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# EXHALED BREATH ANALYSIS - QUANTIFYING THE STORAGE OF LIPOPHILIC COMPOUNDS IN THE HUMAN BODY

## ANALIZA WYDECHU - OZNACZANIE ZAWARTOŚCI ZWIĄZKÓW LIPOFILOWYCH W ORGANIZMIE CZŁOWIEKA

**Abstract:** Real-time analysis of exhaled breath is a promising new method to get quantitative information on lipophilic compounds stored in the human body. Some pilot results are presented on isoprene, which is produced as a by-product of the cholesterol synthesis and appears in exhaled breath at concentrations of about 100 parts-per-billion (ppb). The results have been obtained using proton transfer reaction mass spectrometry (PTR-MS) from healthy volunteers undergoing an ergometer challenge test (with 75 W). Peak exhalation flow of isoprene is about 400 nmol/min shortly after start of the challenge. The setup holds great potential in capturing continuous dynamics of non-polar, low-soluble VOCs over a wide measurement range with simultaneous recording of physiological parameters affecting exhalation kinetics.

**Keywords:** exhaled breath analysis, isoprene, proton transfer reaction mass spectrometry (PTR-MS)

Numerous organic compounds may be stored in the human body, and this is, in particular, so for lipophilic species. Determination of the amounts of such compounds in different compartments of the body (eg a hypothesized homogenous fat compartment or various organs) is an important task. A popular example in this framework is isoprene, a moderately cancerogenous C5 hydrocarbon, whose endogenous origin in mammals has mainly been attributed to the cholesterol synthetic pathway. Isoprene is also produced by plants in huge amounts. Other examples are hydrocarbons (C3-C13), which partly are of exogenous origin (kerosene, petrol) and partly may be due to metabolization of precursors by cytochrome P450 enzymes, by bacteria in the gut or are formed as a result from lipid peroxidation.

Considering their often high volatility, analysis of exhaled breath is the method of choice to reveal the presence of such compounds. Determination of their concentrations in a provided breath sample can be done by gas chromatography with mass spectrometric detection (GC-MS), proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube mass spectrometry (SIFT-MS), laser spectrometry or ion mobility spectrometry (IMS). Particularly, PTR-MS and SIFT-MS allow real-time measurements of exhaled breath, even with breath-to-breath resolution.

The concentration levels in exhaled breath change, depending on the stored amount of the compound in the body, but also depending on various physiological factors such as heart and breathing rate, rate of synthesis in the body, and rate of uptake from external sources.

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Based on the real-time capability of PTR-MS and SIFT-MS, one may model and simulate the continuous flows of volatile compounds within the human body, and validate such simulations by experiments: eg, by measuring concentration levels during physical activity on an ergometer, or by looking at the concentration changes during sleep (with different sleep phases giving rise to different heart and breathing rate).

## Methods

A high-sensitivity proton transfer reaction mass spectrometer (PTR-MS, 3 turbopumps, Ionicon, Innsbruck, Austria) with Teflon rings (instead of Viton rings) was used for our measurements. The count rate of primary ions $H_3O^+$ was around $10^7$ counts per second. Hydronium ions ($H_3O^+$) and water clusters ($H_3O^+$ $H_2O$) are primary ions, the parasitic primary ions $O_2^+$ and $NH_4^+$ are controlled (with counts being less than 2% of the primary ions, respectively). Isoprene (molecular weight 68 g/mol) and acetone (molecular weight 58 g/mol) were measured using their respective protonated forms at mass-to-charge ratios m/z 69 and m/z 59. Due to their contrasting physicochemical properties (isoprene is strongly lipophilic whereas acetone is hydrophilic) we view these two species as paradigmatic examples revealing valuable information on the broad spectrum of possible VOC responses according to distinct physiological conditions.

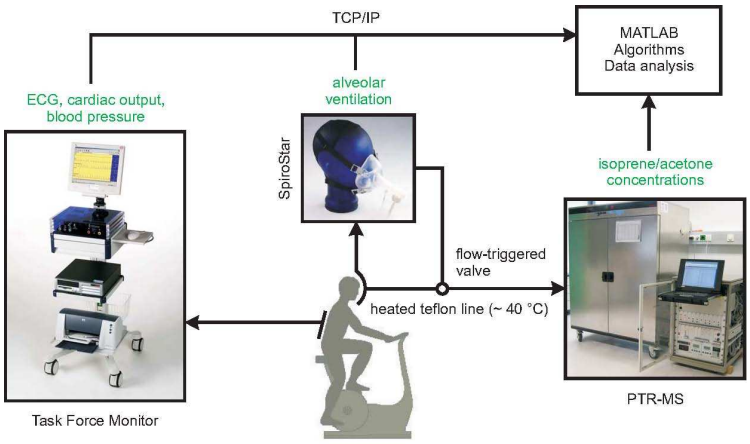


Fig. 1. Schematic presentation of the experimental setup for non-invasive real-time analysis of exhaled breath. Measurement of volatile compounds is performed by proton transfer reaction mass spectrometry (PTR-MS) or by selected flow tube mass spectrometry (SIFT-MS, not shown). Alveolar ventilation is determined by a flow measurement (SpiroStar, Medikro Oy, Kuopio, Finland). The electrocardiogram (ECG), cardiac output and blood pressure are measured by the Task Force Monitor (CNSystems, Graz, Austria)

Respiratory flow is obtained by means of an OEM version of the Medikro SpiroStar USB differential pressure sensor (Medikro Oy, Kuopio, Finland) delivering volumetric flow rates with a sampling frequency of 100 Hz. Inhalation and exhalation occurs through pre-calibrated single-use flow transducers, which can be connected to sterilizable or personal silicone head masks (CORTEX biophysics GmbH, Leipzig, Germany). This

allows the test subject to breath freely through mouth and/or nose while simultaneously reducing the risk of hyperventilation (see Fig. 1).

## Results

The concentration of isoprene in healthy volunteers is around 100 ppb in mixed expiratory exhaled breath [1]. During a challenge experiment at an ergometer its concentration increases ~3-fold (see Fig. 2). Since the alveolar ventilation increases during pedalling at the ergometer by a factor ~5, the absolute concentration of isoprene *decreases* soon after having reached a peak concentration.

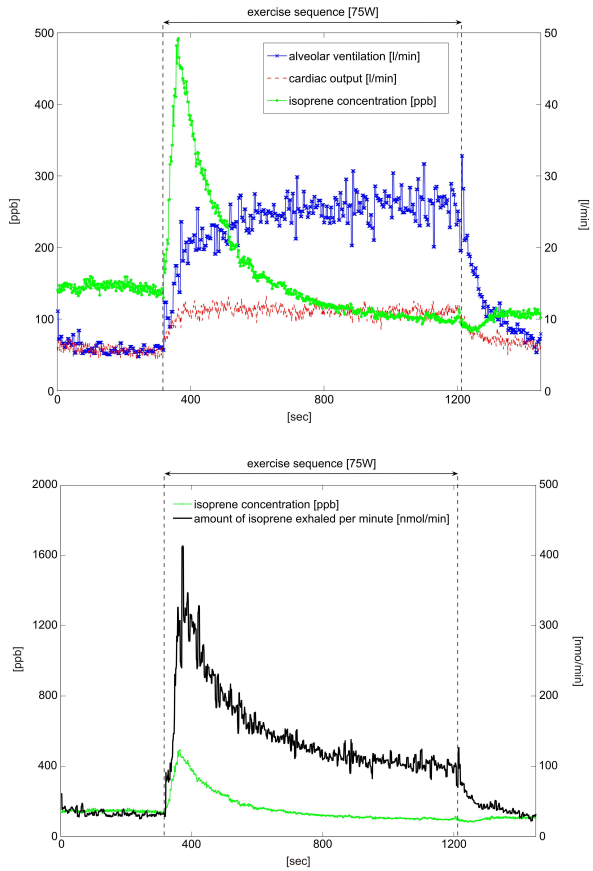


Fig. 2. Isoprene concentration (in ppb) during a challenge at an ergometer of one healthy volunteer (75 W, starting at 317 s, ending at 1213 s). Cardiac output and alveolar ventilation (in $dm^3$/min) are shown. As soon as the volunteers starts pedalling at the ergometer, the concentration of isoprene shows a huge peak, with the concentration increasing ~3-fold. The increase in alveolar ventilation by a factor of ~5 dilutes the amount of isoprene and therefore leads to a *decrease* in absolute concentration. The maximum amount of isoprene excreted per minute is ~12 times the amount per minute at rest. The mean amount of isoprene excreted during pedalling (in this experiment) is ~3.2 times the amount per minute at rest

The production rate of isoprene has been estimated as 0.34 μmol/h/kg B.W., from which 0.31 μmol/h/kg B.W. are metabolized [2]. Only 0.03 μmol/h/kg B.W. isoprene are exhaled unchanged (net isoprene production), amounting to $5\times10^{-5}$ mol/day for a 70 kg person. Elimination through the lungs happens at a concentration of about 100 ppb [1], corresponding to an alveolar concentration of ~140 ppb ($5.2\times10^{-9}$ mol/dm$^3$ at body temperature). Considering an alveolar minute ventilation flow of 5.25 dm$^3$/min [3], this corresponds to ~$4\times10^{-5}$ mol of isoprene/day (~$2.7\times10^{-8}$ mol of isoprene per minute), which is about 80% of the net isoprene production.

The *maximum amount* of isoprene excreted per minute (in the ergometer experiment described in Fig. 2) is ~12 times the amount per minute at rest. The *mean amount* of isoprene excreted during pedalling (in this experiment) is ~3.2 times the amount per minute at rest.

With a partition coefficient of 0.75 between arterial blood and alveolar air [2], the concentration of isoprene in arterial blood is estimated as $3.9\times10^{-9}$ mol/dm$^3$. The distribution constant between fat and blood in humans is ~82 [2]. Hence, in steady state the concentration of isoprene in the fat compartment is estimated to be $3.2\times10^{-7}$ mol/dm$^3$. For an arterial blood volume of 2.8 dm$^3$ [4] and fat tissue volume of 13.3 dm$^3$ (19% of B.W.), the amount of isoprene in this compartments is estimated to be $1.1\times10^{-8}$ mol and $4.2\times10^{-6}$ mol, respectively. Therefore in steady state the amount of isoprene in the fat compartment is ~380 times the amount of isoprene in the arterial blood.

## Discussion

Kinetic modeling and corresponding on-line determination of concentrations of lipophilic compounds in exhaled breath is a new interesting field of research, still in its infancy. In the future it will be a powerful tool for better understanding of the fate of volatile compounds within the body.

## Acknowledgement

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# ANALIZA WYDECHU - OZNACZANIE ZAWARTOŚCI ZWIĄZKÓW LIPOFILOWYCH W ORGANIZMIE CZŁOWIEKA

**Abstrakt:** Przedstawiono wstępne wyniki oznaczenia stężenia izoprenu w wydechu zdrowego człowieka. Izopren jest produktem ubocznym reakcji syntezy cholesterolu. Do oznaczenia zastosowano metodę reakcji przeniesienia protonu przy wykorzystaniu spektrometrii mas (PTR-MS).

**Słowa kluczowe:** analiza wydechu człowieka, izopren, metoda reakcji przeniesienia protonu wraz ze spektrometrią mas (PTR-MS)

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