.988	0.3	0.9	0.977
Acetone	0.6	1.8	0.993	0.3	0.9	0.945
2-Methylbutane	1.7	5.1	0.990	0.8	2.4	0.964
2,3-Dimethylbutane	3.1	9.3	0.989	1.1	3.3	0.929
2-Methylpentane	4.2	12.6	0.992	0.3	0.9	0.98
3-Methylpentane	4.0	12.0	0.899	0.5	1.5	0.988
2,4-Dimethylpentane	–	–	–	0.3	0.9	0.987
Toluene	0.5	1.5	0.993	0.7	2.1	0.917
Benzene	0.7	2.1	0.990	0.7	2.1	0.955
2-Propanol	0.7	2.1	0.570	1.0	3.0	0.986
Acetonitrile	0.1	0.3	0.974	0.3	0.9	0.989
Isoprene	0.4	1.2	0.986	0.1	0.3	0.988

Limit of detection is defined as the smallest quantity of the substance, xL, that can be detected with reasonable certainty by a given analytical procedure. xL is given by equation:

$$xL = xbi\tilde{i} \pm ksbi$$

where $xbi\tilde{i}$ is the mean result obtained for a blank sample, sbi is the standard deviation of the blank, and k is a numerical factor selected according to the required confidence level [13].

The mean of the blank measurements and its standard deviation were estimated using bootstrap methods [14] applied to linear regression models: calibration series were performed for each measurement. The results were used to estimate the parameters a and b in the equation Y = aX + b, where Y is the measured concentrations, and X denotes the injected concentrations (fitting was performed according the least squares method). Using bootstrap methods, standard deviations for the parameters a and b were calculated. Extrapolation to the zero injected concentration gave the offset a value that represents a for a blank measurement. The numerical factor k was set to 3, which ensured that the concentration values above the determined LOD were different from the possible zero signal (confidence level 99%).

High affinity of H_3O^+ ions to the oxygen-containing molecules assured the improved efficiency of ionization. Protonated forms of hydrocarbons occurred seldom. In case of PTR–MS, LOD values for aliphatic hydrocarbons ranged from 4.5 nmol dm^{-3} (hexane) to 5.8 nmol dm^{-3} (pentane) and for aromatic hydrocarbons from 0.5 nmol dm^{-3} (toluene) to 0.7 nmol dm^{-3} (benzene). In GC–MS the lowest LODs were found for *e.g.* 2,4-dimethylpentane, 2-methylpentane, and hexane (0.3 nmol dm^{-3}), and the highest were obtained for butane (1.8 nmol dm^{-3}), 2,3-dimethylbutane (1.1 nmol dm^{-3}), and heptanal and 2-propanol (1.0 nmol dm^{-3}). In general, LOD values for alkanes and branched alkanes obtained in GC–MS were lower than in PTR–MS; for oxygenated species the difference between LODs obtained in both methods was much smaller.

The obtained data set was evaluated by factor analysis. Two main factors were computed on the basis of principal component analysis. The factors were rotated using normalized Varimax technique. The first one was related to LODs and LOQs in GC–MS, while the second one to PTR–MS (Tab. 2). Factor scores (Fig. 3) indicate sensitivity of the system. Higher value of a particular factor is related to the lower sensitivity of the tested method.

Table 2. Results of quantitative analysis of exhaled breath from COPD patients

Number of patient / Compound	1	2	3	4	5	6	7	8	9	10
	Concentration nmol dm^{-3}									
Butane	11.2 ± 0.33	–		–	6.1 ± 0.31					
Pentane	1.2 ± 0.06		1.2 ± 0.05		–					
Hexane	0.9 ± 0.07									
2-Butanone	0.6 ± 0.04	0.5 ± 0.05					0.5 ± 0.04			0.4 ± 0.03
Acetone	41.6 ± 1.11	28.2 ± 1.13	8.7 ± 0.35	35.2 ± 1.21	20.9 ± 0.84	20.0 ± 0.81	38.6 ± 1.16	14.2 ± 0.52	38.7 ± 1.18	13.9 ± 0.51
2-Methylpentane			1.5 ± 0,11	–						
3-Methylpentane				1.5 ± 0,11	–					
Isoprene	7.6 ± 0.37	8.8 ± 0.34	10.7 ± 0.41	8.9 ± 0.34	8.1 ± 0.36	8.7 ± 0.34	6.0 ± 0.36	2.9 ± 0.14	1.3 ± 0.06	5.0 ± 0.25

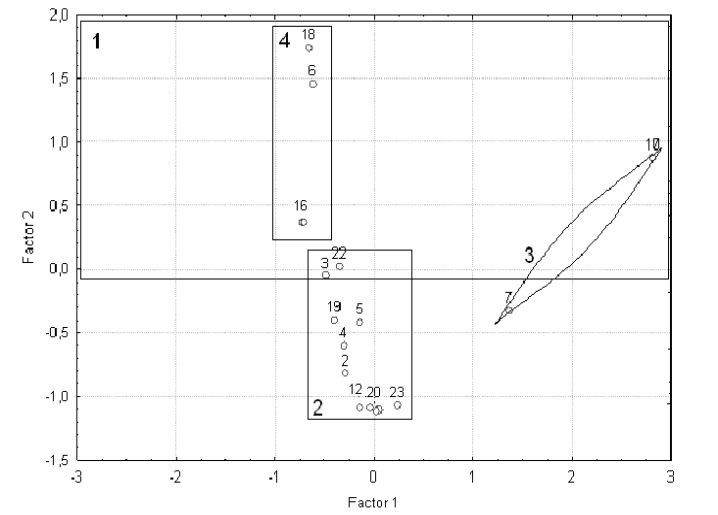

Figure 3. Factor scores for VOCs detected in breath samples

According to the calculated factors, the compounds were divided to three groups. The first group of compounds was characterized by relatively high LODs in PTR–MS measurements. For determination of these compounds GC–MS was thus the preferred method. The second group included the compounds (aldehydes), which could be determined at the lowest level using PTR–MS. The GC–MS method was the method of choice for the third group of compounds. Calibration graphs were used for determination of target VOCs in breath samples from COPD patients. The study was approved by the Ethic Commission (Nicolaus Copernicus University).

Mass spectrum and retention times of the standards were used for the quantitative analysis. Acetone and isoprene were the most abundant components of breath samples of COPD patients (Tab. 2). Concentrations of acetone ranged from 8.7 to 41.6 nmol dm^{-3} and of isoprene from 1.3 to 10.7 nmol dm^{-3}. The content of aliphatic hydrocarbons varied from 0.9 nmol dm^{-3} (hexane) to 11.2 nmol dm^{-3} (butane). Concentrations of aliphatic aldehydes were below LODs of the methods.

CONCLUSIONS

GC–MS method is still considered the best for determination of VOCs in breath samples. Only this procedure allows for unambiguous identification of many chemicals. Although it is highly sensitive, it has several limitations. It requires both sampling and preconcentration steps prior to the introduction of the collected sample into a gas chromatographic column, which may lead to contamination and losses of

analytes. Relatively new PTR–MS technique enables on-line determination of compounds in breath without preconcentration or chromatographic separation. PTR–MS can be an excellent tool for the on-line measurements of target species directly at the patient's bed. However, high sensitivity of PTR–MS is limited only to aldehydes (butanal, pentanal, hexanal, heptanal).

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