Univerzita Karlova v Praze
Přírodovědecká fakulta
Katedra fyzikální a makromolekulární chemie

Ústav fyzikální chemie Jaroslava Heyrovského
Akademie věd České republiky

Mgr. Regina Brůhová Michalčíková

Nové metody hmotnostní spektrometrie pro stopovou analýzu látek v lidském dechu

New mass spectrometric methods for trace gas analysis of human breath

Disertační práce

Školitel: Prof. RNDr. Patrik Španěl, Dr. rer. nat.
Konzultant: Prof. RNDr. Eva Tesařová, CSc.

Studijní program: Fyzikální chemie
Studijní obor: Fyzikální chemie

Praha, 2019

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze dne ……..

……..

Podpis

„Život je nejlepší školou života.“

Jára Cimrman

Acknowledgments

In the following few sentences I would like to express my gratitude to all the people, who were involved in my doctoral study at the Charles University in Prague.

Firstly, I would like to thank to my supervisor and mentor, professor Patrik Španěl. He has been a constant source of scientific and mental support for me and gave me the sufficient encouragement to bring this work to the finish. In the recent years, he has helped me to understand principles of the selected ion flow tube mass spectrometry (SIFT-MS) method and taught me many things from the area of physics, physical chemistry, mathematics, statistics or presentational skills. I would also like to express my thanks professor Eva Tesařová, my supervisor-consultant at Charles University, Faculty of Science for her support starting from bachelor degree up to now. She was always prepared to provide me help, advice and useful comments.

I am also grateful to my colleague Ksenyia Dryahina for her enormous help at all stages of this project. She was not only a great co-worker, but became also my friend.

Let me please to acknowledge also all the remaining colleagues from the department of Chemistry of Ions in Gasous Phase at J. Heyrovský Institute of Physical Chemistry, namely Violetta Shestivska, Kristýna Sovová, Pavel Pásztor, Jiří Kubišta and Anatolii Spesyvyi. It was a pleasure to collaborate with them.

Of course I would like to thank also professor David Smith FRS from Keel University in England, who together with Patrik Španěl developed SIFT-MS method. It was my honour and privilege to collaborate on my project with such an expert.

Most importantly I would like to thank my family and friends for their love, support and reminders of what is really important in life. I love you!

Keywords

Breath analysis, Gas chromatography – mass spectrometry, Selected ion flow tube mass spectrometry, Ion molecule reactions, Proton transfer, Three body associations, Chemical ionisation, Volatile organic compounds, Biomarkers, Carboxylic acids, Alkenes, Heptenes, Inflammatory bowel disease, Ulcerative Colitis, Crohn's disease, *m/z* 99, Nalophan sampling bags, ethylen glycol, methyldioxolane

Klíčová slova

Analýza dechu, Plynová chromatografie – hmotnostní spektrometrie, Hmotnostní spektrometrie v proudové trubici s vybranými ionty, Reakce iontů s molekulami, Transfer protonu, Třístranné asociace, Chemická ionizace, Těkavé organické látky, Biomarkery, Karboxylové kyseliny, Alkeny, Hepteny, Zánětlivá onemocnění střev, Ulcerativní kolitida, Krohnova choroba, *m/z* 99, Nalophanový sáček pro vzorkování, Ethylenglykol, methyldioxolan

Contributions to papers included in the thesis

A. R. Brůhová Michalčíková, P. Španěl, A selected ion flow tube study of the ion molecule association reactions of protonated (MH+), nitrosonated (MNO+) and dehydroxidated (M-OH)+ carboxylic acids (M) with H2O, International Journal of Mass Spectrometry 368, 2014, 15-22.

Conception and design of the study: both authors equally. Supervision of the study: PS. Experimental set-up: RBM. Data collection/analysis: RBM. Interpretation of results: both authors. Drafting the manuscript: both authors. Approval of intellectual content: both authors.

B. R. Brůhová Michalčíková, K. Dryahina, P. Španěl, SIFT-MS quantification of several breath biomarkers of inflammatory bowel disease, IBD: A detailed study of the ion chemistry, International Journal of Mass Spectrometry, 396, 2016, 35–41.

Conception and design of the study: all 3 authors. Supervision of the study: PS. Experimental set-up: RBM, KD. Data collection/analysis: RBM, KD. Interpretation of results: all authors. Drafting the manuscript: RBM, PS. Approval of intellectual content: all authors.

C. R. Brůhová Michalčíková, K. Dryahina, P. Španěl, A detailed study of the ion chemistry of alkenes focusing on heptenes aimed at their SIFT-MS quantification, International Journal of Mass Spectrometry 425, 2018, 16–21.

Conception and design of the study: all 3 authors. Supervision of the study: PS. Data collection/analysis: RBM, KD. Interpretation of results: RBM. Drafting the manuscript: RBM, PS. Approval of intellectual content: all 3 authors.

D. R. Brůhová Michalčíková, K. Dryahina, D. Smith, P. Španěl, Volatile compounds released by Nalophan; implications to SIFT-MS and other chemical ionisation mass spectrometry analytical methods, Rapid Communication in Mass Spectrometry, In press, 2019, Accepted13th September 2019.

Conception and design of the study: all authors. Supervision of the study: KD, PS. Data collection/analysis: RBM, KD. Interpretation of results: all authors. Drafting the manuscript: all authors. Approval of intellectual content: all authors.

Hereby I declare that the actual contribution of R. Brůhová Michalčíková to these publications was as detailed above. In the terms of percentage her contribution was in my estimation as follows: A. 60%, B. 40%, C. 50%, D. 30%.

Prague 16.9.2019 Prof. RNDr. Patrik Španěl, Dr. rer. nat.

List of abbrevations

VOCs	Volatile Organic Compounds
SIFT-MS	Selected Ion Flow Tube - Mass Spectrometry
PTR-MS	Proton Transfer Reaction - Mass Spectrometry
ppbv	parts per billion by volume
ppmv	parts per million by volume
pptv	parts per trillion by volume
GC-MS	Gas Chromatography-Mass Spectrometry
SPME	Solid Phase Microextraction
NTD	Needle Traps
EN	Electronic nose
HBT	Hydrogen Breath Test
BET	Buffered end–tidal
PVF	Polyvinyl fluoride/Tedlar
PTFE	Polytetrafuoroethylene/Teflon
PET	Polyethylene terephthalate/Nalophan
PEA	Polyester aluminium
MMT	Metalcoated multilayer Tedlar
CT	Computer tomography
MRI	Magnetic resonance imaging
BMI	Body mass index
GI	Gastrointestinal
F1P	Fructose 1-phosphate
G6P	Glucose 6-phosphate
G1P	Glucose 1-phosphate
LCFA	Long-chain fatty acids
BCFA	Branched-chain fatty acids
HMG	Hydroxy methyl glutaryl
CYP	Polymorphic cytochrome P450 mixed oxidase enzymes
AS-MLC	Applied Sensors Hotplate carbon monoxide sensor
AS-MLN	Applied Sensors Hotplate nitrogen dioxide sensor
AS-MLX	Applied Sensors Hotplate VOC sensor
UC	Ulcerative colitis

CD	Crohn's disease
IBD	Inflammatory bowel disease
GIT	Gastrointestinal tract
CF	Cystic fibrosis
PA	*Pseudomonas aeruginosa*
ICU	Intensive care unit
BMAC	Breath methylated alkane contour
EI	Ionization by electrons
LOD	Limit of detection
LAS	Laser absorption spectroscopy
IMS	Ion mobility spectrometry
CRDS	Cavity ringdown spectroscopy
QCM	Quartz crystal microbalance
SAW	Surface acoustic wave
FTIR	Fourier transform infrared spectroscopy
TD	Thermal desorption
SPME	Solid-phase micro-extraction
GC-UV	Gas chromatography - UV spectrometry
MIMS	Membrane inlet mass spectrometry
APCI	Atmospheric pressure chemical ionisation
TAGA	Trace atmospheric gas analyser
EESI-MS	Extractive electrospray ionisation - mass spectrometry
SESI-MS	Secondary electrospray ionization - mass spectrometry
FA	Flowing afterglow
SRI	Switchable Reagent Ions
TOF	Time-of-flight
PEEK	Polyether ether ketone
FS	Full Scan mode
MIM	Multiple Ion Monitoring mode
IP	Ionization potential
HPLC	High-performance liquid chromatography
WG5	Working Group 5
HSE CAR	UK Health & Safety's Committee on Anal. Requirements
ISCARE	Clinical and Research Centre for IBD in Prague

CLD	Chronic liver disease
CAR/PDMS	Carboxen/polydimethylsiloxane
RT	Retention time
SIFDT-MS	Selected ion flow-drift tube – mass spectrometry
SSL	Split/Splitless injector

Abstract

This dissertation thesis summarizes results of experiments that have been carried out during my PhD studies related to the new mass spectrometric methods for trace gas analysis of human breath.

The thesis is divided into the theoretical and experimental part. The chapter at the beginning of this dissertation summarizes the current research in the area of breath analysis. It is describing the common breath metabolites, benefits and challenges of the method for therapeutic monitoring and clinical diagnosis and current applications. The next chapter of the theoretical introduction describes the techniques suitable for this area of research, with a special emphasis on mass-spectrometric techniques (in particular the selected ion flow tube mass spectrometry, SIFT-MS, method that allows accurate quantification of trace gases and vapours in humid air/human breath). All these parts are elaborated via the scientific literature review.

The following chapters are then directly related to my own research and describes the conducted experiment, including the results obtained. This experimental part "Results and Discussion" is divided to the individual subsections, which are conceived as the commentaries to the enclosed research papers published in peer reviewed journals. The first is the detailed step by step overview of the kinetics of ion molecule reactions (the basis of SIFT-MS) including the determination of rate constants and product branching ratios for several ion-molecule reactions of H_3O^+, NO^+ and $O_2^{+\bullet}$ reagent ions with carboxylic acids and possible Inflammatory Bowel Disease, IBD, biomarkers. The latter subsections are focused on three-body association reactions related to experiments, where the samples are influenced by the presence of water vapour (case of breath analysis), and on optimization of SIFT-MS kinetic library for accurate quantifications by this method. The last two sections finally concern the application of SIFT-MS for the breath analysis and describe not only results gathered in the area of IBD, but also challenges, which are connected with the off-line analysis and utilisation of Nalophan sampling bags, that have been encountered during my research - mainly the problematic of impurities released from the Nalophan sampling bags (off line analysis), that could significantly involve the SIFT-MS breath analysis, where low-level substances are determined.

Abstrakt

Tato disertační práce shrnuje výsledky experimentů, na kterých jsem pracovala během svého postgraduálního studia zaměřeného na nové metody hmotnostní spektrometrie pro stopovou analýzu látek v lidském dechu.

Disertační práce je rozdělena na teoretickou a experimentální část. Úvodní kapitola shrnuje současný výzkum v oblasti analýzy dechu, přičemž popisuje metabolity běžně obsažené v dechu, benefity a podněty této metody pro terapeutické monitorování, klinickou diagnózu a též současné oblasti aplikace. Další kapitola teoretické části popisuje techniky vhodné pro tuto oblast výzkumu, se zvláštním důrazem na hmotnostně spektrometrické techniky (zejména pak hmotnostní spektrometrii v proudové trubici s vybranými ionty, SIFT-MS, metodu, jež umožňuje přesnou kvantifikaci stopových plynů a par ve vlhkém vzduchu/lidském dechu). Obě kapitoly jsou zpracovány formou rešerše.

Navazující kapitoly se pak přímo týkají mého vlastního výzkumu a popisují provedené experimenty i dosažené výsledky. Experimentální část „Výsledky a diskuze“ je rozdělena do několika podkapitol, jež jsou koncipovány jako komentáře již publikovaných výsledků v impaktovaných časopisech. První podkapitola se detailně zabývá iontovou chemií (základ metody SIFT-MS), včetně stanovení poměrů větvení a rychlostních konstant reakcí reakčních iontů s vybranými karboxylovými kyselinami a možnými biomarkery zánětlivých onemocnění střev, IBD. Další sekce se pak věnují třístranným asociačním reakcím u reakcí, kde hraje duležitou roli přítomnost vodní páry ve vzorku (právě případ analýzy dechu) a optimalizací SIFT-MS kinetické knihovny pro přesnou kvantifikaci pomocí této metody. Poslední dvě sekce se již věnují aplikaci SIFT-MS pro analýzu dechu a popisují nejen výsledky získané v oblasti IBD, ale taktéž problémy, na které jsme během mého výzkumu narazili - zejména problematiku nečistot uvolňovaných z Nalophanových sáčků (off-line analýza), jež mohou významně ovlivnit analýzu dechu pomocí SIFT-MS, kde jsou stanovovány látky o velmi nízké koncentraci.

Contents

1 Aims

The aim of my PhD research project as formulated at the onset of my postgraduate study was:

> "To develop new reaction schemes and methodologies in selected ion flow tube mass spectrometry, SIFT-MS, based on chemical ionization of volatile substances present in the air during a well-defined reaction time using selective ion reagents H_3O^+, NO^+ and O_2^+. This work will include study of reactions of kinetics of ion molecule reactions be the use this method in interdisciplinary research, particularly in breath analysis for clinical diagnosis and monitoring of therapy. The research will be complemented by the development of new physical and chemical extraction methods for pre-concentration of trace substances for GC/MS analysis."

This dissertation shows that I have reached this aim and obtained some interesting and original results during the experimental work directed towards this goal.

2 Introduction

As a species, humans require air to survive, a need we share with most other members of the Kingdom Animalia. Just as the composition of the inhaled air can provide much information about the surrounding environment, the constitution of an exhaled breath can have a highly informative value about a particular individual. Such information can be than very important for many different applications, namely therapeutic monitoring and clinical diagnosis, where breath analysis could nicely supplement the standard methods such as blood and urine analysis.

Human breath is a very complex matrix of volatile organic compounds (VOCs), non-volatile organic compounds (aerosol particles), and inorganic compounds. It is considered, that human breath contains as many as 300 and even 3500 constituents, most of which are present in trace amounts. The typical composition [1] is: unmodified (inhaled) nitrogen (somewhat less than 74% by volume if water vapour is included, 79% of permanent gases) [2], oxygen (reduced from 21% inhaled to about 13.6-16.0% exhaled), water vapour (saturated at 37°C about 6%), carbon dioxide (about 5%, formed by respiration and commonly expressed as end-tidal CO_2 measured in the traditional units of mm Hg, i.e Torr, in the range from 28 to 38) [3] and argon (1%). Along with these major and significantly represented substances, the exhaled breath contains also several parts per million by volume (ppmv) of methane, hydrogen and carbon monoxide, about 1 ppmv of ammonia, less than 1 ppmv of acetone, methanol, ethanol and other volatile organic compounds, VOCs. The final composition is, however, very variable and depends on the inhaled air quality (it is expected that most of these compounds are of exogenous origin) and endogenous biological process. The human breath is therefore absolutely unique, characteristic and VOCs contained therein carry information concerning the biochemical status of an individual [4]. It is also very important to mention, that whereas the large number of volatile compounds appear even in the breath of healthy individuals, other compounds appear in the breath of diseased ones and certain of normally-occurring compounds have been shown to be elevated or decreased in specific diseases [5]. A typical example can be elevated concentrations of acetone in breath of patients suffering with diabetes [6,7] (which can be seriously, even dominantly influenced by diet – the trend common for many different metabolites), added ammonia in renal disease (potentially usable in therapeutic monitoring of dialysis) [8-10], nitric oxide in asthma

[11, 12] or small hydrocarbons, ethane and pentane, in oxidative stress (the fact, that was discussed in numerous studies and in some cases also unproved) [13-15].

The monitoring of such differences and in general, the concentration of individual VOCs, became the subject of a new scientific discipline, Breath analysis. Breath analysis is described as the non-invasive method for gathering the data about the clinical state of investigated individuals.

The area of modern breath testing commenced in 1971, when Nobel Prize winner Linus Pauling and co-workers demonstrated that human breath is a complex gas, containing well over 200 different VOCs in picomolar concentrations that can be separated by gas-liquid partition chromatography [16]. However, physicians are utilising a method of breath analysis, in the form of odour sensing, in their profession since the days of Hippocrates [17].

2.1 Breath analysis of VOCs for therapeutic monitoring and clinical diagnosis

Breath tests, besides a few exceptions, have not been integrated routinely in clinical practice. The complex mechanism underlying pulmonary gas exchange makes breath analysis a challenging subject and there are still many issues, which have to be considered and solved. The examples could be sampling and collection methods, or interpretation of results, which can be sometimes ambiguous. Any analytical methods used in breath analysis have to cope with different issues.

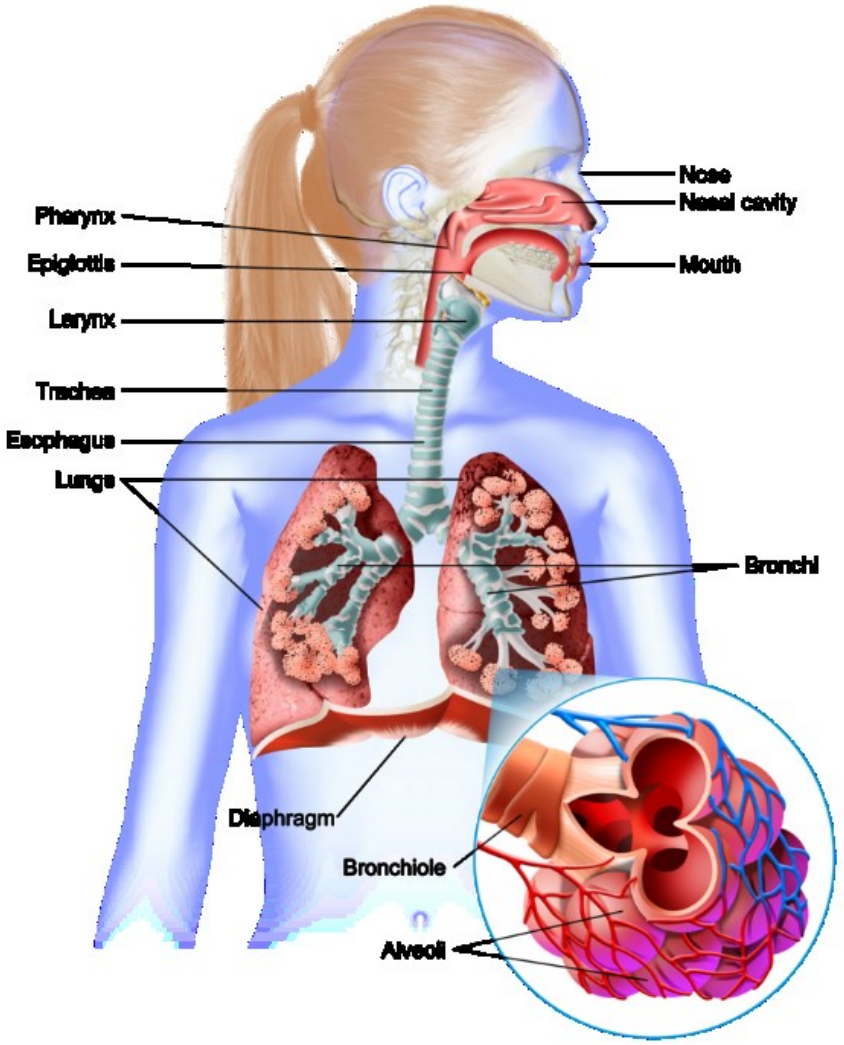


Figure 2.1. Lungs and airways. (https://webstockreview.net/explore/lungs-clipart-breathing-rate/)

Gas exchange [18] during respiration occurs primarily through diffusion. It takes place between the air within the alveoli and the pulmonary capillaries. Nowadays, it is known that exhaled breath concentrations of VOCs may be even under resting conditions significantly influenced by specific physiological parameters such as cardiac output (blood pressure, heartbeat rate) and breathing patterns (alveolar ventilation), depending on the physico-chemical properties of the compound under study [19]. Understanding the influence of these factors is an essential requisite not only for the development of a reliable methodology (achieving accurate sample collection, selection of the suitable analytical method), but especially for correct deduction of the corresponding blood concentration levels.

Breath gas concentration can be related to blood concentrations via mathematical modelling. The simplest model relating breath gas concentration to blood concentrations was developed by Farhi (1) [20]:

$$C_{measured} = C_A = \frac{C_V}{\lambda_{b:air} + \frac{V_A}{Q_C}} \quad (1)$$

Here, C_A denotes the alveolar concentration which is assumed to be equal to the measured concentration. It expresses the fact that the concentration of an inert gas in the alveolar air depends on the mixed venous concentration, C_V, the substance-specific blood:air partition coefficient, $\lambda_{b:air}$, describing diffusion equilibrium between capillaries and alveoli and ventilation-perfusion ratio, $\frac{V_A}{Q_C}$. Ventilation-perfusion ratio ensures that the ideal amount blood and gas is received by the alveoli for efficient gas exchange. It depends on the alveolar ventilation, V_A, controlling the transport of the VOC through the respiratory track, and cardiac output, Q_C, controlling the rate at which the VOC is delivered to the lungs. Blood:air partition coefficient, $\lambda_{b:air}$, is strongly dependent on temperature ranging from 23 °C in the mouth to 37 °C in the alveoli, affecting soluble gas exchange [21]. Unfortunately, this model fails when two prototypical substances like acetone (partition coefficient $\lambda_{b:air}$=340) or isoprene (partition coefficient $\lambda_{b:air}$= 0.75) are measured.

For low blood soluble gases ($\lambda_{b:air} \leq 10$) [21] the measured concentration is dependent on the rates at which blood is pumped through the lungs and ventilation, specifically the ventilation-perfusion ratio, where $C_{measured} = C_A$, meaning that low blood soluble VOCs must exchange completely in the alveoli.

Soluble VOCs ($\lambda_{b:air}$ > 10) [21] tend to be less affected by changes in ventilation and perfusion, however, hydrophilic exhaled trace gases, such as acetone, interact with the water-like mucus membrane lining the conductive airways. The exhaled breath concentrations of these volatiles appear to dilute on their way up from the deeper respiratory track to the airway opening (dilution effect), consequently for these highly soluble volatiles the concentration measured in exhaled breath is different from the alveolar air concentration, $C_{measured} \neq C_A$. There is also evidence that, with highly soluble volatile compounds, gas exchange occurs in the airways rather than alveoli [22].

There was also a lot of investigation assessing the concentration profiles during exercise [19,23-26]. Recently, the influence of exercise on mouth-exhaled and nose-exhaled breath was further investigated [25]. Smith and co-workers reported significant increase of isoprene breath concentrations which are in agreement with previous findings [26].

Based on the above mentioned facts, it was clear that more refined models are necessary to achieve better compliance between VOC breath concentrations and corresponding blood concentration levels. An isoprene gas-exchange model (2) was developed and showed a good fit to the breath isoprene concentrations measured during exercise. Dependency of heartbeat rate and breath rate for isoprene breath concentrations have been assessed, where isoprene levels were measured during exercise [19,24]. Isoprene concentrations showed a dramatic increase within the initials seconds of exercise [19,24], followed by a decline when heartbeat rate reached the maximum value and respiration rate increased, and lastly at the end of the exercise isoprene concentrations reached similar concentrations seen at the beginning. This means that the degree of blood-to-air partitioning of isoprene is very sensitive to heart rate. Such measurements demonstrate a relationship between breath rate volume, Vbr, heartbeat volume, HBV, Henry's law constant, H, and temperature, T, seen in equation below:

$$C_{A0} = C_{V0}\ exp\left(-\frac{1}{HRT}\frac{Vbr}{HBV}\right) \qquad (2)$$

For volatiles such as isoprene, with low solubility in blood and high volatility (Henry's law constant extremely low) a concentration gradient within the lungs is created and governed by the velocity of the bloodstream pumped through the lungs (proportional to heartbeat frequency) and the breathing rate. Namely, with increases in both heart rate

and breathing rate, more efficient partitioning of isoprene to breath air is restored. This means that isoprene evaporates efficiently through the transport via the bloodstream to the lungs, hence, $C_{A0} \neq C_{V0}$, meaning that isoprene venous blood concentration entering the lungs, C_{V0}, is different from isoprene arterial blood concentration leaving the lungs, C_{A0}.
Moreover, measurements taken during sleep showed enhanced blood isoprene concentration due to lower heartbeat rate achieved during the night [24].

All the above mentioned problems and a lack of normalization and standardization are causing a huge variations between results of different studies. Nevertheless in the last few years, there is a significant progress in this field and introducing breath analysis into clinical practice will be challenge of today and tomorrow.

2.1.1 Common breath metabolites and their physiological concentrations

As was already mentioned above, the VOCs presented in the breath of people can be divided to exogenous, originating from current or previous environmental exposures, and endogenous, arisen by internal metabolic (anabolic and catabolic) production.

Exogenous VOCs penetrating the body as a result of environmental exposure can be used to quantify a body burden. The example can be the halogenated organic compounds, which are analysed for environmental or expositional issues. Also breath tests are often based on the ingestion of isotopically labelled precursors, producing isotopically labelled carbon dioxide and potentially many other metabolites.

Endogenous VOCs are released within the human organism as a result of normal metabolic activity or due to pathological disorders (see the scheme in *Figure 2.2*). They can be produced at various organs and places in the body, enter the blood stream and are eventually metabolized or excreted via exhalation, skin emission, urine, etc. Also the production of VOCs by bacteria (oral cavity, gut) is possible. The origins of certain VOCs in breath presumed to be endogenous have been proposed to be useful as preclinical biomarkers of various undiagnosed diseases including lung cancer, breast cancer, and cardio-pulmonary disease. Identification and quantification of potential disease biomarkers can be seen as the driving force for the analysis of exhaled breath. Moreover, future applications for medical diagnosis and therapy control with dynamic assessments of normal physiological function or pharmacodynamics are intended. Nevertheless, in

order to develop a diagnostic breath test, ready to be used in clinical practice, it is necessary to unravel the baseline physiological concentrations of volatiles present in human breath, and their relationship with age, gender, ethnicity, and metabolic changes in the body. Such an effort can be seen in many previous studies and projects, some of which are mentioned below.

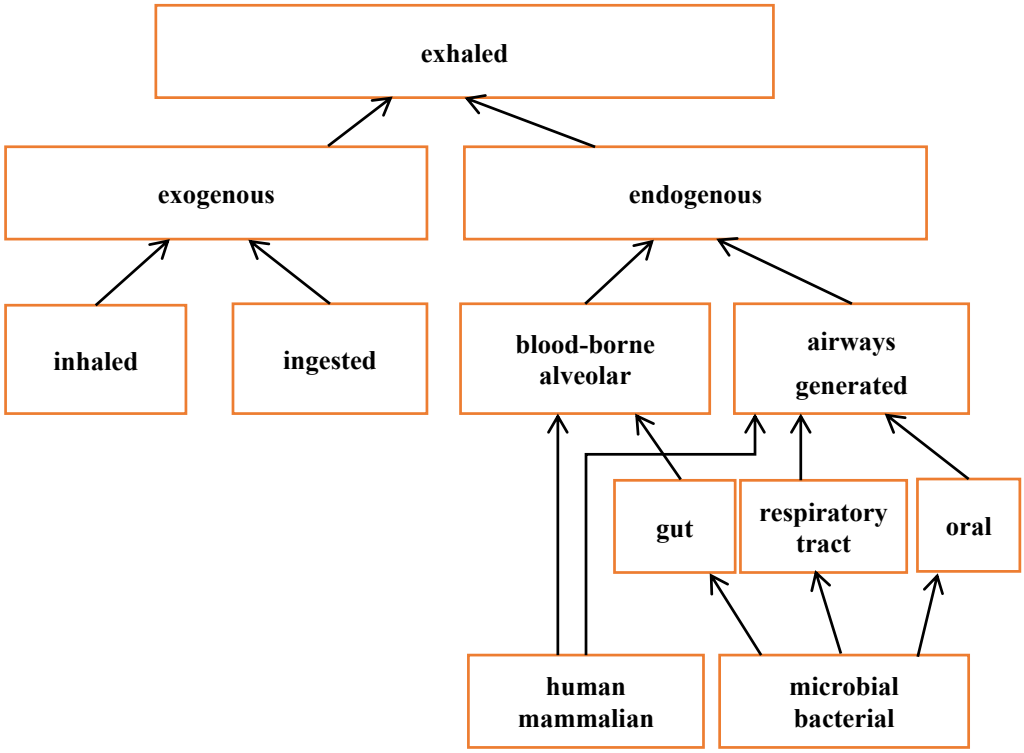


Figure 2.2. A scheme of various origins of VOCs in exhaled breath.

Concentrations of common breath metabolites, ammonia, acetone, isoprene, ethanol and acetaldehyde have been measured in the selected ion flow tube - mass spectrometry (SIFT-MS) in the breath of five healthy volunteers over a period of 30 days by Diskin and co-workers [27]. The mean concentrations were calculated, and meaningful distributions obtained for ammonia, acetone, isoprene, and ethanol [27,28].

Subsequently, the longitudinal SIFT-MS study of common breath metabolites, ammonia, acetone, methanol, ethanol, propanol, acetaldehyde and isoprene in the breath of 30 healthy subjects over a six-month period has been performed by Turner and co-

workers [29-32].The study assessed the biological variability and the concentration distributions for above mentioned metabolites have been determined on-line in single breath exhalations and showed to be a log normal distribution for these metabolites. Ammonia was shown to be a major breath metabolite with a median of 833 parts per billion by volume (ppbv), followed by acetone (477 ppbv), methanol (461 ppbv), ethanol (112 ppbv), isoprene (106 ppbv), propanol (18 ppbv), and acetaldehyde (22 ppbv).

It has been proved that most of the ammonia observed in mouth-exhaled breath has its origin in the oral cavity [33]. Ammonia is also produced systemically, it appears in the body as a breakdown product of proteins, a contribution originated from the bacterial degradation of protein in the intestine [30]. The metabolic pathway is starting in the liver, where the ammonia is converted into urea, which is then eliminated in urine. Some of the ammonia is expelled from the breath and some is emitted by the skin [34].

The metabolic pathways of acetone are well established. The decarboxylation of acetoacetate and the dehydrogenation of isopropanol are the main two sources of acetone [35]. Acetone concentrations are elevated in ketosis states including diabetes, the disease dominated by two metabolic changes: rise of blood sugar concentration and intensive lipolysis [35]. However, acetone was not marked as unique biomarker of diabetes.

The metabolites belonging to the group of alcohols, methanol and ethanol, may arise as anaerobic fermentation products by gut bacteria [36]. This production way is than common for all alcohols in the series from methanol to heptanol. Methanol is contained in some foods, such as apples and drinks, which, when ingested, increases the methanol in the circulation and, hence, in the exhaled breath [36]. Methanol is used industrially as a solvent, pesticide, and alternative fuel source. It also occurs naturally in animals and plants. Methanol can be absorbed into the body by inhalation, ingestion, skin contact, or eye contact. Methanol does not appear to be generated in the mouth and concentrations detected in breath are of systemic origin. Most breath ethanol, however, appears to be due to mouth fermentation of sugars (unless the subject has been consuming alcoholic drinks) [37].

When we will focus on 2-propanol, we can see that it is a product of the enzyme-mediated reduction of acetone. Bacteria present in the gut produce several alcohols, including the structural isomers isopropanol or 2-propanol and it is this isomer that largely exists in the human body [38]. Nevertheless, it is still present in lower concentrations than ammonia or other above mentioned VOCs.

The biochemical origin of isoprene in human breath is not entirely clear. However, it is considered to be a by-product of cholesterol synthesis [39]. Abnormal breath isoprene concentrations are related to end-stage renal failure and increases in isoprene concentrations have been associated with oxidative stress. However, this assumption has not been proved by the work of Lirk and co-workers [40]. As was already mentioned, isoprene concentrations are also very variable based on the physical activity (linked to local variations of gas exchange in peripheral tissues) [26].

Acetaldehyde concentrations result from endogenous ethanol metabolism [5]. As a consequence, acetaldehyde concentrations in breath are invariably lower than the corresponding ethanol concentrations. In healthy individuals, it is rapidly cleared by conversion into acetic acid or acetate ions and thus it is present at low concentrations in the body. However, these concentrations in breath may not be obvious because acetaldehyde can also be produced from cellular activity involving sugars.

2.1.2 Influence of demography (age, gender and diet)

The wide variation of concentrations of volatile metabolites in human breath can be influenced also by many external factors, for example diet and natural intra-individual biological and diurnal variability. Therefore the relationship between baseline concentrations of common breath VOCs and some demography parameters as age, gender, ethnicity and diet has been investigated in many studies.

Age and gender are important factors to be taken into account in breath analysis. Taucher and co-workers recognised that isoprene concentrations in children's exhaled breath are significantly lower than in adults [41,42]. VOCs and particularly isoprene on the breath of 126 volunteers have been measured also by Lechner and co-workers, reporting an increase in isoprene concentration of exhaled air of male subjects [43]. Both studies have been conducted by using proton-transfer-reaction mass spectrometry (PTR-MS). Also the SIFT-MS studies of age influence on VOCs presence in breath have been conducted [38,42,44]. The exhaled breath of several volunteers within the age range 4–83 years was investigated by Španěl and co-workers, whereas a trend of increasing breath ammonia concentration with age has been revealed in this study [44]. Smith and co-workers also reported apparent elevation of breath isoprene concentrations during adolescence, probably due to the onset of puberty, as stated by the authors [42].

Another interesting topic involving concentration of VOCs is the ovarian cycle and ovulation phase. During analysis of the headspace of urine from a number of female volunteers, Smith and co-workers observed acetone and ammonia concentrations occasionally higher than the normal. The urine samples were collected before any food intake. Such findings suggested that it may be caused by metabolic changes occurring during ovulation and related to menstrual cycle length [45,46].

There is an influence of food intake and starvation on the concentrations of breath metabolites [47] and many studies have been focused on this topic in the past. One of them quantified breath metabolites ammonia, methanol, ethanol, propanol, formaldehyde, acetaldehyde, isoprene, and acetone by SIFT-MS for a group of five volunteers, before and after ingesting 75 g of glucose in the fasting state [34]. Also a study of six healthy volunteers following the ingestion of a liquid protein meal [48] and subsequently of a liquid carbohydrate meal [49] was carried out to investigate the influence by food intake. Alveolar breath concentrations of ammonia, acetone, ethanol, and isoprene were taken from single exhalations in the morning following overnight fasting and then for several hours after ingestion of the meals. Prior to feeding, the acetone concentrations were relatively high and the corresponding ammonia concentrations were relatively low in the breath of all six volunteers. Following feeding, the acetone concentrations all decreased as the body was nourished. Initially, the ammonia concentrations all reduced towards a minimum value (near to 200 ppbv) approximately 30 min after feeding by both the protein and the carbohydrate meals and then began to increase in the breath of all six study participants. For the (nitrogen containing) protein meal the ammonia increased to values obviously greater than their respective initial values, whereas for the carbohydrate meal it asymptotically approached the initial values prior feeding. It has been hypothesized by the authors, that the initial dip in the ammonia concentrations was the result of an increase in portal blood flow as the stomach was loaded with the meal [48,49]. The hypothesis above was supported also by an experiment, when the ethanol (three different quantities, namely 7.5 ml after fasting, 17 ml after fasting and 2.5 ml immediately after a lunch meal) was administered to volunteers in 500 ml of tap water (a volume chosen to mimic the large volumes of protein and carbohydrate liquid meals that were used in previous experiments. At blood/body water concentrations greater than 0.1 gL^{-1} it is known that ethanol exhibits saturation kinetics, i.e. the rate of elimination is constant regardless of concentration [50]). The similar decrease in the breath ammonia was observed within this study [37]. There were unexpected increases in the breath ethanol concentrations

following the ingestion of both the protein and carbohydrate meals, which were traced to the presence of ethanol evidences in the liquid meals. The studies of ethanol metabolism were reported by Winkler and co-workers [51]. Metabolic degradation of ethanol was tracked by the ingestion of isotope-labelled ethanol using real-time breath gas analysis with PTR-MS. The findings indicated that, in part, ethanol was metabolized to acetone and isoprene, as deuterated acetone and isoprene were observed in the mass spectra. However, the signal of the deuterium-labelled acetaldehyde was not observed, suggesting that this product did not enter the blood stream but was rapidly further metabolized. There is a lot of foods, such as garlic, onion, mint, banana and coffee, which are known to emit volatiles at trace concentrations [52,53]. Therefore, for the breath analysis, it is always necessary to take the influence of food into consideration before evaluation of the gathered results.

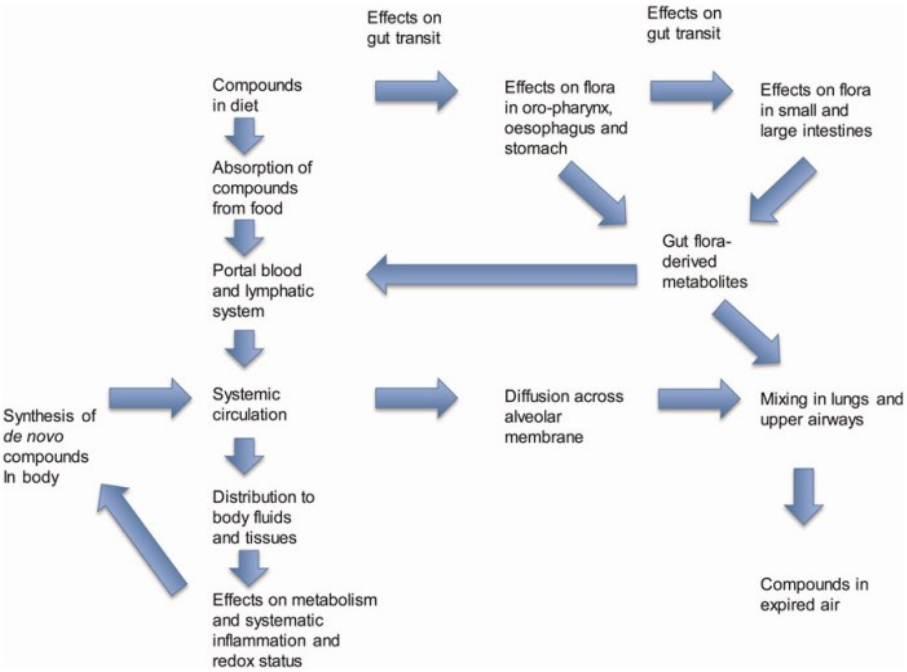


Figure 2.3. The complex interactions between diet and expired breath metabolites. (Reproduced from Ajibola et al., Journal of Nutritional Science, 2013 [140]). In general are volatile metabolites in the breath derived from several sources: environmental inspired air, from cells, including microorganisms that are located throughout the oral/nasal cavities and the pulmonary system, the upper and lower gastrointestinal tracts and from general human metabolism.

2.1.3 Influence of external volatile compounds

It has been shown that the composition of exhaled breath is considerably influenced by exposure to pollution and indoor-air contaminants, for example, smoking-enhanced acetonitrile concentrations were found in the breath and urine of smokers [54-57]. The danger of smoking, and to a less extent passive smoking, have been well documented and publicized in the past and in addition to the already mentioned acetonitrile, compounds like 2,5-dimethylfuran, benzene, toluene, styrene, ethane, pentane, hydrogen cyanide (HCN) and carbon monoxide (CO) have been identified as characteristic in smokers and passive smokers' breath [58-62]. Acetonitrile is a compound presented in the exhaled breath of smokers at measurable concentrations even several days after smoking. After cessation, it takes nearly a week until the concentrations decrease to that of non-smokers [54]. Based on above mentioned fact, this cyanide can be used as good indicator of whether a given subject is a smoker or not. Increased concentrations of exhaled ethane and pentane are probably caused by high concentrations of hydrocarbons in cigarette smoke, as well as oxidative damage caused by smoking [60]. Measuring carbon monoxide in exhaled breath is also a well-established method used to differentiate between smokers and non-smokers [62]. As a constituent of cigarette smoke, CO enters the blood circulation during smoking and forms carboxyhemoglobin (COHb). The elimination of CO is primarily by respiration, thus, there is a strong correlation between CO in breath and COHb [62]. In addition to the above mentioned compounds, also concentrations of isoprene, acetone, ammonia and ethanol have been investigated. Significant increase (70% in mean) has been observed in case of isoprene. The mean increase in acetone concentrations was found to be 22%, in contrast, for ethanol was observed mean decrease of 28% [63].

Aside from smoking, there are other ways of VOCs entering the body. Breath contains a diverse range of VOCs that can be taken up by the body through inhalation or skin, and, depending on distribution kinetics, may be present in exhaled breath for different periods after exposure. For instance, limonene was found in cosmetics, perfumes and cleaning products and is emitted by wooden furniture and floorings. It is known to be soluble in blood and adipose tissue and, therefore, has the potential to be taken up by the body during inhalation [64]. Limonene can be not only inhaled, but also easily ingested. This compound occurs in the greatest abundance in citrus fruits, large variety of other fruit and vegetables and it is a common additive in commercial food and drinks. There is

also a number of studies, which are focusing on exposure of workers to different volatile compounds. This area has and continues to receive considerable attention, as it is an important area of public concern with serious health implications. The examples could be the SIFT-MS studies carried out in collaboration with experienced health and safety scientists following breath from a healthy individual who had been exposed for several hours to a controlled atmosphere of perchloroethylene (C_2Cl_4)[65], exposure of healthy volunteers to controlled amounts of xylene and trimethylbenzene isomers [66], or intake of anaesthetic gases, halothane, isoflurane, and sevoflurane, by operating staff [67].

2.2 Sampling and analysis

The analytical techniques that are available these days for breath analysis can be easily divided on those for real time measurements of concentrations of volatile metabolites in exhaled breath, i.e. SIFT-MS, PTR-MS, ion mobility spectrometry and laser-based spectroscopy [68-78,26,23,47], and the off-line techniques. With regard to drawbacks of on-line techniques (high instrumentation costs, a somewhat less certain identification of compounds, lack of control over the sampling time), the off-line techniques are still the most utilised methods. The off-line exhaled breath analysis is than typically carried out by collecting a sample in a suitable container or trap, concentrating the analytes of interest by different pre-concentration method (e.g., solid phase microextraction (SPME), sorbent trapping, or needle traps (NTD)), and analysing it by thermal desorption gas chromatography coupled to mass spectrometry. To wit, gas chromatography-mass spectrometry (GC-MS) remains the gold standard for the analysis of breath constituents [79-85,5]. For the breath analysis is desirable also monitoring over the time, because of biological variability among subjects (an issue in breath sampling). This approach than serves as a form of self-control [86]. In order to perform breath sampling it is necessary to consider the diffusion of volatile organic compounds from blood to alveolar air, which depends on their physicochemical properties, such as, polarity, solubility in fat, Henry partition constant, and volatility [28].

Concerning the quality of analysis (especially off-line) the sampling and sample stability are the most critical steps in the entire analytical procedure. In fact, preservation of the sample integrity during sampling and sample storage is probably one of the most demanding challenges in analytical chemistry. Different phenomena accompanying these

phases of analysis like interaction with the sampling container (adsorption/desorption processes and release from the container material itself), permeation through the container walls (loss of sample components and contamination of sample by external pollutants), as well as chemical reactions facilitated by high humidity and highly reactive species can irreversibly modify the original sample composition, consequently cause the alteration of the final results of analysis and lead to erroneous conclusions [87]. This is particularly true in the case of exhaled breath analysis. Ultra-low concentrations of volatile organic breath constituents (from ppbv to low parts per trillion by volume (pptv)), presence of highly reactive species and high humidity inducing wet chemistry make breath samples particularly vulnerable to all problems related to storage [82-84,5,88,89] and controlled sampling is therefore a key requirement for reliable analysis of breath biomarkers. Actually, there are no accepted standardized methods for on-line or off-line VOC breath-gas sampling and analysis. The first guidelines concerning sample collection for breath analysis was released in 1999 by the American Thoracic Society for nitric oxide (NO) monitoring in breath [90]. Later on, updated guidelines were published in 2005 for measurement of NO in mouth- or nose-exhaled breath [91]. In 2005, recommendations were also published for exhaled breath condensate sampling and analysis [92]. Hence, reproducibility and reliability of sampling methods and analytical measurement procedures continue to be of critical importance.

Many different aspects related to sampling are debated in scientific community, such as body posture of the subject when providing the breath sample; hyperventilation; control of the flow or volume of breath during collection; sampling via nose or mouth; number of breath samples to be taken to reduce variability (single or multiple breaths); dilution and contamination of the sample; physiological parameters, such as respiratory rate or heart beat rate; alveolar breath or end-tidal volume and dead space; number of subjects per study to avoid over-modelling; and direct analysis or sampling for storage [86]. Also, temperature dependence influencing the composition of breath samples in off-line measurements must be taken into consideration [93,94]. The number of issues to be considered suggests that the development of several protocols for standardized breath sampling may become mandatory.

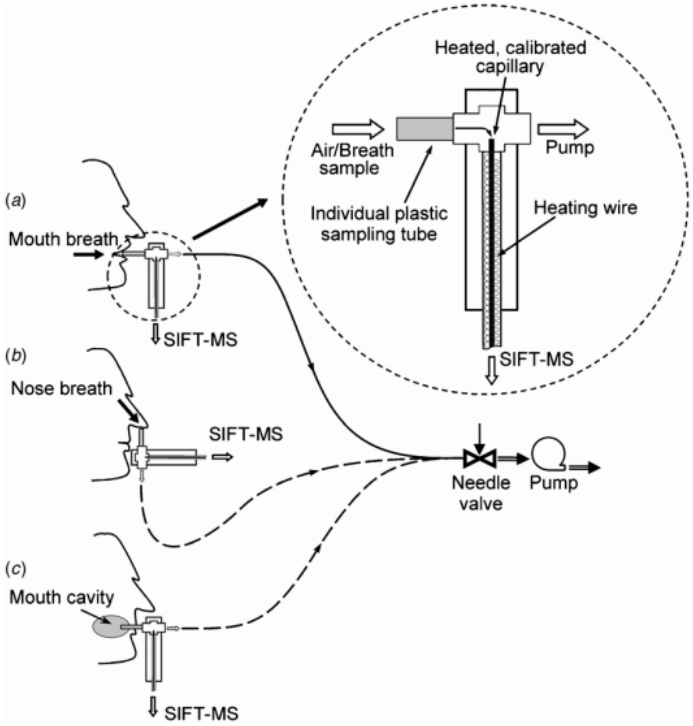


Figure 2.4. A representation of the sampling of (a) breath exhaled via the mouth, (b) breath exhaled via the nose and (c) the air in the mouth cavity. The ambient air, exhaled breath or cavity air samples pass along the individual plastic sampling tubes and are drawn across the sampling calibrated capillary (shown in the enlargement) by the action of the small pump. The in-line needle valve is used to regulate the sample flow rate. SIFT-MS indicates the sampling line to the SIFT-MS instrument. (Reproduced from Smith et al, Rapid Communication in Mass Spectrometry, 2008 [33]).

2.2.1 End-tidal and Alveolar Breath

Concentrations of volatile compounds in blood are reflected by their concentrations in the exhaled air, depending on their blood-gas partition coefficient or solubility. Alveolar breath is air in equilibrium with systemic blood, whereas end-tidal air is the last fraction of expired air, whose composition resembles alveolar air. Because the alveolar breath is not possible to study non-invasively, the general term "end-exhaled breath" is applied because it does not imply that the composition of expired air is always identical to the equilibrated air inside the alveoli. It has long been acknowledged that alveolar gas exchange is dependent on ventilation, pulmonary perfusion, and the blood:air partition coefficient [21], thus, the non-homogeneities in the composition of alveolar air among different lung regions over different blood:air solubilities of volatile organic

compounds [95]. There is evidence that the gas exchange of highly soluble volatile compounds occurs in the airways rather than alveoli [22], meaning that VOCs measured at the mouth depend on expiratory flow. Generally, the term alveolar breath may be applied for low blood soluble VOCs, whereas for highly soluble volatiles such as acetone the term end-exhaled breath should be used due to the evidence that gas exchange occurs in the airways rather than alveoli. Such evidence was quantified for the first time by Španěl and co-workers, who demonstrated the discrepancy between the concentrations in the alveolar region to that in exhaled air [96]. In 1940s W.S. Fowler noted, that the volume of exhaled air is a mixture of dead space and alveolar air [97]. Dead space was than previously defined as the volume of expired air, which acts as a conducting airway (nose, pharynx, larynx, trachea), whereas alveolar air is the expired air fraction that has been exchanged in the alveoli. Breath analysis for medical diagnosis relies on end-tidal sampling [98], involving the collection of only end-tidal air. Alveolar concentration reflects the concentration in blood and consequently, the concentration in blood reflects the metabolic processes occurring in the body. Furthermore, there is evidence that better reproducibility of data is obtained when only the end-tidal fraction of breath is analysed [99].

The initial strategy for selection of the right part of the exhaled breath for analysis was discarding first part (500 mL) of exhaled breath, to avoid dilution of the sample by dead space. Nevertheless, because it incorrectly assumes that all subjects have the same volume of dead space [100], there was an effort to define another strategy and approach. For the on-line analysis has been developed method called buffered end–tidal (BET) breath sampling [98]. The purpose of this method is to extend the end-tidal fraction of single exhalation. To do so, patient is asked to exhale through a tailored tube, in which the end-tidal breath is buffered.

Nevertheless, in addition to the facts above, it is still difficult to unravel concentration of breath molecules prior to mouth appearance, because, in many cases, we do not have enough knowledge about the parameters and processes which could affect the VOCs´ final concentration in the mouth. For example, highly soluble compounds are diluted on their way up from the deeper respiratory track to the airway opening, leading to a dilution effect on VOCs' concentrations (see the chapter below *2.2.2 Dilution and Contamination*). In addition, changes in ventilation, body posture and stress can have a significant impact on the observed breath concentration [3].

2.2.2 Dilution and Contamination

It was found out, that the exhaled breath concentrations of water soluble substances appear to dilute on their way up from the deeper respiratory track to the airway opening, leading to discrepancies between the true alveolar breath and the measured concentrations, demonstrating a dilution effect. It is because hydrophilic exhaled trace gases, such as acetone, interact with the water-like mucus membrane lining the conductive airways, an effect known as wash-in/wash-out behaviour [101]. Thanks to this, highly soluble gases are present in large concentrations in the airway tissue and mucus as compared to less blood-soluble gases for a given partial pressure. In the breath analysis the absorption-desorption phenomenon must be also taken into consideration. The first step in this process is absorption of soluble gases from the airway wall to the inspired air during inspiration. By the time the air reaches the alveoli, the air is saturated with the soluble gas and no additional gas exchange occurs. During the next step, expiration, a gradient air-to-mucus is established promoting the deposition of soluble gas on the mucus and delaying the rise in soluble gas partial pressure at the mouth. For this type of gases an anatomic dead space cannot be defined [21,22].

Perfusion (diffusion through the airway wall) and temperature are another important phenomena influencing the airway gas exchange. Perfusion is driven by the bronchial blood flood, meaning that an increase in blood flow increases the amount of blood soluble gas in the exhaled breath. The relationship between the exhaled and inhaled breath concentrations have been investigated in the past for seven different compounds, whereas gathered results were in compliance with previous models [96].

2.2.3 Sampling of single or multiple breaths

Number of breath samples is another important aspect discussed in connection with the breath analysis, whereas breath sampling can be performed for single breath or multiple breath cycles [98,102,103]. The second option is that more preferred in order to acquire reproducible breath samples. Composition of a single breath may not be a representative alveolar gas sample for the reason that breaths may considerably vary from each other due to different modes and depth of breathing.

Breathing patterns [3] have been studied and measurements, such as mouth pressure, tidal volume, respiration rate, end-tidal carbon dioxide, and mixed expired carbon dioxide, were recorded. Paced breathing [3] profiles showed reduced breath

variability, according to mass and respiration rate. The authors suggested that controlled breathing would prevent hyperventilation, reducing variability in ventilation.

2.2.4 Storage, stability and sampling containers for breath analysis

Direct sampling is preferable to storage for later analysis. This way the decomposition of samples or loss of compounds by diffusion is avoided. When direct analysis is not possible, the appropriate storage of exhaled breath is an important issue to consider, as was already mentioned above.

Several types of materials, such as rigid sample containers (gas tight syringes, glass bulbs, stainless steel canisters) or flexible sampling bags, can be used for sampling and storing of breath samples. Each of these containers is than characterized by certain advantages, but also disadvantages.

In the recent years are for the breath analysis increasingly preferred polymer sampling bags, e.g. Tedlar (PVF, polyvinyl fluoride), Tefon (PTFE, polytetrafuoroethylene), Nalophan (PET, polyethylene terephthalate) and metal-coated multilayer bags (Flexfoil and polyester aluminium (PEA)) [104,105]. This is due to their moderate price, inertness, relatively good durability and reusability. Unfortunately, there are also some challenges in connection with these containers, especially the phenomena affecting sample composition like permeation through the bag walls, reversible/irreversible adsorption and pollutants emission from bags film. The pros and cons of individual polymer sampling bags are, of course, slightly different depending on the material characteristic. Tedlar, currently one of the most popular and commonly accepted materials (especially in the USA) [82,83,104,106-113] is very robust in use, but suffer from background odor concentrations of typically >80 ou_E /m^3 (European odour unit = One ou_E is the mass of pollutant that, when evaporated into 1 m^3 of odourless gas at standard conditions results in a mixture with concentration equal to the olfactory threshold for that pollutant). This is caused by use of a solvent during production [114]. Teflon bags are quite inert but not very robust in use and are quite costly, which in practice almost rules out one-time use. Nalophan bags have been evaluated in the previous studies as best choice for the collection of breath samples in terms of contaminants released during storage (Nalophan sampling bags are considered to be containers with the perfect background, because in the previous studies focusing on this aspect, no or just few impurities have been detected), good sample stability (up to 24 hours for both dry and

humid samples), and very limited costs (great advantage, allowing one-time use followed by disposal) [112,115]. Unfortunately, the material is not always easy to obtain in smaller quantities and needs to be stored for some time to reduce background odours. Nalophan impurities have been investigated also as part of my work and are in detail discussed in section 4.5 *Challenges in connection with breath analysis - Nalophan sampling bags*.

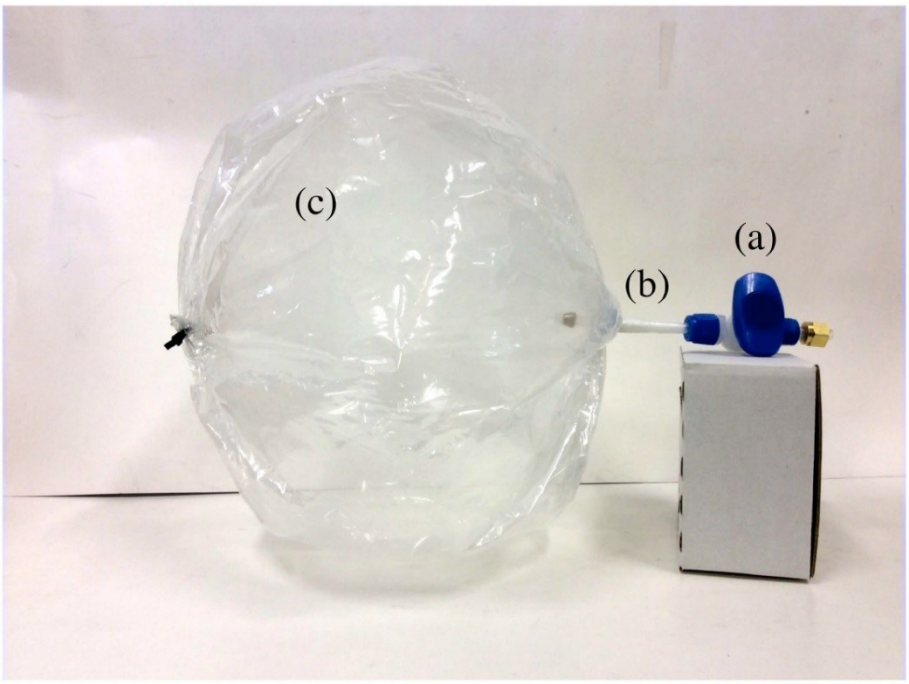


Figure 2.5. Nalophan sampling bag composed of (a) stopcock, (b) PTFE tube, and (c) Nalophan bag. (Reproduced from Ghimenti et al, Journal of Breath Research, 2015 [115]).

In addition to these polymer sampling bags are on the market available also multilayer bags, claiming reduced diffusion. These bags use one of the chemically inert materials mentioned previously as an inner layer, combined with metalized film layers to reduce diffusion and permeation. Examples are the metalcoated multilayer Tedlar (MMT) bag and the Cali-5-bond were bag [116,117].

Number of VOCs have been previously assessed in term of their stability in individual polymer sampling bags by PTR-MS, SIFT-MS or GC-MS [93,112,113,115,118] to determine optimal solution and material for any category. It was investigated that smaller samples are more vulnerable to VOCs losses by permeation. Additionally, the volume of the sample collected affects the stability of the sample, thus,

Mochalski and co-workers recommended sample collections as large as possible to prevent background emissions of contaminants [118].

Humidity also affects the species recoveries, and the high humidity in exhaled breath might cause significant decrease in vapour concentrations for those compounds highly miscible with water [118,113]. Water vapour diffuses through most bags at a speed dependent on the temperature of the bag material [93]. Such findings can easily be tracked by exploiting the full capabilities of SIFT-MS to measure the water vapour in air/breath samples. Condensation affects the sample authenticity, especially for water-soluble compounds. The loss of volatile compounds to condensed water in Tedlar bags used for breath sampling has been previously evaluated [119] showing differences between dry and wet matrices smaller than 10%. For VOCs with molecular mass above 110 amu, higher losses were detectable (20%–40%) [118]. It is therefore recommended , to store breath samples in pre-conditioned Tedlar bags up to 6 h at the maximum possible filling volume.

2.3 Applications

Although the majority of breath tests has not been integrated in clinical practice, there are some applications of breath gas analysis, which are part of the routine practice.

They include for example breath ethanol test for blood alcohol concentration detection (widely used by law enforcement officials world-wide) [120], and additional approved tests falling into two basic categories: tests that quantify molecules in breath after administration of a drug or substrate; and tests that quantify molecules in breath without any prior administration of a drug or substrate.

Table 2.1. The most developed clinical breath tests.

Breath Carbon Dioxide Test for capnography
Breath Carbon Monoxide Test for neonatal jaundice
Breath Ethanol Test for blood ethanol (law enforcement)
Breath Hydrogen Test to detect disaccharidase deficiency (thus for example lactose, maltose or sucrose intolerance), fructose malabsorption, gastrointestinal transit time, bacterial overgrowth, intestinal stasis
Breath Nitric Oxide Test for asthma therapy
$^{13}CO_2$ functional Breath Tests

The first group of tests is based upon the detection of a metabolite of the drug or substrate. Carbon dioxide is the most popular metabolite; however, since it is one of major products of cellular metabolism, the drug or substrate must be labelled with carbon (^{13}C or ^{14}C). Spectroscopic or radiochemical methods can be used to separate and analyse labelled carbon dioxide in the presence of unlabelled carbon dioxide. The relatively large natural abundance of ^{13}C limits the sensitivity of this method. Breath tests based upon this approach require that the metabolism of the substrate and the excretion of carbon dioxide are well characterized. Moreover, the breath test must be performed under defined conditions based upon the time of administration of the substrate and the time the breath test is administered. Additionally, the ventilation pattern of the patient must be carefully controlled. *Table 2.2* summarizes clinical breath tests that have been proposed based upon the quantification of labelled carbon dioxide. Most of breath tests are diagnostic for liver function or for gastrointestinal tract function. The breath hydrogen test (*Table 2.1*) to detect disaccharidase deficiency, gastrointestinal transit time, small bowel bacterial overgrowth [121] and intestinal stasis is similar to these tests (*Table 2.2*), since it involves the metabolism of a substrate (a carbohydrate, usually lactose) by colonic bacteria. In people, who are lactase deficient, lactose is not absorbed in the small intestine and is therefore, metabolized in the colon. Since the production of hydrogen in a fasting state of normal subject is low (resulting in a breath concentration of less than 42 ppmv) it is not necessary to isotopically label the diagnostic disaccharide. However, it is important to conduct the breath hydrogen test under carefully controlled conditions, since the time for the maximum evolution of hydrogen will depend upon gut motility and this time is critical to the test. The breath ethanol test is also similar to the other breath tests contained in *Table 2.2*, since it involves the measurement of a molecule whose origin is supposed to be exogenous. Metabolism by enteric bacteria can also produce breath ethanol, although the amount generated by this source is orders of magnitude less than an exogenous source.

Table 2.2. The clinical breath tests based upon labelled substrates.

Substrate	Clinical test
Acetate	Orocecal transit time
Aminopyrine	Liver function
Caffeine	Liver function
Erythromycin	Liver function
Galactose	Liver function
Glucose	Insulin resistance
Glycosyl ureides	Orocecal transit time
Ketoisocaproate	Liver mitochondrial function
Linoleic acid	Fatty acid metabolism
Methacetin	Liver function
Methionine	Liver mitochondrial function
Phenylalanine	*Phenylalanine hydrolase* activity
Triolein	Fat malabsorption
Uracil	*Dihydropyrimidine dehydrogenase* activity
Urea	H. pylori bacterial infection [122]

The second group of breath tests is based upon the detection molecules that are produced endogenously as a result of normal or abnormal physiologies. *Table 2.3* has listed a number of molecules with known biochemical pathways that have been detected in human breath and yet only few of these endogenously produced molecules are monitored in the tests listed in *Table 2.1*. The interesting example can be capnography, real time measurement of the carbon dioxide concentration profile (most widely used clinical breath test, which is used to monitor every patient undergoing surgery or in an intensive care unit). Capnography provides important information on cellular metabolism, carbon dioxide transport and pulmonary ventilation (exchange of carbon dioxide for oxygen, and assessment of airway integrity). Each of these processes is critical to monitoring the well-being of a patient. To this group belongs also measurement of nitric oxide originating from cells present in the airway, the application important for asthma detection [11, 12].

Table 2.3. Physiological origin s of some endogenous breath molecules

Compound	Physiological basis
Acetaldehyde	Ethanol metabolism
Acetone	Decarboxylation of acetoacetate
Ammonia	Protein metabolism, oral bacteria
Carbon disulfide	Gut or oral bacteria
Carbon monoxide	Production catalyzed by *heme oxygenase*
Carbonyl sulfide	Gut bacteria
Ethane	Lipid peroxidation
Ethanol	Gut bacteria
Ethylene	Lipid peroxidation
Hydrocarbons	Lipid peroxidation/metabolism
Hydrogen	Gut bacteria
Isoprene	Cholesterol biosynthesis
Methane	Gut bacteria
Methanethiol	Methionine metabolism
Methanol	Metabolism of fruit
Methylamine	Protein metabolism
Nitric oxide	Production catalyzed by nitric *oxide synthase*
Pentane	Lipid peroxidation

A great effort is also being made to integration of new diagnostic tests. Some interesting examples of application, divided into categories as per the disease aetiology (liver and inflammatory and infectious diseases, cancer, food intolerances), are discussed and summarized below. Revelation of diabetes via elevated concentrations of acetone in the breath of patients suffering by this disease [6,7], monitoring of renal disease and potentially also dialysis via added ammonia [8-10] are the examples already mentioned in the introduction.

2.3.1 Cancer

Very interesting investigation in connection with breath analysis is focused on several cancers, for example lung cancer, colorectal cancer, esophago-gastric, bladder, breast or prostate cancer. For most of these cancers it is common, that detection by standard methods as computer tomography (CT) scanner, magnetic resonance imaging (MRI) or biopsies is not effective at an early stage due to the lack of sensitivity. Therefore, the determination of possible cancer biomarkers for these diseases would be very

desirable and SIFT-MS and PTR-MS have, therefore, been tested to determine whether they have potential for their identification.

2.3.1.1 Lung Cancer

Lung cancer is the most common cancer worldwide (2 million new cases each year) and it has one of the lowest survival outcomes of any cancer. It is because over two-thirds of patients are diagnosed at a late stage when curative treatment is not possible. Lung cancer needs to be recognized at a very early stage of growth of the tumours if therapeutic intervention is to cure the condition or improve the prognosis. The growing interest is therefore being given by physical scientists and clinicians, in harness, to the search for volatile biomarkers of tumours in exhaled breath. Over the last two decades, most use has been made of GC-MS by Phillips at all [123-125]. This group has advanced this topic to the point, that the patterns of trace compounds, especially long-chain and branched-chain alkanes, have been observed in the breath of patients suffering from lung cancer in different patterns than for healthy control. Methylated hydrocarbons are proposed for lung or breast cancer biomarkers [126]. One of the additional biomarkers mentioned in connection with lung cancer is the acetaldehyde. The presence of this compound in the breath above physiological concentrations (in mean 22 ppbv) [32] could have major clinical importance. However, these concentrations in breath may not be obvious in most of the cases. Acetaldehyde is an intermediate in the metabolism of ethanol in the liver, however, intake of alcohol will greatly elevate acetaldehyde concentrations in breath [37]. In addition, acetaldehyde can also be produced from cellular activity involving sugars.

To support lung cancer studies, cells in vitro studies were performed in order to analyse the molecular emissions from cancer cells lines SK-MES and CALU-1. The earliest study revealed, that acetaldehyde is present in the headspace above incubated SK-MES and CALU-1 lung cancer cell cultures at concentrations significantly higher than physiological levels [127]. However, studies of Filipiak and co-workers led to conflicting results - it was seen that acetaldehyde was produced by the CALU-1 and NL20 cells, but absorbed from the medium by the 35FL121 Tel+ cells [128]. Based on this fact the SIFT-MS studies on CALU-1 cells have been repeated and extended to include NL20 normal lung epithelial cells and 35FL121 telomerase-positive (Tel+) lung fibroblast cells. Thus,

SIFT-MS has been used to quantify acetaldehyde and, in addition carbon dioxide in the headspace of the cell cultures [129]. The results showed that acetaldehyde was generated by the CALU-1 and NL20 cell cultures in proportions to the number of cells in the medium. However, following incubation, the acetaldehyde concentrations in the headspace of the 35FL121 Tel+ cell cultures were much lower than those present in the headspace of the medium alone. The amount of CO_2 generated by the CALU-1 and 35FL121 Tel+ cells indicated that they were respiring normally, but much less were produced by the NL20 cells, presumably indicating that normal cell metabolism was inhibited. These rather perplexing results indicated that acetaldehyde release is not specifically the action of malignant cells and so this compound alone cannot be used with confidence as a tumour diagnostic. However, an interesting discovery was made for acetic acid (elevated in the breath samples of the cancer patients compared with healthy control) [130]. The 3D model for cell lines cultivation has been also tested in which the cells were cultured in 3D scaffolds composed of collagen type I hydrogels, compared to 2D models where cells are grown on surfaces such as plastic or glass. Quantification by SIFT-MS of cells lines headspace CALU-1 and non-malignant lung cells NL20 revealed that the amount of acetaldehyde released by both cell types grown in a 3D model is higher when compared to that of the same cells grown in 2D models [131]. The latest investigation on the field of cells in vitro studies was focused on quantification of volatiles, propanal, butanal, pentanal, hexanal, heptanal and malondialdehyde (propanedial), that are expected to be products of cellular membrane peroxidation [132]. All six aldehydes were identified in the culture headspace, each reaching peak concentrations during the time of exposure to reactive oxygen species and eventually reducing as the reactants were depleted in the culture. Pentanal and hexanal were the most abundant, reaching concentrations of a few hundred parts-per-billion by volume, ppbv, in the culture headspace.

2.3.1.2 Colorectal Cancer

Colorectal cancer is again a kind of common cancer (1.4 million new cases each year), where the breath analysis could be very promising diagnostic tool. It is attributed to individual genetic predisposition and environmental factors, including lifestyle (elevated body mass index, BMI, obesity, low physical activity) and diet. The

investigation concerning influence of diet revealed, that it can significantly influence and promote the growth of malignant cells. Particularly processed and red meat intake, where protein is the major constituent leading to protein fermentation metabolites potentially carcinogenic and possible linked to colon cancer [133–137], or alcohol's consumption, where metabolites can have multiple molecular consequences that can again instigate colon carcinogenesis [138-139], because its oxidative and non-oxidative metabolism, and formation of by-products, such as reactive oxygen species and metabolites, can lead to a constellation of genetic, epigenetic, cell signalling, and immune processes [139]. The diet has therefore complex effects on the generation of breath compounds [140] and therefore is very important to understand the normal metabolism and describe the VOCs generated under standard body conditions and effect of dietary constituents, whereas the effect of diet can be again mediated by many factors (changes of gastrointestinal flora, gastro-caecal transit time, metabolism, systemic inflammation, redox state). The dietary and metabolic sources of the main metabolites in human breath are available in *Figure 2.5.*

In the past there were studies evaluating breath samples from patients with colorectal cancer, concluding that the pattern of VOCs in patients suffering from colorectal cancer were different from that in healthy controls, particularly concentrations of some specific VOCs such as 1,3-dimethylbenzene, 1,2-pentadiene, cyclohexene and methylcyclohexene [141]. However, much more research is necessary to support this investigation. In connection with this topic is also important to mention, that VOCs generated by the metabolism may emanate also from faecal matter. Thus, analyses of human metabolites as end products of intestine may rely on faecal samples or on breath.

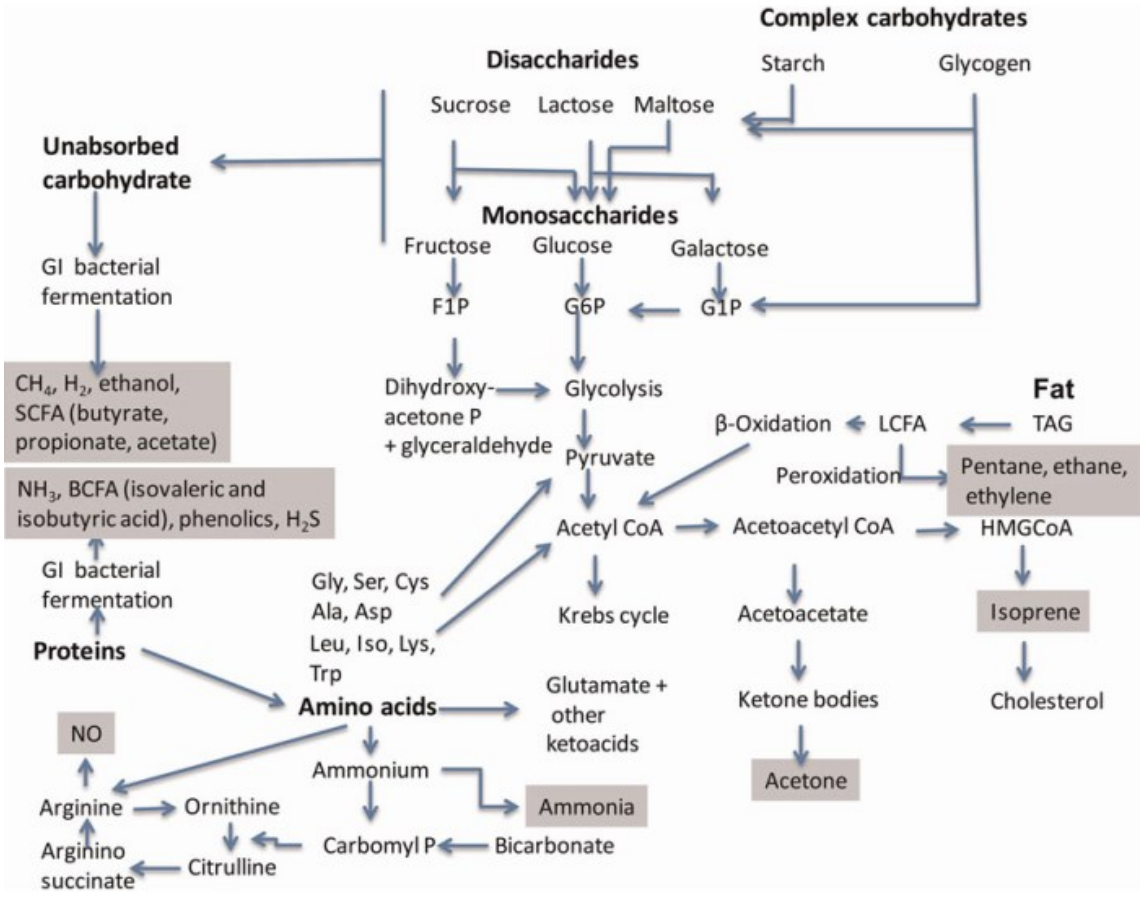


Figure 2.6. Dietary and metabolic sources of the major metabolites in human breath. (Reproduced from Ajibola et al., Journal of Nutritional Science, 2013 [140]). Gastrointestinal (GI); fructose 1-phosphate (F1P); glucose 6-phosphate (G6P); glucose 1-phosphate (G1P); long-chain fatty acids (LCFA); branched-chain fatty acids (BCFA); hydroxy methyl glutaryl (HMG); carbomyl P, carbomyl phosphate. The grey boxes represent compounds that have been identified in breath.

2.3.1.3 Esophago-Gastric Cancer

Gastro-esophageal cancer is the 7th most commonly occurring cancer in men and the 13th most commonly occurring cancer in women. There were over 500,000 new cases in 2018. Same as lung cancer, gastro-esophageal cancer remains a disease with poor patient outcomes. It is again because of above mentioned fact that red flag symptoms often occur at a more advanced stage of the disease, and therefore, people with such cancers most commonly go to their doctor later in the disease process. This delay in diagnosis results in only 20% of patients being suitable for potentially curative treatment at first presentation. One of the main methods for improving these survival rates lies in the earlier detection of these cancers, and VOC analysis represents a promising area to

explore in relation to gastro-esophageal cancer. In this field, four important studies have been conducted, investigating interesting VOCs in breath or headspace vapour of biofluids at different patient groups and healthy controls. The first study on the headspace of gastric content, conducted by Kumar et al in 2012, indicated that the combinations of the following 7 VOCs, acetone, formaldehyde, acetaldehyde, hexanoic acid, hydrogen sulphide, hydrogen cyanide, and methyl phenol are significantly different between cancer patients and healthy control group [142]. In the follow-up study investigating VOCs in the headspace of urine, the concentrations of seven VOCs, acetaldehyde, acetone, acetic acid, hexanoic acid, hydrogen sulfide, methanol, and phenol, were found to be significantly different between cancer, positive control, and healthy groups [143]. The results of these studies identified potential compounds that may be important in VOC profiling of esophago-gastric cancer and thus warrant further investigation. In the next steps, VOCs within exhaled breath have been identified, because it was hypothesized that several of the VOCs detected at increased concentrations in the headspace of biofluids from esophago-gastric cancer patients may be present in the exhaled breath of these patients. In the first study focused on breath the compounds for analysis (a total of 17 VOCs) have been selected based on their presence in biofluids in previous studies on esophago-gastric cancer or if they have been detected in other cancer states. The concentrations of 4 VOCs, hexanoic acid, phenol, methyl phenol, and ethyl phenol, were found to be significantly different between cancer and positive control groups [144]. Further study with large patient cohorts has been conducted by Kumar et al in 2015. Exhaled breath samples were analysed from 81 patients with esophageal or gastric adenocarcinoma and 129 controls. Twelve VOCs from the family of fatty acids, phenols and aldehydes (namely pentanoic acid, hexanoic acid, phenol, methyl phenol, ethyl phenol, butanal, pentanal, hexanal, heptanal, octanal, nonanal, and decanal) have been identified as potential breath biomarkers in esophageal adenocarcinoma and it was concluded that distinct exhaled breath VOC profiles can distinguish patients with esophageal and gastric adenocarcinoma from non-cancer controls [145].

2.3.1.4 Breast Cancer

Breast cancer (1.7 million new cases per year) is accompanied by increased oxidative stress and induction of polymorphic cytochrome P450 mixed oxidase enzymes

(CYP). Both processes affect the abundance of VOCs in the breath because oxidative stress causes lipid peroxidation of polyunsaturated fatty acids in membranes, producing alkanes and methylalkanes, which are catabolized by CYP. Compounds from this group, among others, nonane, 5-methyltridecane, 3-methylundecane, 6-methylpentadecane, and 2-methylpropane, 3-methylnonadecane, 4-methyldodecane, 2-methyloctane, have been suggested as potential biomarkers by Phillips and co-workers. They performed a pilot study (GC-MS) of breath VOCs in women with breast cancer, whereas results have been compared with abnormal mammograms and biopsies. The breath test distinguished between women with breast cancer and healthy volunteers with a sensitivity of 94.1% [146]. Their recent findings were consistent with previous studies, as they indicated that breath contains volatile biomarkers of breast cancer and that these biomarkers can identify woman with disease. However the biochemical origin of volatile biomarkers of breast cancer remains speculative [147]. As another promising breast cancer biomarker has been identified also pentane, which has been found in increased concentrations in the breath of woman with this disease - an initial case-control study performed by Hietanen and co-workers [148].

2.3.1.5 Bladder and Prostate Cancer

Exhaled breath testing for prostate (1.1 million new cases per year) and bladder cancer (0.4 million new cases per year) is another topic that deserves attention. Even in case of this cancer disease, there is a pressing need for non-invasive diagnostic tool that would be suitable for discrimination between patient with and without this illness. As a potential biomarker for tumours identified in 1999 Španěl and co-workers formaldehyde. They conducted a SIFT-MS study, which showed that this compound is elevated in the headspace of urine donated by bladder and prostate cancer patients, whilst being absent from the headspace of the urine from healthy controls [149]. It was also found, that the formaldehyde is at a greater concentration in the urine from the bladder cancer patients than in the urine from prostate cancer patients. This is perhaps not surprising in that bladder tumours are in closer contact with the urine whereas the formaldehyde from prostate tumours must generally progress into the urine via the blood stream [150]. In the recent years, there is also an effort to simplify the breath testing methods and therefore electric nose devices have been tested as possible devices for non-invasive diagnostic. As

an example, we can mention the study of Waltman and co-workers, where the prostate cancer diagnostic have been conducted by utilisation of Aeonose, a handheld, battery-powered electronic nose (EN). The exhaled breath is in this device guided over three hotplate metal–oxide sensors, namely carbon monoxide (AS-MLC), nitrogen dioxide (AS-MLN), and VOC (AS-MLX) sensors, where the VOCs induce a redox reaction on the hotplate of sensor surface, causing the change in conductivity. This redox reaction is dependent on the compound, surrounding gas, sensor surface and surface temperature. In order to distinguish between different VOCs more accurately, the hotplate sensors go through a sinusoidal temperature cycle (260–340°C) during an interval of approximately 20 s. A measurement consists of 36 of these intervals, creating a temperature-specific VOC profile. A metal-oxide sensor measures the response of redox reactions over a certain time period in a thermal cycle, which causes a three-dimensional data matrix (time vs thermal cycle vs response value). Exhaled-breath analysis using an EN (Aeonose) in order to differentiate between Prostate Cancer patients and healthy individuals yielded very promising results [151].

2.3.2 Liver disease

Sulphur containing compounds like methyl mercaptan, ethyl mercaptan, dimethylsulfide or dimethyldisulfide and nitrogen containing compounds such as amines/ammonia are proposed to be characteristic biomarkers for this area, as they correlate respectively with liver diseases [152,153].

Sulphur containing compounds are generated in humans by incomplete metabolism of methionine in the transamination pathway [153]. Their concentrations in human blood and breath are very low under healthy conditions, nevertheless, impairment of liver function increases their level [154]. Sulphur containing compounds are responsible for the characteristic odour in the breath, for example at cirrhotic patients [152]. The characteristic odour of dimethylamine and trimethylamine has been known for a long time [155]. Significant concentrations of ammonia will appear in the blood if ammonia through conversion to urea is limited due to an impairment of liver function (for example cirrhosis or severe hepatitis. The majority of ammonia seen in mouth-exhaled breath is, however, largely generated in the oral cavity [33].

The possible use of VOCs in patients with liver cirrhosis is not fully explored area. An initial study performed by Van den Velde and co-workers [156] showed

discrimination between the cirrhotic group and healthy subjects. They analysed the breath of 50 patients with established liver cirrhosis. Recently, a pilot study using PTR-MS equipped with a time-of-flight mass analyzer was conducted in liver cirrhosis patients by sampling the breath of the subjects [157]. The authors were able to distinguish between healthy and disease subjects. They identified twelve VOCs significantly different between cirrhotic and healthy subjects (ketones, terpenes, sulphur compounds, nitrogen compounds and alcohols). Volatile biomarkers in breath associated with liver cirrhosis have been studied also by Fernández del Río and co-workers [158]. This working group compared pre- and post-liver transplant breath samples and identified five volatiles with statistically significant decrease post-transplant (limonene, methanol, 2-pentanone, 2-butanone and carbondisulfide). On an individual basis limonene showed the best diagnostic capability, but it was improved by combining methanol, 2-pentanone and limonene. Following transplant, limonene shows wash-out characteristics [158].

Another liver disease, where attempts have been made to use breath analysis for detection purposes, is hepatic encephalopathy [159], neuropsychiatric syndrome with symptoms varying depending on the severity of the condition. This disease results from the accumulation of compounds not cleared by the liver and again ammonia is known to be involved in hepatic encephalopathy [159]. However attempts to use breath ammonia measurements for diagnosis have failed, probably because most exhaled ammonia is generated within the oral cavity by bacterial and/or enzymatic [33]. Additionally, some confounding factors have proven to be tricky in VOCs detection and quantification. For example, pulmonary gas exchange abnormalities can be present in patients with advanced liver disease, such as high cardiac output and abnormal dilation of pulmonary capillary vessels, leading to incorrect conclusions [159].

2.3.3 Inflammatory bowel disease

Ulcerative colitis (UC), together with Crohn's disease (CD), are a part of the spectrum of inflammatory bowel diseases (IBDs) - the class of autoimmune disorders in which the body’s own immune system attacks parts of the digestive system [160-163]. It is a chronic inflammatory condition with unknown aetiology and only a partially understood pathogenesis. The consensus is that both diseases are a response to environmental triggers (infection, drugs, or other agents) in genetically susceptible individuals [164].

This group of disorders causing inflammation of the gastrointestinal tract (GIT), is recently in the focus of several areas of research, besides others also breath analysis [165-169]. The reasons are increasing incidence and prevalence of these inflammatory conditions in Europe [170], lack of a single gold standard and multimodal approach for the diagnostics and differentiation. Moreover, most of the currently used diagnostics methods are either invasive or have limited sensitivity or specificity [171-174].

CD is characterized by chronic discontinuous transmural inflammation that may involve any portion of the gastrointestinal tract, but most commonly the terminal ileum, whilst UC is characterized by inflammation of the colonic mucosa, extending to a variable extent from the rectum to the proximal colon. With regard to clinical features and pattern of IBD, the cardinal symptom of UC is bloody diarrhoea. Associated symptoms of colicky abdominal pain, urgency, or tenesmus may be present as well. Symptoms of CD are more heterogeneous, but typically include abdominal pain, diarrhoea, and weight loss. To date there is no cure for IBD, but current medical therapy alleviate the distressing symptoms in the most patients and reduce the mortality, which is greatest in the 2 years after diagnosis for both, UC and CD [175-178].

In the past, there was an effort to utilize breath analysis as a method for IBD detection – differentiation between IBD suffering people and healthy control, but also between CD, UC and between active disease and disease in remission.

One of the pilot studies in this area, conducted by Dryahina and co-workers has been focused on detection of exhaled pentane concentration as a biomarker of lipid peroxidation [168]. The results of this study revealed that breath pentane is significantly elevated in the breath of both, CD patients and UC patients relative to healthy controls, being at somewhat lower mean concentration in UC than CD breath. However, as pentane is a nonspecific inflammatory marker, it was important to add other volatile metabolites to the panel of compounds used for breath test of the IBD disease activity.

The method for the diagnosis of IBD, differentiation between CD and UC and active/remission states by detecting different VOCs in exhaled breath has been patented by the group of inventors leaded by Dallinga [179-182]. These patents have prompted further research in this area, and SIFT-MS study of selected VOCs previously identified by other research groups as being present in modified concentrations in the breath of patients with IBD, has been conducted [165] by our investigative team (see Appendix B for additional details). Data obtained in this experiment, aimed besides others at quantification of some hydrocarbons, have shown some interesting results for alkenes,

specifically elevated ion signal at *m/z* 99 observed in the H_3O^+ SIFT-MS analyses of breath of IBD patients [183] that may be characteristic for heptene. These observations in conjunction with the study of Patel et al. [167], which supported the hypothesis that alkenes are possible IBD biomarkers, caused that a detailed study of the ion chemistry of alkenes focusing on heptenes has been conducted [169]. The study resulted in ion-molecule kinetics data (effectively addition of several entries to a SIFT-MS library) for alkenes, which would ultimately allow fast SIFT-MS analyses of breath samples and the quantification of alkenes for the purpose of IBD diagnostics (see Appendix C for additional details). The additional research is ongoing in this area and some recent results are discussed in the experimental part of this thesis (see section *4.4 Breath analysis and searching for the new biomarkers*).

2.3.4 Bacterial infections - Tuberculosis, Cystic fibrosis complications

Pulmonary tuberculosis (TB) is an infectious disease derived from the organism *Mycobacterium tuberculosis*. The primary detection technique is the Ziehl-Neelsen staining combined with microscopy. It only allows detection of pulmonary disease in an advanced stage, meaning that often the disease has already been transmitted to close contacts. Breath analysis may offer a method for diagnosing pulmonary tuberculosis [184]. Some compounds potential biomarkers have been found by breath sampling, such as methyl phenylacetate, methyl p-anisate, methyl nicotinate, and o-phenylanisole [185]. However, contradictory studies presented different marker compounds, possible due to the fact that Mycobacterium tuberculosis is a slow growing organism. This means that if VOCs are produced they may be released or modified by the host, hence, they may well be present at low concentration and not be detected by the real-time analytical techniques. The best approach should be looking at clinical samples in order to get potential biomarkers of pulmonary tuberculosis [5].

Breath analysis offers great potential as a non-invasive method for diagnosis of lower respiratory tract infections without the need for bronchoscopy or alveolar lavage [186]. Therefore could be utilized as a diagnostic tool with a great value also in connection with cystic fibrosis (CF), an inherited genetic disease caused by mutations in a gene that is responsible for the transfer of ions and salts across the cell membrane [187-189]. In CF the early detection of respiratory pathogens such as *Pseudomonas aeruginosa*

(PA) and *Burkholderia cepacia*, which are the major cause of morbidity and mortality in patients with this illness [190-191], increases the chance of their eradication. The reason why a non-invasive breath test would be of a special benefit (particularly to children) is that they are often unable to reliably expectorate sputum, cultivation of which is the gold standard method for PA diagnosis. This approach offers a way since the detection by SIFT-MS of HCN emission from cultures of PA *in vitro* [192,193]. It was shown that *in-vitro* cultures of PA released several VOCs including, and especially, HCN. Subsequently, SIFT-MS was used for the analyses of breath of 40 children suffering from CF. The concentration of HCN in the exhaled breath was in these patients increased significantly in comparison with children who suffered from asthma but not with CF [194]. However, in 2011 a SIFT-MS study of HCN in the headspace of 96 genotyped PA samples showed that different strains of this bacterium produced different amounts of HCN. Also it was observed that the non-mucoid phenotype samples produced more HCN than the mucoid phenotype samples [193,195,196]. Based on these facts, the group of Shestivska et al. investigated presence of methyl thiocyanate in the headspace of PA cultures and subsequently in the breath of CF patients by SIFT-MS [197], as this compound has been previously confirmed by the pilot experiment using SPME and GC-MS analyses. To wit, cyanide produced by bacteria is toxic and PA avoids its toxicity by several enzymatic pathways. One of the recently described mechanisms of detoxification of HCN by PA is its metabolism into thiocyanate involving the enzyme *thiosulphate sulphurtransferase*. The study of the exhaled breath of CF patients finally indicated that the concentration of methyl thiocyanate is within the range from zero up to 28 ppbv and that the combination of methyl thiocyanate and hydrogen cyanide could be a more robust biomarker of PA and could possibly differentiate different PA strains. The research in the field is nevertheless still ongoing. HCN as a CF biomarker has been further investigated by Gilchrist and co-workers, whereas studies have been focused for example on selection of suitable sampling methodology (mouth vs. nose exhaled breath) [198], or on early detection of PA infection detection [199]. In addition, further respiratory pathogens connected with CF, as *Pseudomonas aeruginosa, Staphylococcus aureus, Stenotrophomonas maltophilia* and the *Burkholderia cepacia* complex, and VOCs emitted from these cultures have been investigated [200,201]. It was shown, that several volatile biomarkers were distinct for one of the four major bacterial taxa that are involved in pulmonary infections of patients with CF. This result may reflect substantial differences in the cellular metabolisms of these taxa, which are phylogenetically distant

from each other [201]. The newest study focused on CF than revealed, that acetic acid (AcOH) is elevated in the exhaled breath of CF patients. Breath acetic acid could be therefore used as a non-invasive biomarker of the acidity of the airways mucosa, which could help to assess the risk of bacterial infection (none of the bacterial pathogens et al. are producing AcOH and that its concentration is not related to mucus build-up, it is most likely that the airway mucosa has a lower pH relative to normal respiratory tract mucus due to the loss of the cystic fibrosis transmembrane conductance regulator gene) and ultimately improve the efficacy of CF therapy. It may be that measurement of AcOH in exhaled breath could support CF diagnosis alongside the standard tests. Clearly, further work is needed to substantiate these findings using greater cohorts of healthy volunteers and CF patients [202,203].

2.3.5 Food Intolerances, autoimmune disorders

Food intolerance, also known as non-immunoglobulin E (non-IgE) mediated food hypersensitivity or nonallergic food hypersensitivity, refers to difficulty in digesting certain foods. It is important to note that food intolerance is different from food allergy (food allergies trigger the immune system, while food intolerance does not). Foods most commonly associated with food intolerance include dairy products, grains that contain gluten, and foods that cause intestinal gas build-up, such as beans and cabbage.

Recently, hydrogen breath test (HBT) has become popular in clinical practice as it is useful for diagnosis carbohydrate intolerance such as lactose and fructose malabsorption. [204-206], a condition in which the patients are unable to absorb or digest certain carbohydrates due to the lack of some intestinal enzymes, leading to bacterial sugar fermentation in the gut.

Coeliac disease is a long-term, under-diagnosed autoimmune disorder that primarily affects the small intestine. Classic symptoms include gastrointestinal problems such as chronic diarrhoea, abdominal distention, malabsorption, loss of appetite and among children failure to grow normally. Coeliac disease is caused by a reaction to gluten, a group of various proteins found in wheat and in other grains such as barley and rye. A preliminary investigation of the concentrations of alcohols in the breath of 10 patients with coeliac disease compared to that in 10 healthy controls using SIFT-MS has been conducted for this disease [81]. No significant conclusions were drawn. Such results are in agreement with recent findings performed by Aprea and co-workers [207], where

real time breath analysis was performed in patients diagnosed with coeliac disease under gluten free diet. As expected, exhaled breath of patients with coeliac disease was similar to the exhaled breath of healthy people and no reliable marker was found.

2.3.6 Transplant rejection

The utilisation of breath analysis for early transplant rejection is a topic very often mentioned in relevant papers focused on breath analysis applications. From our point of view are results achieved in this area a little uncertain. Distrust is supported also by the fact, that no recent studies or works focused on this area are available. Nevertheless, to achieve completeness of the theoretical part of my work, the research conducted with regard to transplant rejection is summarised below.

After organ transplantation, patients have to be treated in the intensive care unit (ICU) because of chronic or acute rejection of the transplanted organ and to avoid complications due to infection following immunosuppression. Allograft rejection following organ transplantation has been linked to alkane exhalation. Pentane concentration were found to be elevated in acute rejection of transplanted hearts, however, it is important to remember, that pentane as a lipid peroxidation marker is linked to inflammation rather than being a specific marker for allograft rejection [208,209]. Carbonyl sulfide (OCS) seems to be generated as a by-product of methionine metabolism and may act as an acute marker of organ rejection after lung transplantation [210]. This volatile marker is not found in the exhaled breath of healthy individuals. A crucial problem is the distinction between organ dysfunction due to infection or any other complication and organ dysfunction due to organ rejection. Up to now, invasive procedures such as biopsy (heart, liver, kidney) or bronchoscopy (lung) were necessary for that purpose. Non-invasive breath test could, therefore, be of considerable benefit to these patients. In connection with heart rejection has been also evaluated larger repertoire of biomarkers of oxidative stress in breath - the breath methylated alkane contour (BMAC) [211,212]. The breath test divided the heart transplant recipients into 3 groups: positive for grade 3 rejection, negative for grade 3 rejection, and intermediate. The test was 100% sensitive for grade 3 heart transplant rejection when the p value was >0.98, and 100% specific when the p value was <0.058; in the intermediate group, the breath test determined the probability of grade 3 rejection and the predictive value of the result [212].

3 Techniques for breath analysis

Volatile metabolite research relies on analytical methods that offer high sensitivity, precision and resolution (see *Table 3.1*). In the last years, great importance is given especially to the on-line, real-time analytical techniques SIFT-MS and PTR-MS. These methods exhibit limit of detection ranging from ppbv to pptv, making them ideally suited to breath analysis [213]. Proton transfer reactions are characteristic for both these techniques and are encouraged by the chemical ionization process that allows a very efficient ionization for many organic compounds in the gas phase. Product ion generation in SIFT-MS and PTR-MS is managed using chemical ionization, arising from ion-molecule reactions rather than electron impact or photoionization, with much less fragmentation of the molecules. Thus, these techniques are called soft ionisation techniques.

In the past, the analysis of volatile trace compounds in air and exhaled breath has most commonly been achieved using mass spectrometry (MS) coupled with some form of sample preparation and compound separation, as in gas chromatography (GC), and exploiting compound ionization by electrons (EI), that is most commonly used in GC-MS. The analytical mass spectra produced by this ionisation process are nevertheless considered as more difficult to interpret (in comparison with analytical mass spectra produced by soft ionisation techniques). GC-MS is considered as a gold-standard analytical method for breath analysis.

Table 3.1. A comparison of the characteristics of the available breath research techniques.

Analytical Method	Mode of operation	Limit of detection (LOD)	Sensitivity	Specificity
SIFT-MS	Direct/Real time	ppbv	High	High
PTR-MS	Direct/Real time	pptv	High	Medium-High
IMS	Real-time	ppbv	Medium	Medium
Sensor arrays	Reference to a database	ppbv	Medium	Medium
GC-MS	Pre-concentration	pptv-ppbv	Very-high	Very-high
LAS	Real-time	ppbv	High	High

Other analytical techniques that are widely used and play important roles in breath analysis are laser absorption spectroscopy (LAS), ion mobility spectrometry (IMS), and electronic noses, containing a variety of gas sensors and semiconductor-based sensor arrays.

Concerning the IMS technique, the ions are generated by a beta emitter or by a corona discharge and they are separated according to their mobilities through the gas, which is usually air at atmospheric pressure [214]. Such devices are not operated at high vacuum conditions and therefore ion-molecule collisions occur, limiting the speed of the ions along the drift tube. Hence, the number of ions reaching the detector is lower compared to the theoretical value. This is an important factor limiting the sensitivity of IMS. During the last ten years it has been applied in medical research, such as detection of skin volatiles [215], detection of volatiles in exhaled breath of patients with lung cancer [216], and determination of anaesthetics concentration in exhaled breath [217].

For the LAS technique, the amount of light absorbed by a sample is related to the concentration of the target specie in the sample. The LAS-based technique cavity ringdown spectroscopy (CRDS) has been successfully applied to measure NO concentration in exhaled breath [218]. This technique enables quantification of volatiles in exhaled breath down to below parts-per-billion by volume levels. It is particularly useful for monitoring purposes, and, recently, the exhaled breath of healthy volunteers was assessed by CRDS [219].

In order to analyse different VOCs, many applications have combined various sensors and materials into a single array, leading to the development of an "electronic nose" [220]. Sensor technology has been used for many years in clinical testing. Currently, the goal consists of finding materials with high sensitivity and good selectivity to the VOCs to be detected. Up to now, the existing materials are mostly conductive polymers, semiconducting metal oxides, or a combination of these two materials [221]. Unique sensors, based on nanoparticles appear as a reliable alternative tool for breath analysis, proving to be inexpensive and easy-to-use. The quartz crystal microbalance (QCM) and the surface acoustic wave (SAW) device are mass-sensitive sensors which have been used in breath analysis [5]. In QCM, gas molecules are adsorbed on the crystal's surface during sensor exposure to a gaseous medium, changing its mass and resonant frequency. A selective adsorption of the gas mixture strongly influences the degree of crystalline order and by the nanostructure boundaries. The polymer overall characteristics, length, planarity of the conjugate polymer chain, and the side chain composition may influence the polymer conductivity [221]. Recently, Cr- or Si-doped WO3 nanoparticles have shown high sensitivity to acetone, leading to the development of a portable chemo-resistance sensor suitable for real-time breath acetone detection [222]. Breath acetone concentration of five test persons at rest or during physical activity

was measured and compared to that measured by PTR-MS. Si-WO3 sensors were selective to acetone in realistic conditions (90% Relative Humidity), and able to detect differences in breath acetone concentrations between 880 to 980 ppbv, in agreement with PTR-MS measurements. However, the combination of specificity, selectivity, robustness in operation, reproducible manufacturing uniformity, and long-life stability is not offered by current sensors at an acceptable cost level. These technologies are unable to identify individual compounds, although they can be used to compare samples to see whether they have similar VOC profiles.

Finally is also important to mention for the purpose of breath analysis also Fourier transform infrared spectroscopy (FTIR), a chemical analytical technique, which measures the infrared intensity versus wave number (wavelength) of light [223].

3.1 Mass spectrometry

The origins of mass spectrometry can be traced back to the 19th century to the Lord Thompson's early work on cathode rays – the discovery of electrons for which he received in 1906 Nobel Prize in Physics. Later, in 1913 J.J. Thompson, with the help of Francis Aston built the first mass spectrometer and resolved neon isotopes and thus they are considered to be the founders of mass spectrometry [224]. These fundamental developments helped towards a better understanding of the elements and their physical properties.

An important step in the analytical use of mass spectrometry was the combination of mass spectrometry with gas chromatography (GC). The modern GC was invented by Martin and James in 1952 [225].GC has become a standard analytical method in many fields, especially petrochemical manufacture, environmental, biological and food sciences, and also in drug residue and forensic analysis. The field of GC rapidly expanded in the 1980s.

As already mentioned above, despite some weaknesses (comparison with standards required for absolute quantification, matrix effects, need of extraction or adsorbing the VOCs onto a suitable adsorbent [85] and long-term analysis) GC-MS is considered to be the gold standard method for analysis of VOCs. The primary objective of chromatographic analysis is to achieve the desired separation of compounds in a mixture in the shortest possible time. Reductions in analysis time have been achieved by

fast GC [226].The principles and theory of fast GC were established in the 1960s, but for routine analysis fast GC were used later in 1990s when the adequate commercial instrumentation was available [227].Currently is gas chromatography mass spectrometry (GC-MS) for the purpose of breath analysis commonly combined with the extraction methods of thermal desorption (TD) [228] and the above mentioned solid-phase micro-extraction (SPME) [85]. GC can be for the purpose of breath analysis connected also with UV spectrometry (GC-UV). Nevertheless, GC-MS is most common.

In 1972 Membrane inlet mass spectrometry (MIMS) was described and gradually became a relatively well established technique for monitoring gases and VOCs directly from aqueous solutions [229]. But this technique can be considered to be more qualitative than quantitative. For quantitative analysis, soft ionisation techniques, like chemical ionisation, are preferred. One of the methods for direct sample analysis is atmospheric pressure chemical ionisation (APCI) followed by mass spectrometry that was originally developed for analysis of trace components in the gas phase [230]. In 1980 trace atmospheric gas analyser (TAGA) based on APCI was used in several environmental applications, explosive detection and even breath analysis [231] and monitoring of CO_2 [232]. Today, APCI is widely used in analysis of trace gases released by foods [233] and many other trace gas analyses down to the parts-per-trillion by volume (pptv) concentrations [231]. In spite of their great sensitivity a weakness of APCI methods is again in its lack of absolute quantification and also due to matrix effects that compromise reproducibility. The state of the art methods stemming from this heritage are extractive electrospray ionisation (EESI) [234] and secondary electrospray ionization-mass spectrometry (SESI-MS) [235].

In the mid-1990s two techniques based on chemical ionization were introduced capable of direct real time trace gas analysis: SIFT-MS and PTR-MS. Both will be described in more detail below.

3.1.1 Proton transfer reaction mass spectrometry

Proton transfer reaction mass spectrometry (PTR-MS) was developed in Innsbruck by Lindinger and co-workers [236] mainly for the purpose of detection of both, biogenic VOCs and anthropogenic VOCs in atmospheric science, environmental research, food and flavour analysis and also breath analysis [237]. Several reviews have already been published about this method. The origin of this method is similar to origin

of SIFT-MS, both methods can be traced back to flowing afterglow method (FA) [238]. PTR-MS is based on chemical ionisation of molecules present in gas sample by reagent ions inside a drift tube. It allows real-time, on-line determination of absolute concentrations of VOCs, with detection sensitivity greater than SIFT-MS. The first PTR-MS instruments developed only used H_3O^+ as reagent ion, a disadvantage compared to SIFT-MS. Currently, the latest instruments use a switchable reagent ion capability, alternating between the three reagent ions like SIFT-MS. This so-called "Switchable Reagent Ions" (SRI) variant of PTR-MS was introduced in 2009. Since then it is possible to switch between H_3O^+, NO^+ or $O_2^{+\bullet}$ reagent ions [239], though the switch takes several seconds.

There are usually overlapping ions in clinical sample headspace and the use of PTR–MS equipped with a time-of-flight (TOF) mass analyzer improves mass resolution to assist ion identification [240,241]. PTR-MS employs an electric field, E, along the flow tube axis to increase the velocities of the ions. The change of the ratio E/N, where N is the number density of the drift tube buffer gas molecules, will affect the reagent ion hydration and product ion fragmentation. Under normal operating conditions E/N is in the range 120–130 Td (1 Td ~ 1 Townsend $\sim 10^{-17}$ V cm^2) representing a compromise between reagent ion hydration on the one hand, and molecular (product) ion fragmentation on the other. The electric field also prevents the formation of substantial quantities of cluster ions. In contrast to SIFT-MS, PTR-MS operates at higher effective temperatures and the underlying ion chemistry is often not known [242,243]. The instrument is much shorter, due to the existence of a shorter drift tube, has a typical length 10–20 cm, and, consequently, the pumping system is reduced in size, making PTR-MS suitable for transport. However, the latest advances in SIFT-MS have surpassed this issue, in which the instrument has become much smaller and suitable for transport [244]. The recent advances in PTR-MS technology have demonstrated a diverse range of applications, especially for breath gas analysis [245].

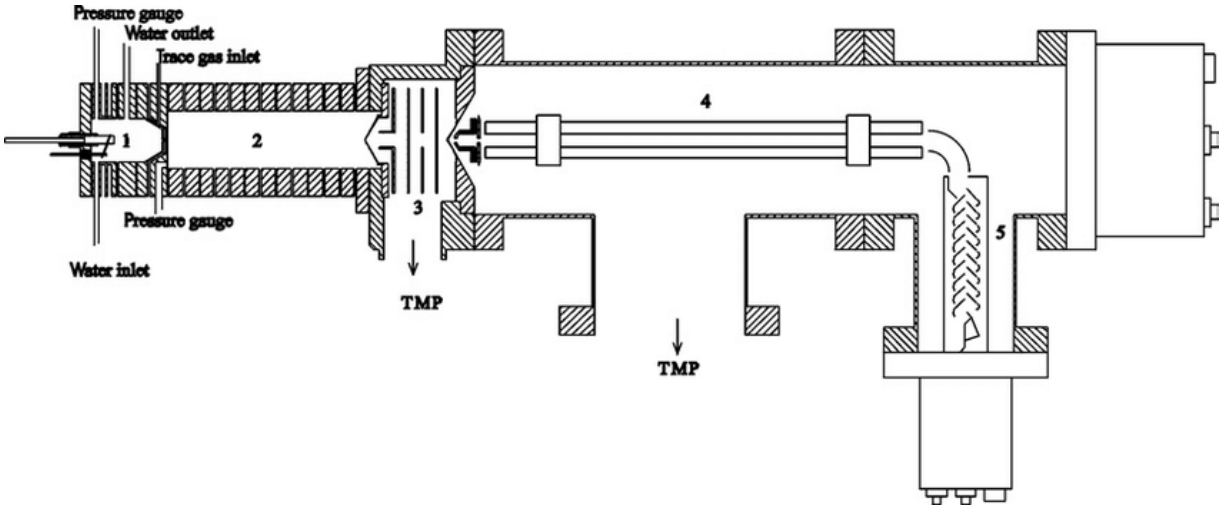


Figure 3.1. Schematic view of the PTR-MS instrument. (1) is the hollow cathode ion source, (2) the drift tube, (3) the transition chamber, (4) the detection chamber containing the quadrupole and the secondary electron multiplier (5). Reprinted with permission from F.Harren. (2007).

3.1.2 Selected ion flow tube mass spectrometry

3.1.2.1 Selected ion flow tube

Selected ion flow tube mass spectrometry (SIFT-MS) is an analytical technique derived from the selected ion flow tube (SIFT), the flow-tube method, conceived and developed in 1976 by N. G. Adams and D. Smith [246] for the study of gas-phase reactions between ions and molecules. SIFT method is in comparison with the flowing afterglow (FA) – the first application of a flow tube, described in 1963 by Ferguson, Fehnsfeld and Schmeltekopf [238] – characterised by presence of a quadrupole mass filter after the ion source. Thanks to this can be reagent ions selected according to their mass to charge ration (*m/z*) before injection into fast flowing neutral carrier gas. The SIFT technique became a standard tool for the study of the kinetics of the reactions between ions and neutral molecules in the gas phase under truly thermalised conditions [247,248]. Research in this area was focused on the study of ion-molecule kinetics important to atmospheric and interstellar ion chemistry [249]. This work has resulted in a large amount of experimental kinetic data for ion-molecule reactions, including rate constants and product ion distributions. These data have been a foundation for the use of SIFT-MS for analytical purposes, especially for determination of trace gas concentrations in air, with a

focus on quantitative analysis of gases in human breath [47,75]. The principles of the SIFT technique are illustrated in *Figure 3.2*.

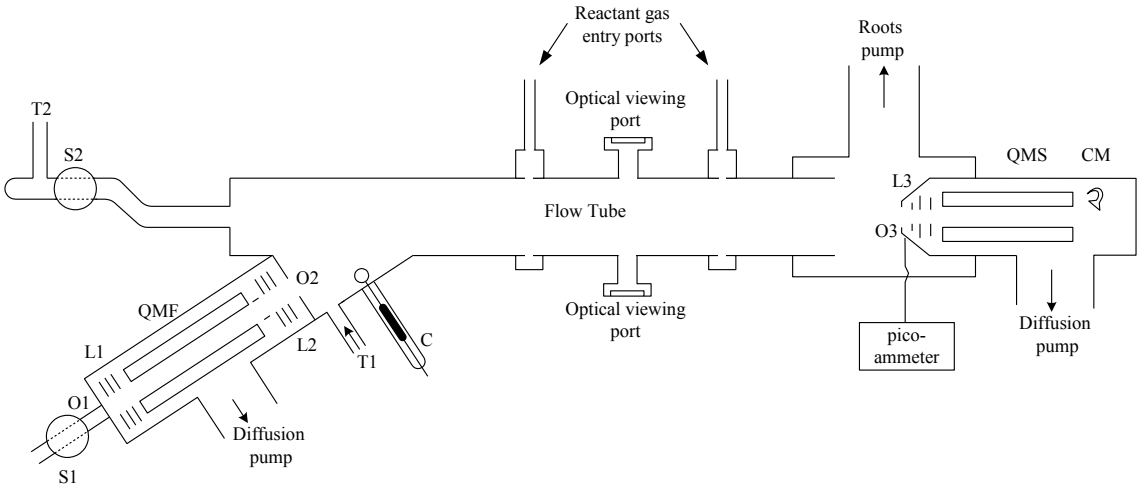


Figure 3.2. SIFT apparatus – historical scheme of the first SIFT instrument build in Birmigham university.. (Reproduced from Hyšpler et al., Journal of Chromatography B: Biomedical Sciences and Applications, 2000 [39]).

3.1.2.2 Selected ion flow tube mass spectrometry

The SIFT-MS has been developed in 1995 by D. Smith and P. Španěl as a method for the analysis of trace gases at ppbv concentrations in atmospheric air, with the focus on the detection and quantification of trace gases of biological origin (human breath, headspace of biological fluids). The main applications of this method have become clinical diagnosis, therapeutic monitoring [250-253] and physiological studies [9,47,253,254]. Traditionally, the SIFT instruments were large apparatuses filling the whole laboratory. In 1997, a Transportable Selected Ion Flow Tube (TSIFT) instrument was constructed at Keele University with a short flow tube of about 40 cm long. Further developments have enabled construction of a compact SIFT-MS instrument, *Profile 3*, with flow tube of only 5 cm long, easily transportable with a weight of 120 kg.

As already mentioned above, the SIFT-MS method is based on chemical ionization, in which the "soft" ionization of neutral molecules is achieved by selected species of reagent ions. This approach minimizes fragmentation of product ions of reactions and thus simplifies the analytical mass spectrum [250].The selection of the appropriate reagent is than an important step of the overall analytical process, whereas

reagent ions must be relatively unreactive with the major components of the air and breath sample, e.g. N_2, O_2, H_2O, Ar and CO_2 in comparison with the trace gases to be analyzed (10-1000 ppbv). Otherwise the reagent ions would be consumed immediately in the reactions with major gases [255]. The reagent ions involved in SIFT-MS ionization were therefore chosen on the basis of understanding of ion chemistry occurring in terrestrial atmosphere (*see Figure 3.3*) [256]. It has been established using rocket-borne and satellite-born mass spectrometers (upper atmospheric regions) that NO^+ and $O_2^{+\bullet}$ ions are dominant in the thermosphere and that H_3O^+ $(H_2O)_n$ are the dominant ions in the altitudes around 70 km. This is explained by low reactivity of H_3O^+, NO^+ and $O_2^{+\bullet}$ with air molecules. Therefore, these ions are ideal as reagents for selective chemical ionisation of reactive compounds present in air matrix. It has been proved by subsequent research that the use of these reagents either separately or in combination is the real strength of SIFT-MS [75].

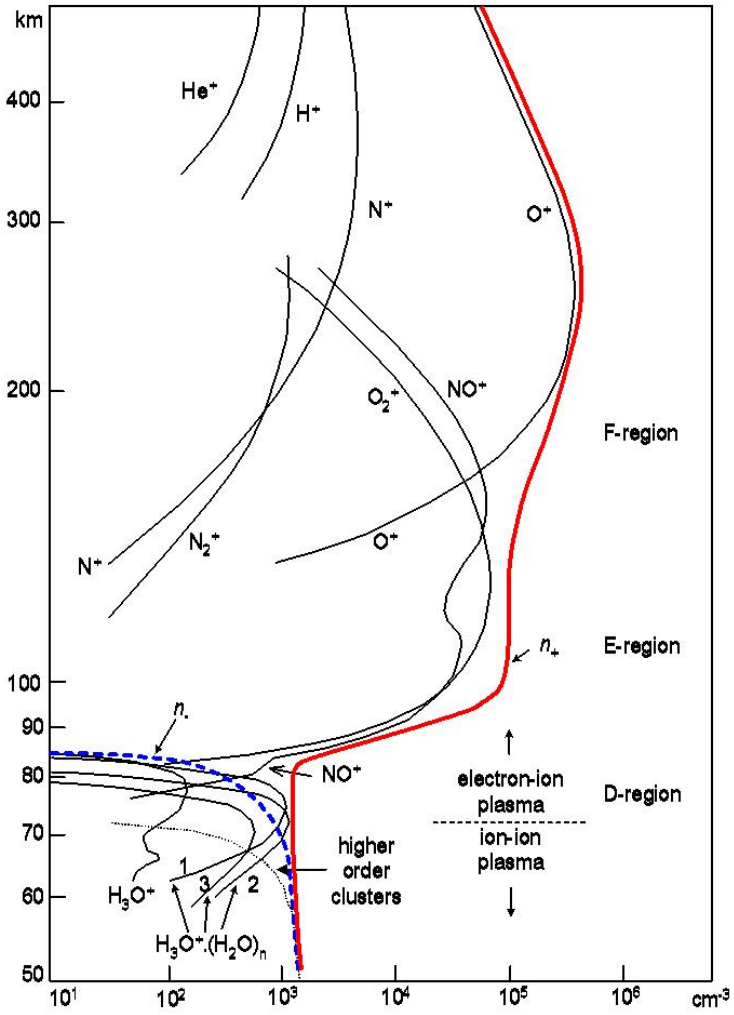


Figure 3.3. The positive ion composition of atmosphere as a function of altitude. (Reproduced from Španěl et al., The Journal of Chemical Physics, 1996 [255]).

3.1.2.2.1 Profile 3 instrument

Currently used, compact and transportable version of a SIFT-MS instrument, *Profile 3*, with flow tube of only 5 cm long and weight of 120 kg, has been first manufactured in 2006 by Instrument Science Limited. This instrument was also used in all experiments discussed in this work and is schematically described in *Figure 3.4*.

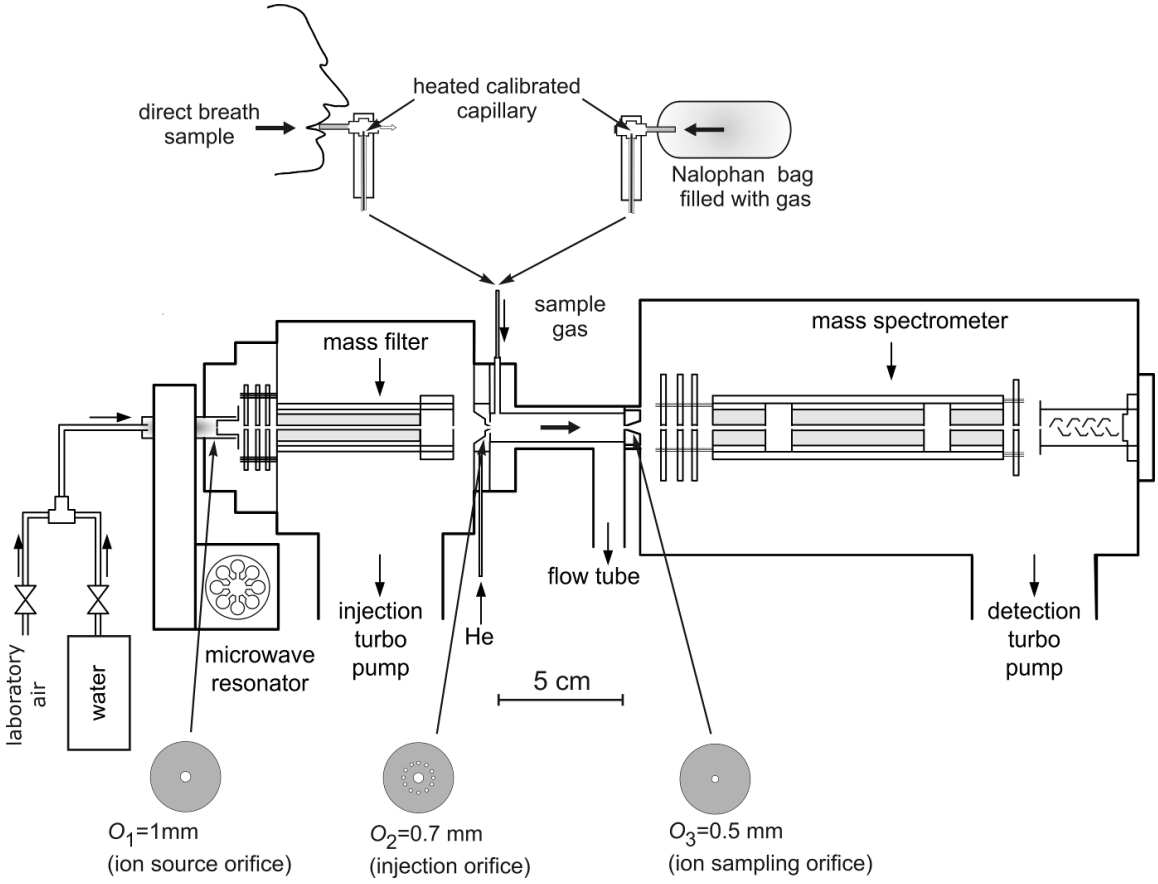


Figure 3.4. Schematic diagram of the *Profile 3* SIFT-MS instrument showing the microwave discharge ion source, injection mass filter and the detection quadrupole mass spectrometer and the three metal discs to which ion current can be measured and which support the orifices O_1, O_2 and O_3 through which, respectively, ions pass from the ion source into the injection mass filter, mass selected ions enter the flow tube and via which ions are sampled into the analytical quadrupole mass spectrometer. Picture finally illustrates both sampling methods - direct breath sampling into the instrument and sampling from bag samples. (Reproduced from Smith et al., International Journal of Mass Spectrometry, 2009 [257]).

Positive ions are generated in an electric discharge ion source (usually microwave glow discharge) from a mixture of water vapour and air [258] maintained at total pressure of 0.3 mbar. From the mixture of ion species extracted through the orifice O_1 a current of

ions of a given mass-to-charge ratio, m/z, is obtained using a quadrupole mass filter. Selected precursor/reagent ions (H_3O^+, NO^+ or $O_2^{+\bullet}$) are then injected into a fast-flowing inert carrier gas (usually helium at a pressure of 100 Pa ~ 1 Torr) via the orifice O_2 and convected along the flow tube (diameter of 1 cm and 5 cm long). Concurrently, the neutral analyte molecules of a sample vapour can be introduced into the flow tube (a continuous flow of the gas sample, typically 20 to 40 mL/min at standard atmospheric pressure and temperature) via a heated and calibrated sampling capillary. The actual flow rate can be measured by a mass flow meter connected to the sample inlet. The capillary and the connecting tubes (all made from polyether ether ketone (PEEK)) are all heated to about 80ºC to prevent condensation of water and other condensable species and memory effect.

The reagent ions finally meet the gas sample in the area of flow tube and may react - undergo chemical ionisation, depending on their chemical properties (proton affinity or ionisation energy). The reaction is allowed during a defined reaction time (0.6 ms), which is determined by the carrier gas flow rate and the reaction length. The reactions of reagent ions with trace gases in the sample diluted by the carrier gas form the product ions that are characteristic of each trace gas compound. The product ions are finally analysed using the detection (analytical) quadrupole mass spectrometer and are counted by a channeltron multiplier/pulse counting system. Thus the obtained count rates are used in calculation of the concentrations of volatile compounds.

The SIFT-MS instrument can be operated in two modes: The Full Scan (FS) mode or Multiple Ion Monitoring (MIM) mode [244]. In FS, a complete mass spectrum is obtained by sweeping the detection quadrupole over a selected *m/z* range for a chosen time whilst a sample of air or breath is introduced into the carrier gas at a steady flow rate. In the MIM mode, only the count rates of the reagent ions and those of selected product ions are monitored as function of time. This real-time monitoring is possible because of the fast time response of SIFT, approximately 20 ms.

The software that controls the instrument allows switching of the injection mass filter between the selected *m/z* values of the reagent ions (19, 30 and 32). Worthy of note from the point of view of analytical sensitivity is the effective dwell time, t_d, during which the product ions of a given *m/z* are accumulated. In the FS mode the total measurement time, t_d, for each ion (*m/z* value) is:

$$t_d = 0.3\frac{n_s t_s}{(m_1 - m_0)} \quad (3)$$

Here, n_s is the number of full scans, t_s is time of each scan, m_0 and m_1 are limit *m/z* values of the scan. So, for example, for a single scan (n_s=1) across the range *m/z* 10-130 with each scan lasting 60 s, the dwell time for a single product ion is 150 ms. Therefore, at least 7 such scans (whilst maintaining the sample flow for 7 minutes) would have to be integrated to achieve 1 s of integration time. Often it is practical to cycle the reagent ions between the individual full scans (e.g. *m/z* 19; then 30; then 32; and repeat ….) and obtain 5 full scans for each reagent ion (15 scans total). In this way, representative mass spectra can be obtained even when the composition of the sample is slowly changing with time.

In the MIM mode, which provides more precise quantification of the targeted trace compounds than does the FS mode, the total measurement time per product ion, t_m, is related to the total sampling time, t_i, the reagent and product ion dwell times, t_{reag} and t_{prod}, the number of reagent and product ions recorded, n_{reag} and n_{prod}, the wait time before counting on each ion, t_w, and the fly back time, t_f, by the expression:

$$t_m = \frac{t_i t_{prod}}{\left[n_{reag}\left(t_{reag} + t_w\right) + n_{prod}\left(t_{prod} + t_w\right) + t_f\right]} = \frac{t_i t_{prod}}{t_{cycle}} \quad (4)$$

Here, t_{cycle} is the time taken to collect the data for all ions, effectively giving the resolution of the time profiles along the *x*-axis.

The available count rate of the reagent ions determines the sensitivity and precision of SIFT-MS analysis; 10^6 counts per second is a desired value. Reduction of this count rate by a factor of two results in the precision of the measurement to be lowered 1.4 times (square root dependence). However, a reagent ion count rate that is too large can result in non-linearity of the ion detector (due to its dead time); this can be compensated for [259] and can be checked by the relative intensities of the ^{18}O isotopologues of the H_3O^+ and $O_2^{+\bullet}$ reagent ions.

As mentioned above, the SIFT-MS method enables analysis by using three different precursor/reagent ions (H_3O^+, NO^+ or $O_2^{+\bullet}$). According to the selected reagent ion we finally speak about the H_3O^+, NO^+ or $O_2^{+\bullet}$ reactions.

3.1.2.2.2 H_3O^+ reactions

The reactions between H_3O^+ reagent ions and organic molecules (M) proceed predominantly via proton transfer and usually result in only one characteristic (MH^+) product ion. However, these MH^+ ions sometimes dissociate to M-OH. This fragmentation results from elimination of water after protonation of some alcohols or larger aldehydes or carboxylic acids. The important point is that the proton affinity of the molecules must be greater than that of H_2O for proton transfer to occur. The ion chemistry of the H_3O^+ reagent ion with many different compounds has been previously comprehensively described in the literature.

Except of "primary" reactions are for H_3O^+ reagent ion characteristic also "secondary" reactions, because in the presence of water molecules the MH^+ product ions can associate with H_2O forming hydrated ions $MH^+(H_2O)_{1,2,3}$. The H_3O^+ reagent ions are also partially converted to hydrated hydronium ions $H_3O^+(H_2O)_{1,2,3}$, that can act as additional reagent ions, and contribute to the production of $MH^+(H_2O)_{1,2,3}$ via ligand switching reactions [260,261]. Therefore, the ion chemistry, which is important for real time and accurate quantification, is influenced by the presence of water vapour and it is necessary to account for this. This topic will be covered in detail in the following chapter: *4.2 Three-body association reactions – ion chemistry involving water molecules*.

3.1.2.2.3 NO^+ reactions

The reactions of NO^+ are more diverse in comparison to those of H_3O^+, but usually also result in one or two primary product ions. Due to the low recombination energy (RE) of NO^+ ions (9.25 eV) they cannot ionize the major air components. Thus, only molecules with ionization potential (IP) less than 9.25 eV can be ionized by charge transfer. Another reaction mechanisms characteristic for NO^+ reagent ions are then hydride ion transfer, producing $(M\text{-}H)^+$ ions, and association reaction, producing NO^+ M ions. NO^+ is in practical SIFT-MS analyses mainly used to quantify aldehydes, ketones and carboxylic acids. The use of NO^+ can in most cases help when minimising the effects of isobaric compounds. When using NO^+ reagent ions to analyse humid samples, the role of water must be again accounted for, because a fraction of product ions can again associate with

H_2O molecules forming hydrated ions [75,246]. See chapter *4.2 Three-body association reactions – ion chemistry involving water molecules* for additional details.

3.1.2.2.4 $O_2^{+\bullet}$ reactions

For a radical cation, $O_2^{+\bullet}$, a characteristic reaction with VOCs is dissociative charge transfer [258]. The resulting primary product ion is a radical cation. The charge transfer is usually dissociative with the fragmentation patterns similar to electron ionisation, EI. Similar fragmentation follows, usually favouring a closed shell ion product. Because the energy is well defined in the charge transfer (in contrast to the wide energy distribution in EI) there are fewer types of fragments. However, the spectra of VOCs obtained using the $O_2^{+\bullet}$ reagent ions are typically more complicated than those obtained using H_3O^+ or NO^+. The choice of reagent ion depends on the compounds to be analysed, in the case of $O_2^{+\bullet}$ this is the reagent of choice for very small molecules including NH_3 [262] and CH_4 [263] or hydrocarbons like isoprene [264] or pentane. Different product ions are produced in the reactions with the different reagent ions. Such can be used in identification of isobaric compounds [75].

3.1.2.2.5 Absolute quantification

The theoretical background for the absolute quantification of the trace gases in real time is based on the first order kinetics. In general, the absolute concentrations are calculated based on knowledge of the rate constants, reagent and product ion count rates and the known reaction time.

As an illustrative example can be used the proton transfer reaction (only one compound, M, reacts with reagent ion to produce product ion, MH^+).

$$H_3O^+ + M \rightarrow MH^+ + H_2O \qquad (5)$$

the reaction proceeds during a well-defined reaction time t_r (typically 0.6 ms) with the rate constant k. MH^+ is assumed to be the only product ion and if $[H_3O^+] >> [MH^+]$, then

the kinetics can be approximated by the *Equation 6* (note the [ ] brackets represent count rates):

$$[MH^+] = k[M][H_3O^+]t_r \quad (6)$$

[M] can be finally expressed as:

$$[M] = \frac{1}{t_r}\frac{[MH^+]}{k[H_3O^+]} \quad (7)$$

The measured ion signals are proportional to the ion concentrations. Therefore the measurement of the MH^+/H_3O^+ signal ratio analysed by the mass spectrometer allow the absolute quantification of M.

However, in some cases the reaction scheme is more complicated and involves ion clusters. Then the following general equation must be used to calculate the concentration of the trace gas molecule, [M]:

$$[M] = \frac{1}{t_r}\frac{f_{p1}I_{p1}/D_{ep1} + f_{p2}I_{p2}/D_{ep2} + \cdots}{f_{i1}I_{i1}k_1 + f_{i2}I_{i2}[(k_1 + k_2)/2]/D_{ei2} + \cdots} \quad (8)$$

In the case of larger concentration of [M] and assumption of the secondary reactions of the product ions, *Equation 8* should be expanded to the logarithmic form:

$$[M] = \frac{1}{k_1 t_r}\ln\left(1 + k_1\frac{f_{p1}I_{p1}/D_{ep1} + f_{p2}I_{p2}/D_{ep2} + \cdots}{f_{i1}I_{i1}k_1 + f_{i2}I_{i2}[(k_1 + k_2)/2]/D_{ei2} + \cdots}\right) \quad (9)$$

- I_{p1}, I_{p2} etc. presents product ion signals (count rates are corrected for the detector dead time and for mass discrimination in the downstream quadrupole mass spectrometer [259]);
- I_{i1}, I_{i2} etc. are reagent ions signals (e.g. H_3O^+, $H_3O^+(H_2O)$, again corrected for mass discrimination and dead time);
- $k_{1,2}$ are rate constants of ion-molecular reactions between reagent ions and neutral molecule [M];
- $D_{ep1}, D_{ep2}, D_{ei2}$ etc. are differential diffusion coefficients of ion products and reagent ions.

The absolute concentration of [M] in the flow tube can be converted to the relative concentration of the molecule in the gas sample, p_M/p_0, from the direct consideration of

continuous flow dilution of the sampled air (flow rate, Φ_a) in to the carrier gas (flow rate, Φ_c):

$$\frac{p_M}{p_0} = \frac{[M]p_0}{n_0 p_g}\frac{T_g}{T_0}\frac{\Phi_c + \Phi_a}{\Phi_a} \qquad (10)$$

- n_0 is a physical constant - Loschmidt's number (2.687 x 10^{19} cm^{-3}). It is a reference value of concentration at standard atmospheric pressure, p_0 = 760 Torr and temperature T_0 = 273.15 K [259];
- p_g is the flow tube pressure;
- Tg is the flow tube temperature.

The relative concentration p_M/p_0 is expressed in the units of ppbv by multiplying by 10^9.

The SIFT-MS is thus allowing the calculation of concentrations of the trace amounts of VOCs by using the physical quantities as flow rate of a sample (Φ_a) and helium carrier gas, flow tube pressure (p_g) and temperature (Tg) and reagent and product ion signals (c/s), I_i, I_p (their ratio). All the calculations are done by the analytical software automatically and immediately during each analysis, by using the kinetics data entries (set of reagent ions and product ions together with the rate constants and factors, f), which are stored in the so-called kinetic library in the special format (*Table 3.2*). Factors (f_i and f_p) are simply used to multiply the acquired raw count rate of the ion. Usually, values of 1.0 are used, but in some cases would such a simple calculation not provide valid results and values other than 1.0 should be used to account for all the circumstances (see chapter *4.3 Construction and optimization of kinetics library*).

Table 3.2. Sample kinetics library entries describing straightforward calculations of 2-hexanone concentrations using the three reagent ions indicated. Rate constants (k) are given in units of cm^3s^{-1}. NOTE: Because of historical reasons, the format of SIFT kinetic library requires the use of term "precursor" for the reagent ion.

compound(ion)	2-hexanone(H3O+)	2-hexanone(NO+)	2-hexanone (O2+)
number of precursors	4 precursors	3 precursors	2 precursors
m/z k f_i	19 4.0e-9 1.0 37 3.05e-9 1.0 55 2.66e-9 1.0 73 2.44e-9 1.0	30 3.0e-9 1.0 48 1e-9 1.0 66 1e-9 1.0	32 3.1e-9 1.0 50 1e-9 1.0
number of products	2 products	1 product	4 products
m/z k f_p	101 1.0 119 1.0	130 1.0	43 1.0 58 1.0 85 1.0 100 1.0

3.2 Extraction methods for pre-concentration of trace substances for GC/MS analysis

3.2.1 Solid-phase microextraction

As already mentioned above, solid-phase microextraction (SPME) is one of the most frequently used methods for sample preparation in the area of breath analysis and in connection with GC-MS. This technique has been developed in the late 1980s by Arthur and Pawliszyn [265, 266] and is considered as a convenient, universal, rapid, inexpensive, and efficient technique for sampling solid, liquid, or gaseous samples. It requires no solvents or complicated apparatus and provides linear results over a wide range of analyte concentrations. Analysis of the extracts can be performed not only by using GC or GC/MS, but it can be also performed by High-performance liquid chromatography (HPLC) [85,267-269]. This technique was successfully used for the determination of volatile compounds from environmental, toxicological, and pharmacological specimens with different matrices. SPME has been also applied to analysis of VOCs in human breath [270].

In the SPME technique, a fused silica fiber coated with a polymeric stationary phase is contained in a specially designed syringe whose needle protects the fiber when septa are pierced. The fiber is directly exposed to a liquid or gaseous sample to extract and concentrate the analytes. After the equilibration of the sorption process is attained, the fiber is withdrawn into the needle and introduce into an injector of a gas chromatograph, where the extracted compounds are thermally desorbed and analysed. SPME can be performed manually or by an autosampler. The method is economical, because one single fiber can be used repeatedly. Unlike in conventional methods for analysis of gaseous samples, modified equipment like a complex valve injection system, a thermal desorption device, or a cooling trap is not required by SPME. The SPME fiber is easily cleaned by desorbing any contaminants in a hot GC injector. The SPME technique coupled with GC has been used to analyze volatile and semivolatile air compounds [271]. The fiber can be exposed directly to the air or to a sample collected in a gas sampling container.

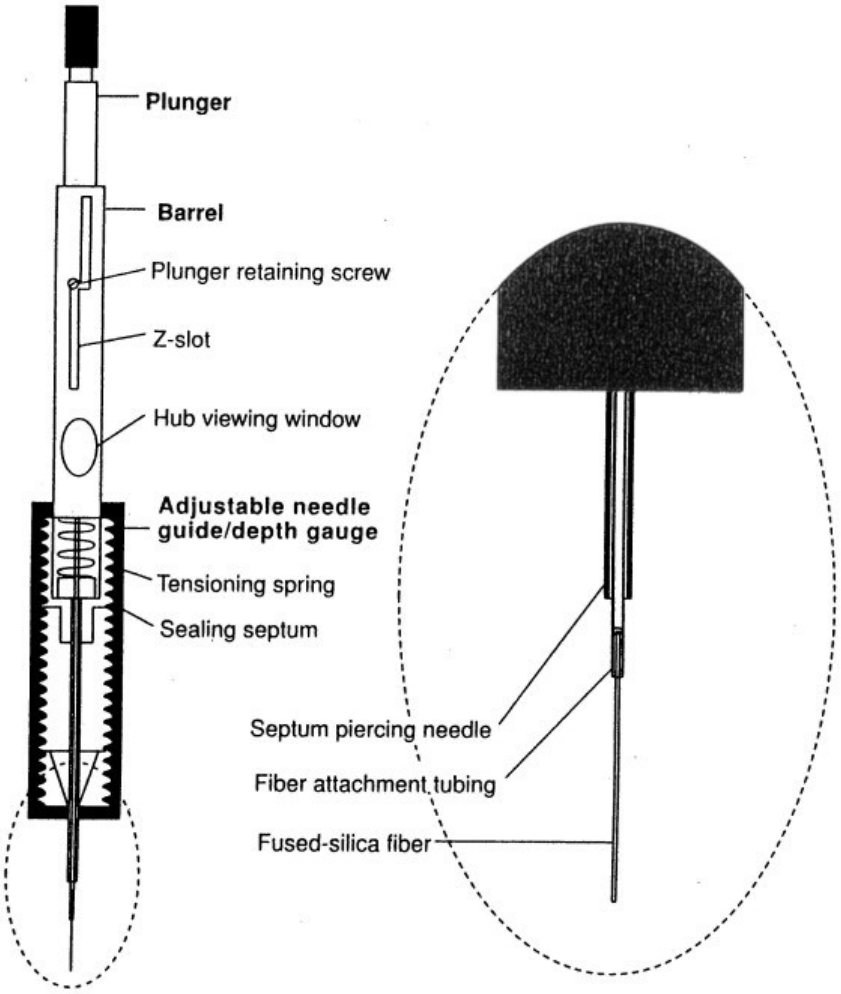


Figure 3.5. Commercial SPME device made by Supelco. (Reproduced from Kataoka et al., Journal of Chromatography A, 2000 [272]).

3.2.2 Thermal desorption

Analytical thermal desorption, known within the analytical chemistry community simply as thermal desorption (TD) is a versatile technique that concentrates volatile and semi-volatile organic compounds (VOCs and SVOCs) in gas streams prior to injection to gas chromatograph (GC). By extracting organic vapours from a sample and concentrating them into a very small volume of carrier gas, TD maximises sensitivity for trace-level target compounds, helps to minimise interferences, and routinely allows analyte detection at the ppbv concentration or below [273].

This method originated in the mid-1970s as an adaptation to the injection procedure for GC. Injector liners were packed with a compound able to adsorb organic compounds, used to sample air or gas, and then dropped into the inlet of the GC. This principle was first widely employed for occupational monitoring, in the form of personal badge-type monitors containing a removable charcoal strip [274]. Such monitors offered the advantage of being amenable to analysis without a separate solvent-extraction step. In the 1970s was also developed a method by which volatiles in the air were collected by diffusion onto tubes packed with a sorbent, which was then heated to release the volatiles into the GC system. These were first introduced for monitoring sulfur and nitrogen dioxide [275] but the analyte scope later widened as the sorbents became more advanced. Another early method (closely related to the modern purge-and-trap procedure) involved passing a stream of gas through a water sample to release the volatiles, which were again collected on a sorbent-packed tube [276]. Such axial-type samplers, which later became known as 'sorbent tubes', were laid out as an industry standard in the late 1970s, by Working Group 5 (WG5) of the UK Health & Safety's Committee on Analytical Requirements (HSE CAR). At the same time, various basic functionality requirements for thermal desorption have been specified by the same group, and in the years since then, a number of improvements have been made to instrumentation for thermal desorption, including two-stage operation, splitting and re-collection of samples, improved trap-cooling technology, standard system checks, and automation.

Thermal desorption fundamentally involves collecting volatile organic compounds onto a sorbent, and then heating this sorbent in a flow of gas to release the compounds and concentrate them into a smaller volume. Early thermal desorbers used just single-stage operation, whereby the volatiles collected on a sorbent tube were released by heating the tube in a flow of gas, from where they passed directly into the

GC. Modern thermal desorbers can also accommodate two-stage operation, whereby the gas stream from the sorbent tube (typically 100–200 mL) is collected on a narrower tube integral to the thermal desorber, called the focusing trap or a cold trap. Heating this trap releases the analytes once again, but this time in an even smaller volume of gas (typically 100–200 μL), resulting in improved sensitivity and better GC peak shape [269]. Modern thermal desorbers can accommodate both single-stage and two-stage operation, although single-stage operation is now usually carried out using the focusing trap to collect the analytes, rather than a sorbent tube. It is normal for the focusing trap to be held at or below room temperature, although a temperature no lower than 0 °C is sufficient for all but the most volatile analytes. Higher trap temperatures also reduce the amount of water condensing inside the trap (when transferred to the GC column, water can reduce the quality of the chromatography).

The sorbent tube and the focusing trap may be packed with one or more sorbents. The type and number of sorbents depends on a number of factors including the sampling setup, the analyte volatility range, analyte concentration, and the humidity of the sample [277,278]. One of the most versatile and popular sorbents for thermal desorption is poly(2,6-diphenyl-p-phenylene oxide), known by its trademark Tenax®.[279]

4 Results and Discussion

The section "Results and Discussion" is divided in five subsections that summarize or complement published work that I have worked on during my PhD study. Each subsection thus represents comments on peer reviewed journals included in my dissertation, having the objectives, methods, results and discussion and conclusions.

4.1 Determination of rate constants and product ion branching ratios

Objectives

It is a generally accepted fact that before proceeding to deeper investigation in the area of SIFT-MS analysis, the relevant knowledge of ion chemistry for compounds of interest are necessary. Therefore the first subsection of my thesis is focused on the details of studies (carried out during the PhD project) concerning the ion chemistry and overview of the whole experimental process, starting from observed mass spectra and leading to determination of the rate constants. In summary, the step by step activities conducted on the beginning of each project.

The data presented in this subsection will relate to the research articles published in impacted peer reviewed journals, as included in the Appendices A [280] and B [165] dealing with a group of carboxylic acids and a group of possible IBD biomarkers. The chapter is not only summarising the published work, but also provides some additional details. At the onset, it is nevertheless useful to outline the motivation for the choice of compounds:

A selected ion flow tube study of the ion molecule association reactions of protonated (MH^+), nitrosonated (MNO^+) and dehydroxidated $(M\text{-}OH)^+$ carboxylic acids (M) with H_2O (see Appendix A)

The ion chemistry study has been carried out of the reactions of H_3O^+, NO^+ and $O_2^{+\bullet}$ with four carboxylic acids, namely formic acid, acetic acid, propionic acid and butyric acid. Carboxylic acids are VOCs important in many areas of research: from the medical diagnostics and therapeutic monitoring, where the carboxylic acids are used for diagnostics of oral malodour [281,282], esophago-gastric cancer [142-144] or severe asthma [283], to food science [284], where is SIFT-MS analysis of these compounds used

for monitoring of biochemical reactions [285], but mainly for quality assessment (in this content analysis of food aromas) [286-287].

SIFT-MS quantification of several breath biomarkers of inflammatory bowel disease, IBD: a detailed study of the ion chemistry (see Appendix B)

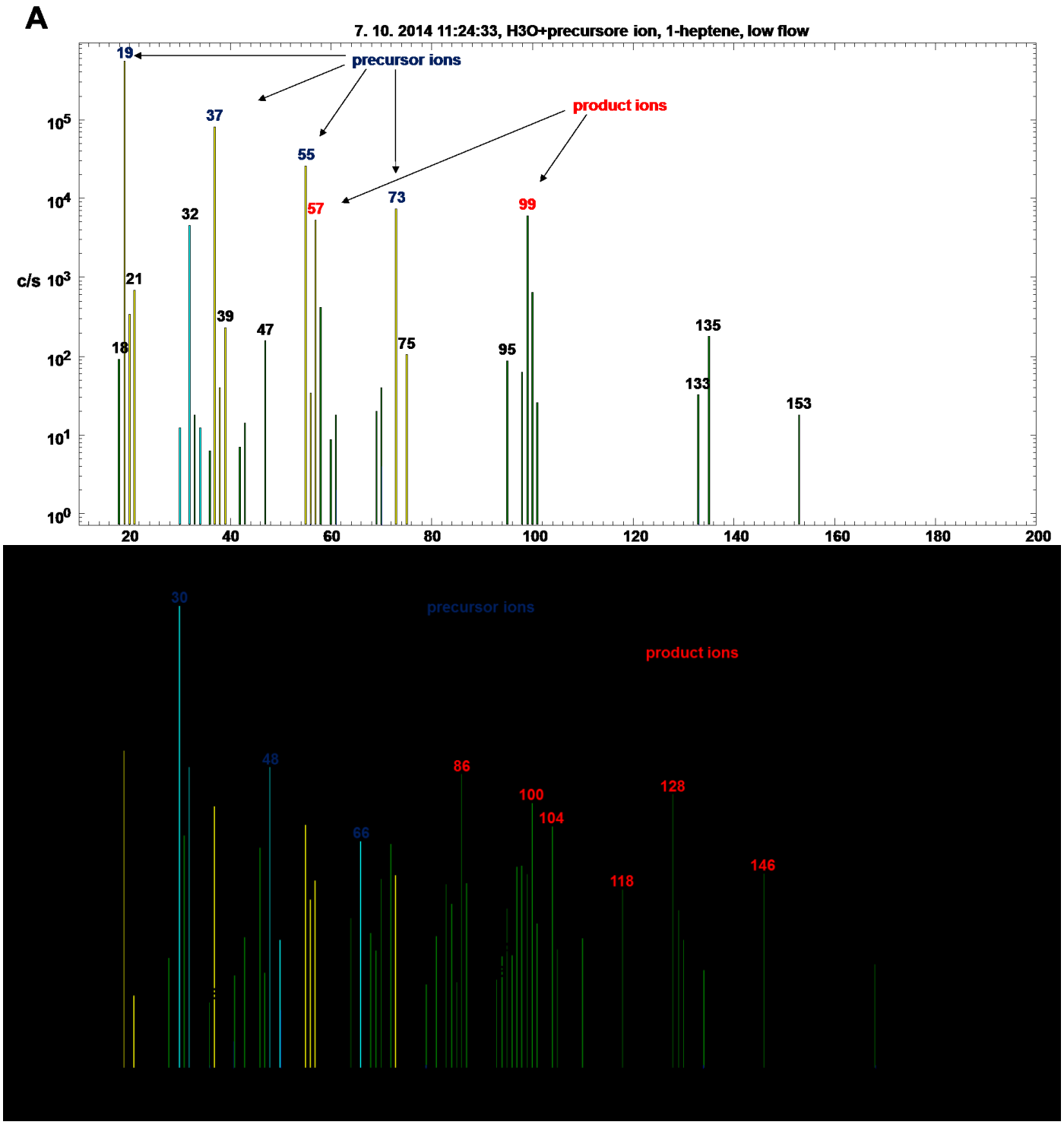
This work was focused on several compounds (2-methylpentan, 1,3,5-triazine, trans-2-octene, cis-2-nonen-1-ol, 2′-methylacetophenone, 1-heptene and 2-hexanone) previously identified to be present in modified concentrations in the breath of patients with inflammatory bowel diseases, IBD. For additional details regarding this illness and background concerning investigation in this area see chapter *2.3.3 Inflammatory bowel disease*. A selected ion flow tube, SIFT, of the reactions of H_3O^+, NO^+ and $O_2^{+\bullet}$ with these volatile organic compounds has been carried out with the ultimate goal to obtain the rate constants and product ion branching ratios of the primary reactions including three-body association rate constants, describing the rate of hydration (see the next chapter *4.2 Three-body association reactions*). The results of this study allow accurate SIFT-MS quantification of these compounds in exhaled breath via the non-invasive way.

Methods

Determination of branching ratios

In order to determine the product ions and their branching ratios, mixtures of dry air/ambient air and a trace amount (less than 10 parts per million by volume, ppmv) of vapour of a compound were introduced into the SIFT instrument (Profile 3 SIFT-MS manufactured by Instrument Science Limited, Crewe, UK) via a heated calibrated capillary and full scan (60 s integration time) mass spectra were acquired whilst each of the three selected reagent ions were alternately injected into the helium carrier gas. Note that switching between the three reagent ions H_3O^+, NO^+ and $O_2^{+\bullet}$ was controlled electronically by the data system. The *m/z* mass spectral range (typically *m/z* 10-200) was chosen on the basis of the molecular weights of compounds included in the study, allowing for possible formation of adduct ions (hydrated ions – additional details are available in the chapter *4.2 Three-body association reactions – ion chemistry involving water molecules*). The major ion products were identified by inspection of these mass spectra (see *Figure 4.1* as example of SIFT-MS spectra obtained for 1-heptene; *m/z* 57

and 99 have been identified as major product ions when the H_3O^+ reagent ion has been used; *m/z* 86, 100, 104, 118, 128, 146 by utilising NO^+ reagent ion and *m/z* 56, 69, 70 and 98 for $O_2^{+\bullet}$ reagent ion) and their count rates were precisely determined in separate experiments using the multi-ion monitoring (MIM) mode [47].



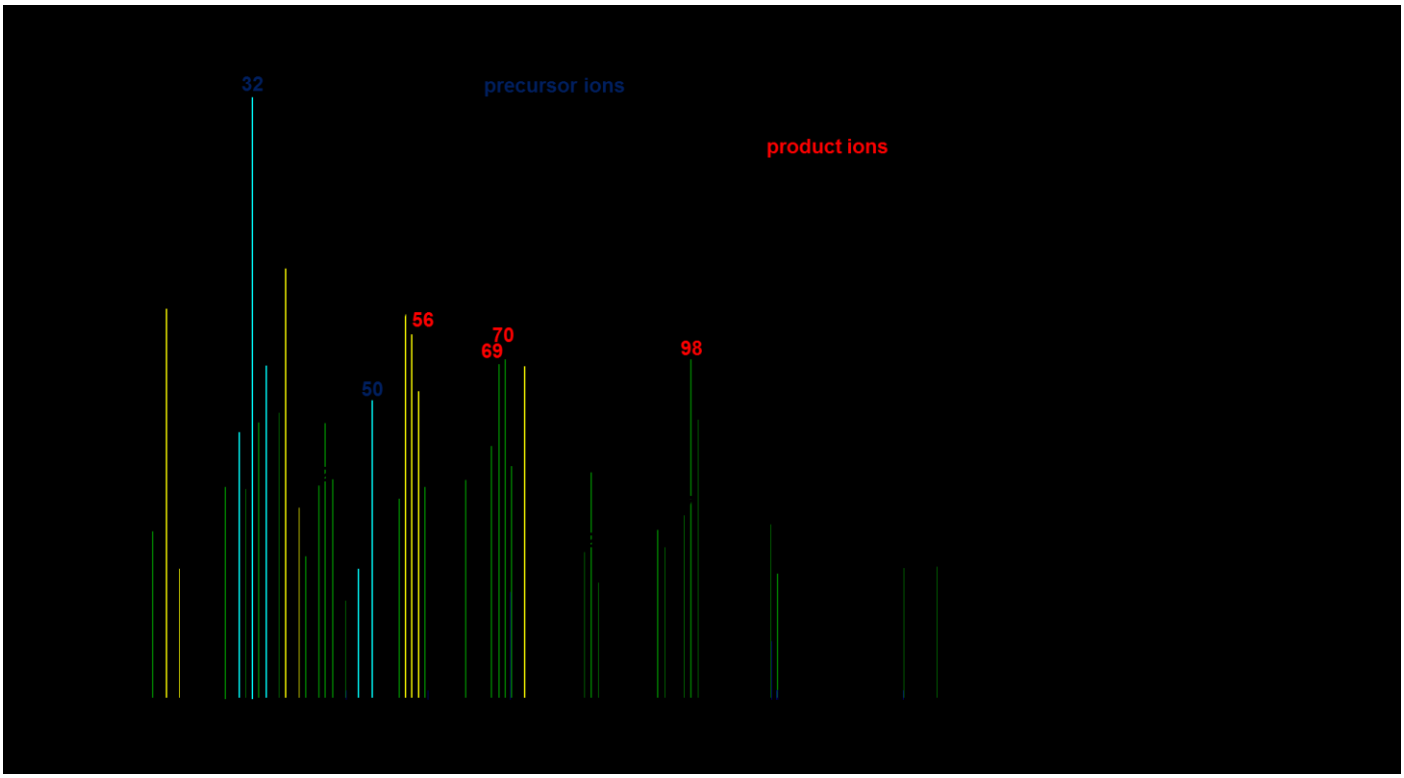


Figure 4.1. The SIFT-MS spectrum obtained for 1-heptene. The arrows indicate the ions resulting from the reactions of the H_3O^+ reagent ion (A); NO^+ reagent ion (B) and $O_2^{+\bullet}$ reagent ion (C).

When looking at the *Figure 4.1 – C*, it is worthy to mention again, that the SIFT-MS spectrum has the fragmentation patterns similar to MS spectrum gathered by electron ionisation (see the *Figure 4.2* illustrating the mass spectrum gathered for 1-hepten).

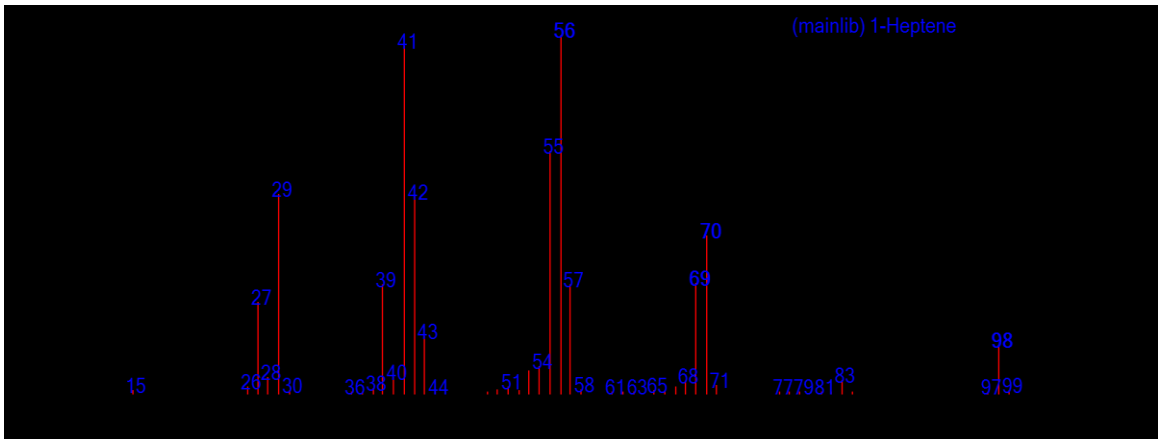
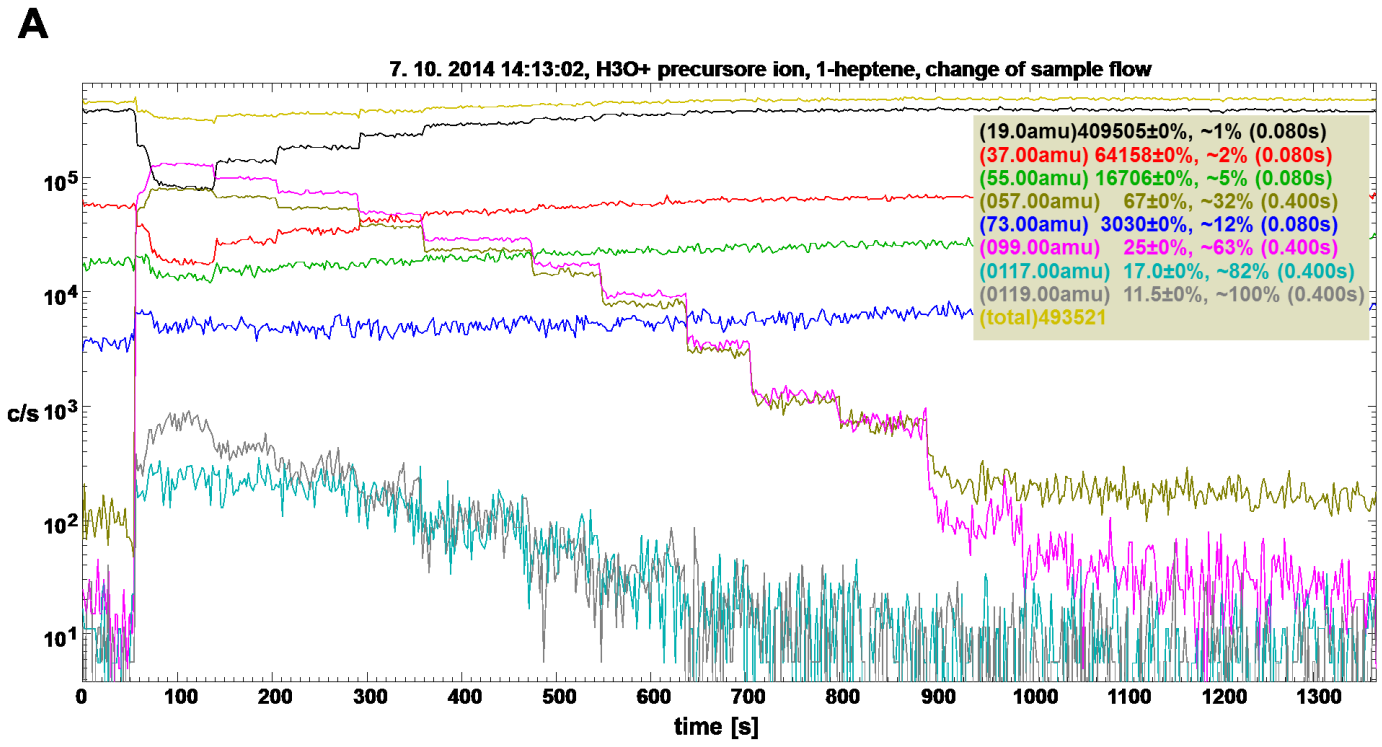


Figure 4.2. The EI mass spectrum for 1-heptene. The spectrum has been obtained from the NIST MS Search 2.0 library.

In the MIM mode, the appropriate reagent ion and all the identified product ions were included. In case of the study focused on carboxylic acids, the variable concentrations required for the experiment were achieved by gradually adding vapour of a compound of interest to the Nalophan bag with dry air by a syringe, whereas in case of IBD possible biomarkers study, the dependence of ion count rates on the changing amount of the molecules of the VOC/air mixture flowing into the He carrier gas was obtained by varying the flow rate of the sample (see the example of 1-heptene data in *Figure 4.3*). Flow rate of the injected gaseous sample was than varied by a needle valve and monitored by a flow meter (manufactured by Voegtlin, Aesch, Switzerland).



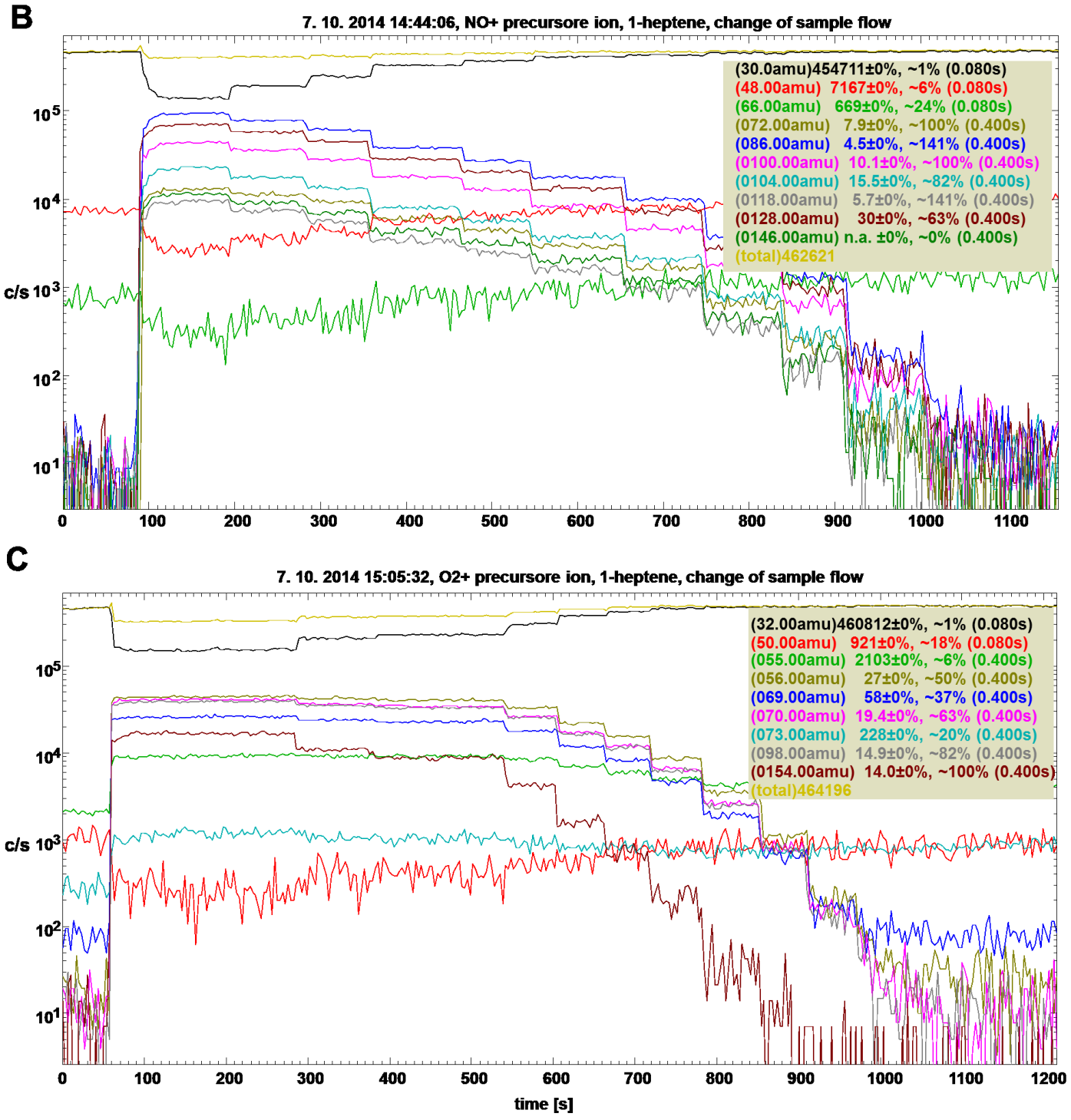


Figure 4.3. The time profile of the decay of signal in c/s of the 1-heptene product ions together with reagent ions - H_3O^+ (A); NO^+ (B) and $O_2^{+\bullet}$ (C). As can be seen from these time profiles, to avoid omitting of any possible product ions, usually more interesting *m/z* have been investigated than finally confirmed (f.e. *m/z* 119 in case of H_3O^+ reagent ion or *m/z* 72 in case of NO^+ reagent ion).

The primary product branching ratios of the reactions were then determined by plotting the percentages of the individual product ions on a linear scale as a function of the sample mixture flow rate determined accordingly to the exponential reduction of the reagent ion count rate. The example of this type of a plot for 1-heptene is shown in *Figure 4.4*. By extrapolating to zero sample gas/vapour flow (i.e., estimating the limit at zero sample concentration) the true primary branching ratios, excluding any secondary reactions were obtained. In our experiments, only product ions with resulting branching ratio greater than 5% were reported.

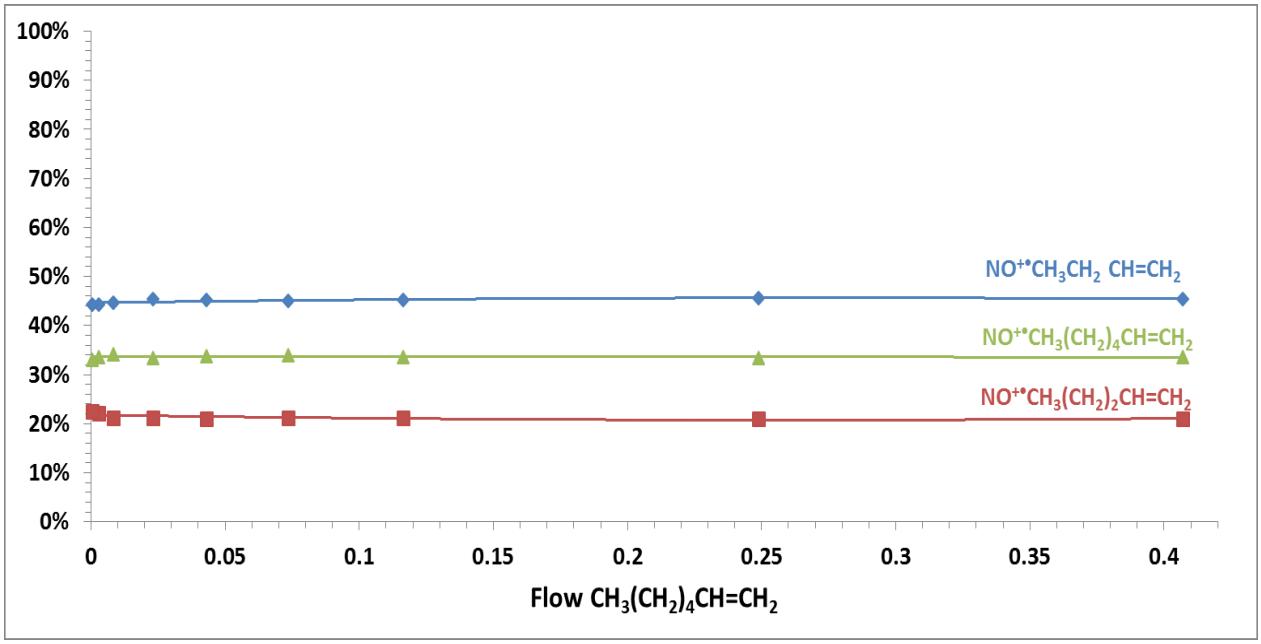


Figure 4.4. A plot of percentages of individual product ions as a function of the flow rate of a mixture containing heptene in humid air introduced into SIFT when NO^+ reagent ions are injected. The primary product branching ratios are obtained by extrapolating the lines to zero flow.

Determination of the rate constants

SIFT-MS quantification requires knowledge of rate constants, which can be obtained either from original papers, eventually from the Anicich index of the literature for bimolecular gas phase cation-molecule reaction kinetics [288]. Nevertheless, in some cases the required rate constant may not available and then must be determined experimentally by the following procedure, that has been utilised for example in case of work focused on IBD possible biomarkers.

It is well established that proton transfer reactions of H_3O^+ proceed at collisional rate when these reactions are exothermic by more than 40 kJ/mol [289]. Thus, the rate constants (k) for the reactions of H_3O^+ can be calculated as collisional (kc) according to using the Su and Chesnavich parameterized trajectory theory [290] using the dipole moments (μ_r) calculated according to the density functional theory (DFT, B3LYP, 6-31G*) using Abalone software (NWChem, version 1.8.88, Agile Molecule) and polarisabilities (α) calculated according to empirical method from the chemical structure of the molecules [291]. The example of the dipole moment calculation result by using the Abalone software is available in the *Figure 4.5*. First of all, it is necessary to draw properly the molecule of interest. In the following step the geometry must be optimised by the selected method (in our case DFT/b3lyp/6-31G*). Finally, the software provide information about the dipole moment (tab „electrostatics“).

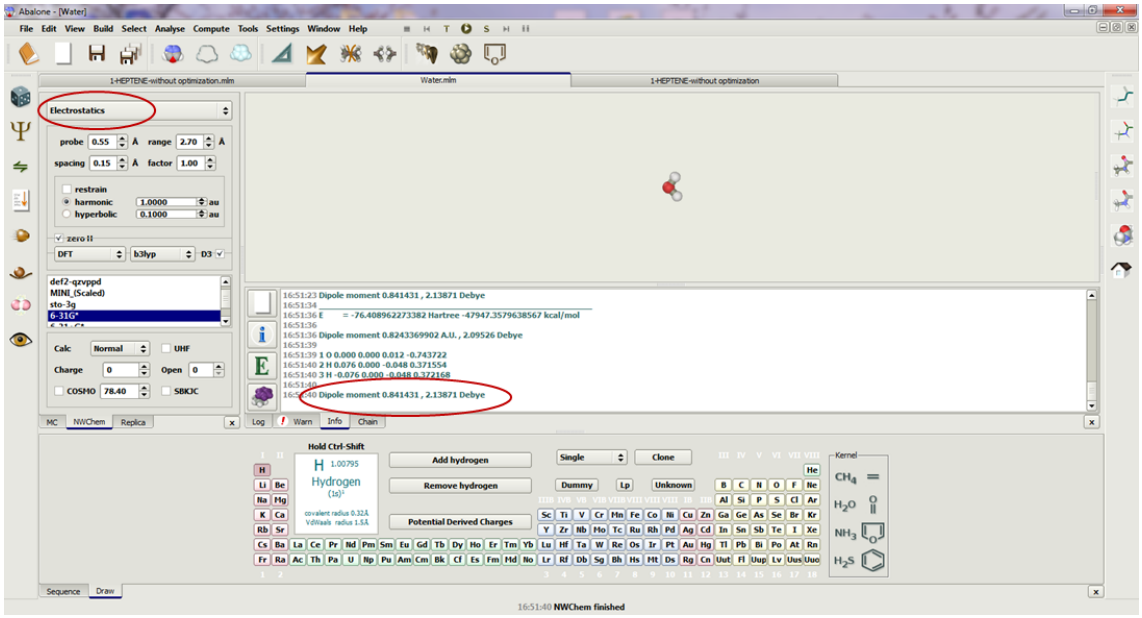


Figure 4.5. Print screen from the Abalone software – calculation of dipole moment by the DFT/b3lyp/6-31G* method.

The rate constants for the reactions with NO^+ and $O_2^{+\bullet}$ (k) are then determined based on their experimentally derived decay rates relatively to that for the H_3O^+ reaction by injecting all three reagent ions simultaneously and allowing them to react with the neat vapours of the compounds introduced at varied concentrations [257]. In these experiments, SIFT-MS instrument (still in the MIM mode) is set up to allow all three reagent ions to react simultaneously with the product ions (*Figure 4.6*). This can be

achieved by adjusting the mass setting of the injection mass filter to zero value effectively setting the voltage differential between the quadrupole rods to 0 V. The flow rate of injected sample is again controlled by opening and closing the needle valve and is monitored by a flow-meter. The maximum flow rate in our experiments was 33.4 mL/min at standard atmospheric pressure and temperature, whereas the minimum flow rate was 0 mL/min. Uncertainty in the absolute value of the determined rate coefficients is +/-20% [47].

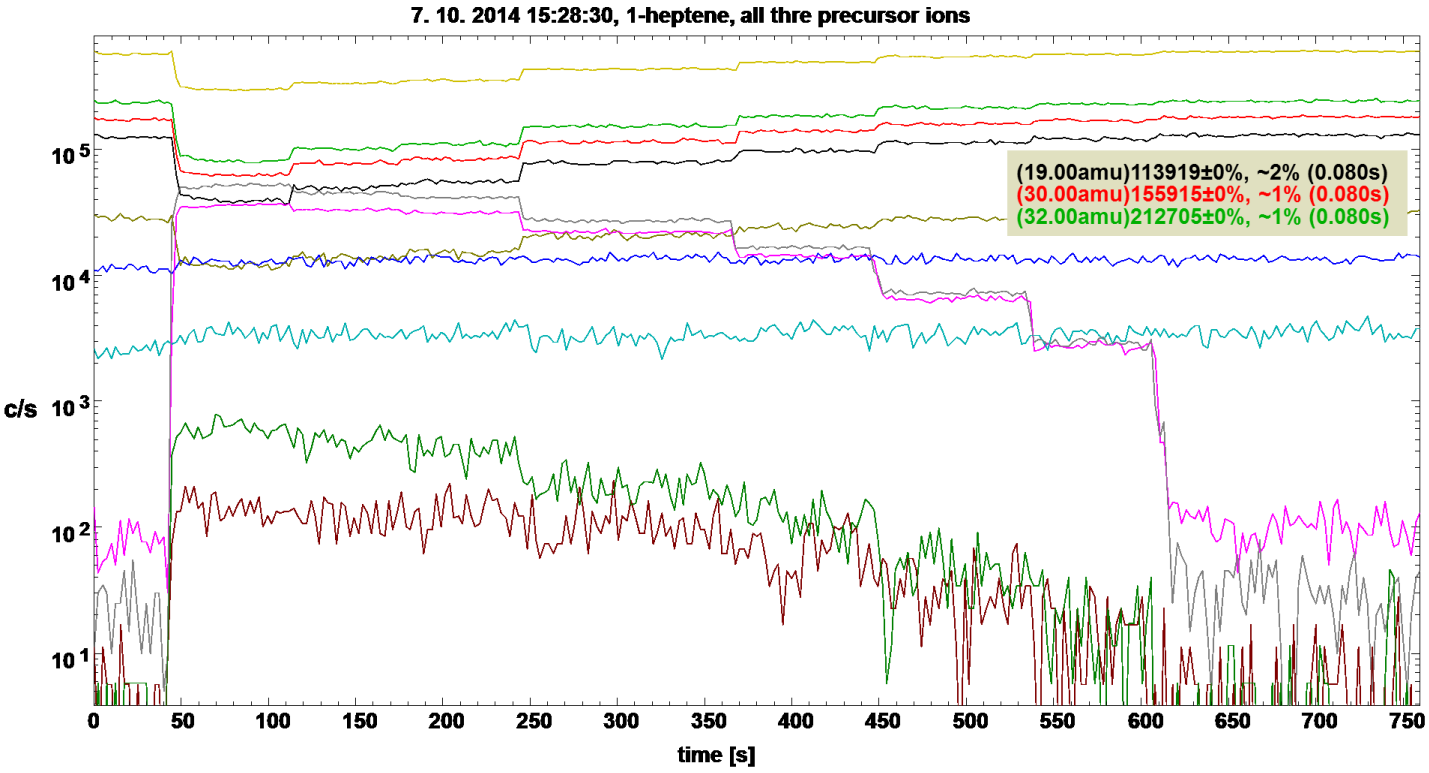


Figure 4.6. MIM profile when all three reagent ions are injected simultaneously into the flow tube where they react with the sample (1-heptene) introduced at varied concentration.

The plot of experimental dependence of the reagent ion count rates on the sample flow rate is shown in *Figure 4.7*. The slope reflects the experimental derived relative rate constant which is then multiplied by the theoretically calculated value of k_c of H_3O^+ in order to obtain absolute values for the NO^+ and $O_2^{+\bullet}$ reactions.

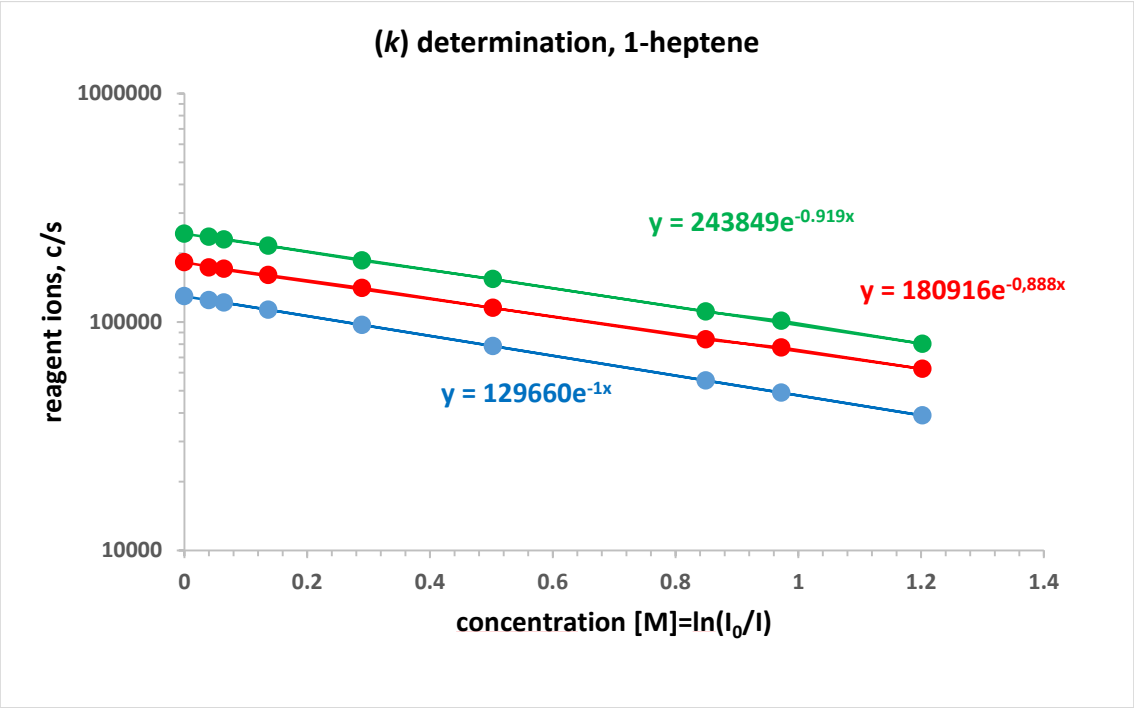


Figure 4.7. The count rates of H_3O^+, NO^+ and $O_2^{+\bullet}$ plotted on a semi-logarithmic scale as functions of the sample flow rate for 1-heptene. The rate constants (*k*) for the NO^+ and $O_2^{+\bullet}$ reactions are finally determined from the relative slopes of these plots.

Results and Discussion

In this part, the results gathered within the experiments focused on carboxylic acids and IBD possible biomarkers are summarized and presented. Not only data published in the relevant manuscripts (Appendices A and B), but also results not explicitly included in these works - predominantly, examples of individual mass spectra of IBD possible biomarkers are presented in this section, because these are new and original data. In the following text the product ions resulting from the ion-molecule reactions are discussed.

1. Carboxylic acids

Product branching ratios for the primary H_3O^+, NO^+ and $O_2^{+\bullet}$ reactions

Primary product ions and the corresponding branching ratios observed in the experiments are summarized and available in form of a table in the relevant manuscript (Appendix A).

In general, the main primary product for all the H_3O^+ reactions is the protonated parent molecule, MH^+. In the case of formic acid and acetic acid we are speaking about the only primary ionic product. On the contrary, in case of propionic acid and butyric acid reactions, there are two primary product ions - the protonated parent molecule, MH^+, and the molecular ion which is created through the way of water molecule lost from the protonated parent molecule. The data obtained within this study are in agreement to those previously published [292].

The reactions of NO^+ with the carboxylic acids proceed via three-body association reactions forming $M{\cdot}NO^+$ adduct ions or via hydroxide ion transfer. It is important to highlight, that reactions of formic acid and acetic acid are in comparison to the collisional rate constant significantly slower and the only product is the $M{\cdot}NO^+$ adduct ion. This fact can be explained by the lack of any available exothermic bimolecular reaction channels. The higher carboxylic acid, such as propionic or butyric acid, proceed via both reaction paths, three-body association reaction and hydroxide ion transfer, nevertheless, the adduct ion is always the major product (see the attached manuscript – Appendix A for additional details).

The reactions of carboxylic acids with $O_2^{+\bullet}$ reagent ions are (with the exception of the reaction of formic acid, which has proven to be slower than previously anticipated) fast with no obvious trends within the ionic products. The most frequent reaction pathway is the non-dissociative charge transfer reaction, producing the parent radical cation $M^{+\bullet}$. The other common reactions pathway is also dissociative charge transfer resulting in several fragment ions.

Rate constants for the primary H_3O^+, NO^+ and $O_2^{+\bullet}$ reactions

The calculated rate constants (k_c) for H_3O^+ reactions and the rate constants (k) for the NO^+ and $O_2^{+\bullet}$ reactions are available in the relevant manuscript and are in compliance with the previously published values [292]. More accurate rate constants for the primary NO^+ and $O_2^{+\bullet}$ reactions of formic acid were obtained in our experiment, where we compared the product ion count rates in the H_3O^+, NO^+ and $O_2^{+\bullet}$ reactions. We have found, that both reactions were slower than the previously published upper limits of $0.1x10^{-9}cm^3s^{-1}$ and $2x10^{-9}cm^3s^{-1}$ respectively [292]. The rate constant for reaction with H_3O^+ reagent ion was thus estimated as $0.05x10^{-9}$ cm^3 s^{-1} and the rate constant for reaction with $O_2^{+\bullet}$ reagent ion as $0.4x10^{-9}$ cm^3 s^{-1}.

2. IBD possible biomarkers

Product branching ratios for the primary H_3O^+, NO^+ and $O_2^{+\bullet}$ reactions

Same as in the case of carboxylic acids, primary product ions and the corresponding branching ratios observed in the experiments are summarized in the form of a table, which is available in the relevant manuscript (Appendix B).

The major ion product of the H_3O^+ reactions with most of the investigated IBD possible biomarkers is again the protonated molecule, MH^+, whereas minor channels involving fragmentation are also evident. Nevertheless, in some cases the protonated molecule has not been observed at all.

The first exception is the reaction of 2-methylpentane, where two parallel reaction channels are observed: the three-body association, that is possible because the proton transfer reaction is endothermic [293] and the channel that corresponds to a loss of a H_2 from the protonated molecule):

$$H_3O^+ + CH_3CH_2CH_2CH(CH_3)_2 \rightarrow CH_3CH_2CH_2CH(CH_3)_2H^+H_2O \quad (11a)$$

$$\rightarrow CH_3CH_2CH_2CHCH_3CH_2^+ + H_2 + H_2O \quad (11b)$$

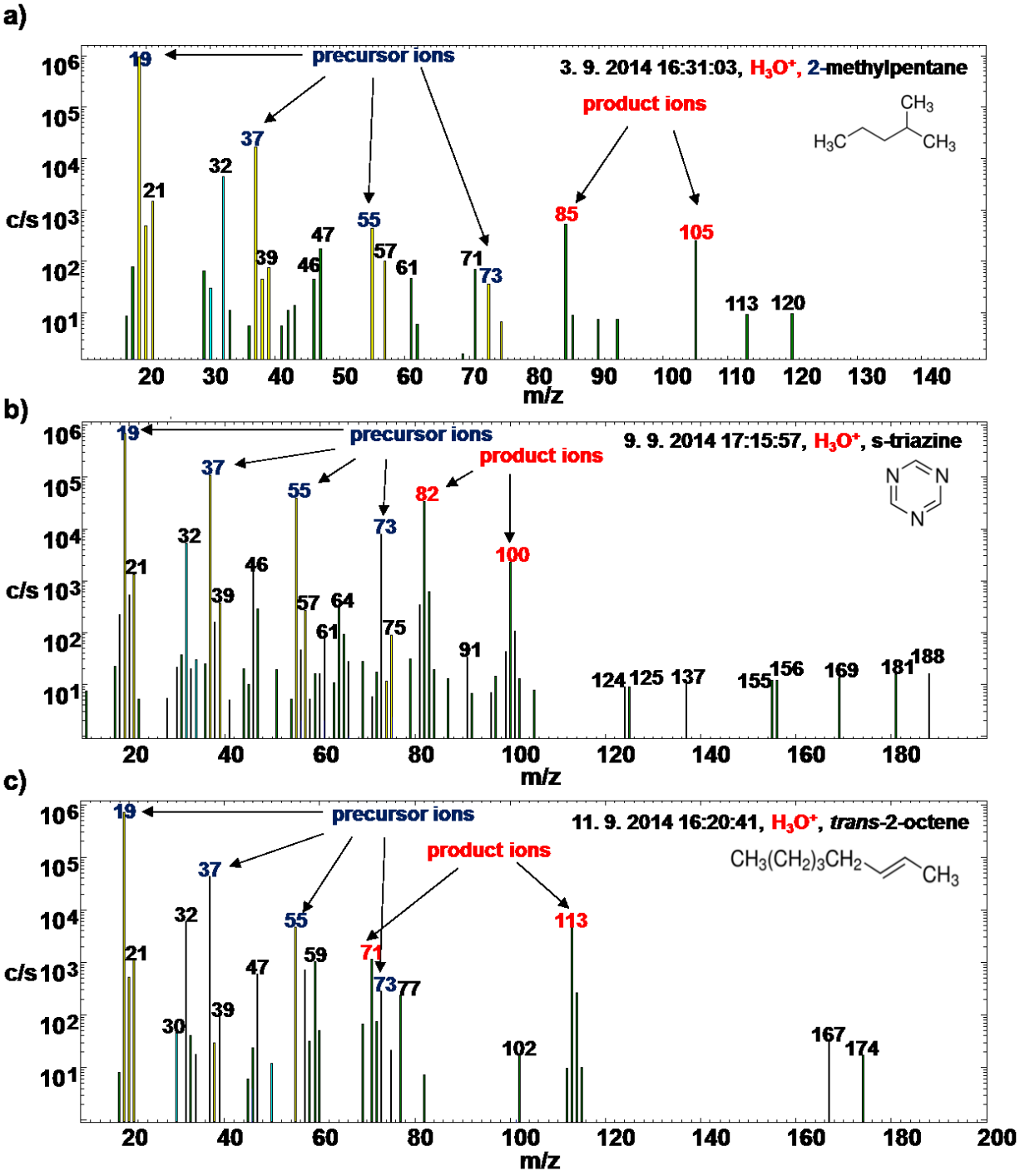
The second reaction channel described by the *Equation 11b* is not a reaction commonly observed in SIFT-MS studies. Based on this fact, reaction enthalpy has been calculated to determine if the reaction is exothermic. Using the data from [294], it was finally confirmed, that the product is energetically possible, as the reaction enthalpy, $\Delta_r H_T^{\emptyset}$, is -66 kJ/mol.

The second exception is than reaction of ***cis*-2-nonen-1-ol**, where the reaction proceed only via dissociative proton transfer – the reaction channel common for larger alcohols, resulting in production of the hydrocarbon fragment ions, $M\text{-}H_2O^+$.

$$H_3O^+ + CH_3(CH_2)_5CH{=}CHCH_2OH \rightarrow CH_3(CH_2)_5CH{=}CHCH_2^+ + 2\,H_2O \quad (12)$$

In the terms of primary product attractiveness, 1,3,5-triazine is very interesting compound, as the protonated parent molecule has even mass, which is not very common and can be easily recognized on SIFT-MS spectra as a signature for nitrogen containing compounds. The individual mass spectra of IBD possible biomarkers are available in *Figures 4.8 a-e*.

In case of some investigated IBD possible biomarkers, formation of the hydrated protonated molecules resulting from the secondary reactions with water present in the reactant mixture has been observed. For additional details see the next chapter (*4.2 Three-body association reactions*).



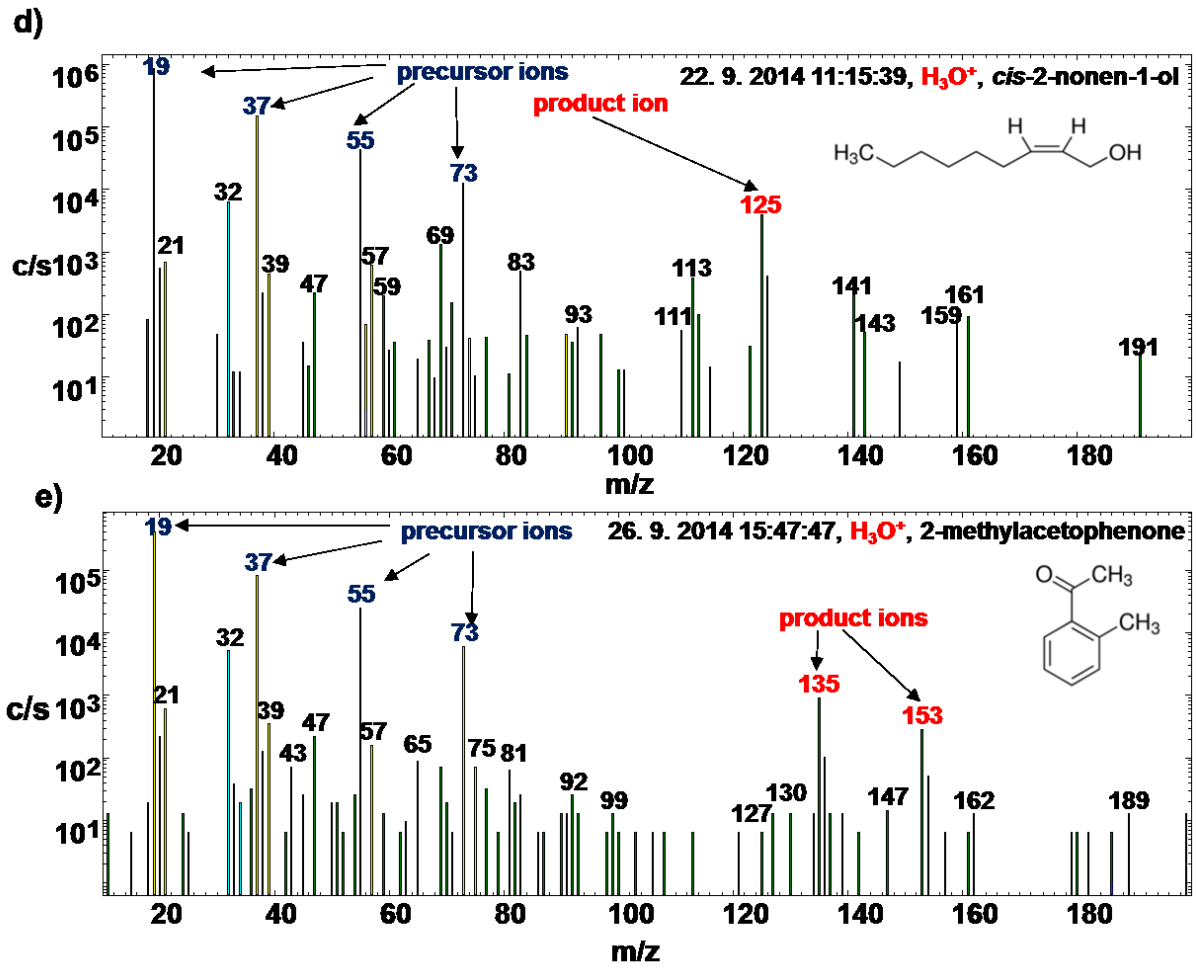
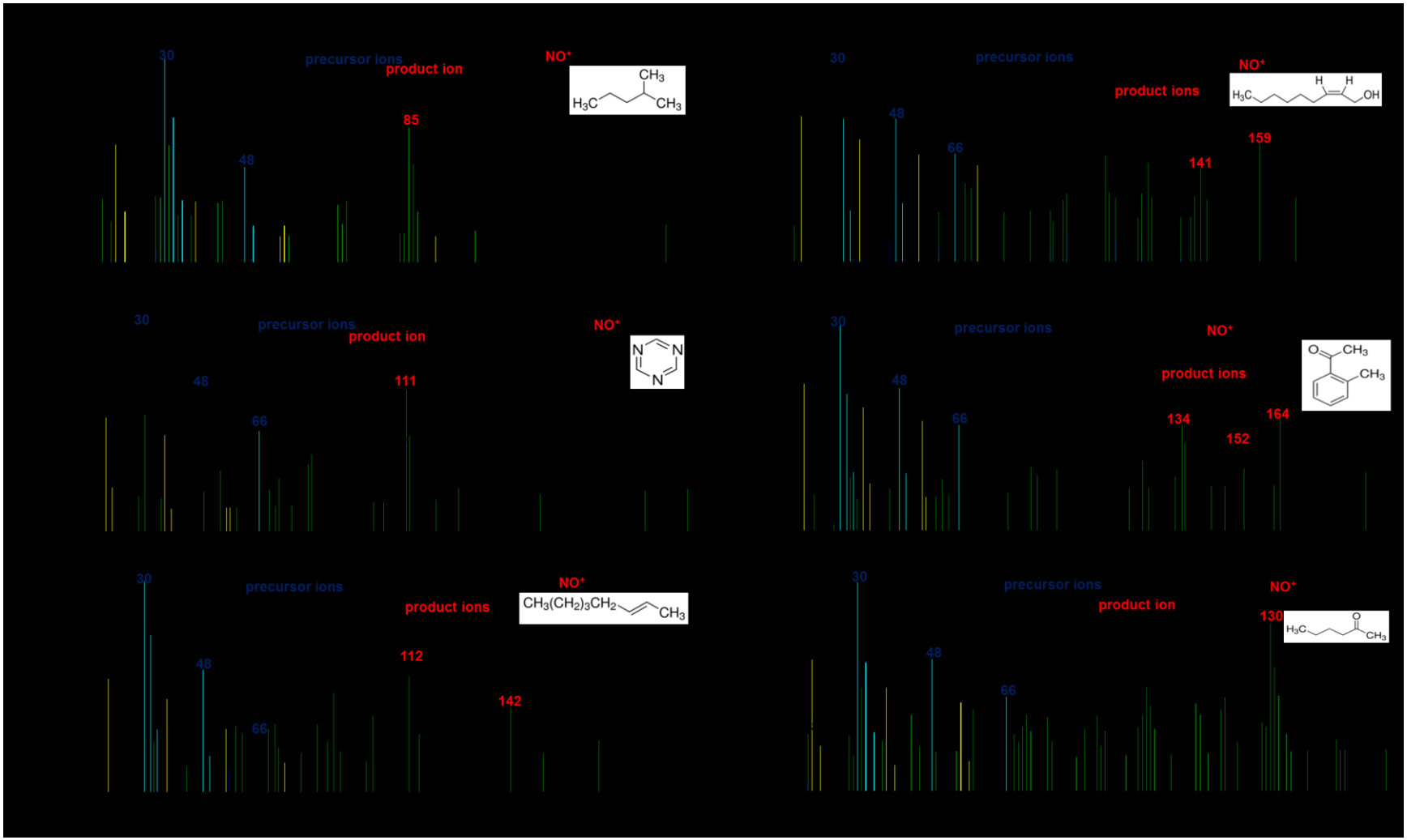


Figure 4.8 a)-e). The SIFT-MS spectra obtained when the headspace above a) 2-methylpentane, b) *s*-triazine, c) *trans*-2-octene, d) *cis*-2-nonen-1-ol and e) 2-methylacetophenone has been sampled. The arrows indicate the ions resulting from the reactions of the H_3O^+ reagent ion. The SIFT-MS spectrum for 1-heptene is not included, as it was presented in the introduction of this chapter (see above). The SIFT-MS spectrum is not included, as it was presented in earlier studies.

The ion products of the NO^+ reactions with some IBD possible biomarkers are shown in the mass spectra in *Figures 4.9 a-f*. The reactions of NO^+ with the IBD possible biomarkers proceed via three-body association, forming MNO^+ adduct ions, via hydride ion, H^-, transfer, or via charge transfer producing nascent M^+ ions. The hydration of the primary products by the association reactions with H_2O present in the reactant mixture is again quite common and is described in detail in the chapter below (*4.2 Three-body association reactions*).



4.9 a)-f). The SIFT-MS spectra obtained when the headspace above a) 2-methylpentane, b) *s*-triazine, c) *trans*-2-octene, d) *cis*-2-nonen-1-ol, e) 2-methylacetophenone and 2-hexanone has been sampled. The arrows indicate the ions resulting from the reactions of the NO^+ reagent ion. The SIFT-MS spectrum for 1-heptene is not included, as it was presented in the introduction of this chapter (see above)

Majority of the reactions of IBD potential biomarkers with $O_2^{+\bullet}$ reagent ions proceed via charge transfer producing the parent radical cation $M^{+\bullet}$. Another common reaction pathway is the dissociative charge transfer resulting in several fragment ions, which are very often radicals. The spectra obtained using the $O_2^{+\bullet}$ are in comparison to those gathered by using H_3O^+ or NO^+ more complicated (see *Figure 4.10 a-c*). The observed fragment are also formed by electron ionisation which proceeds via a similar mechanism involving transient molecular radical cation.

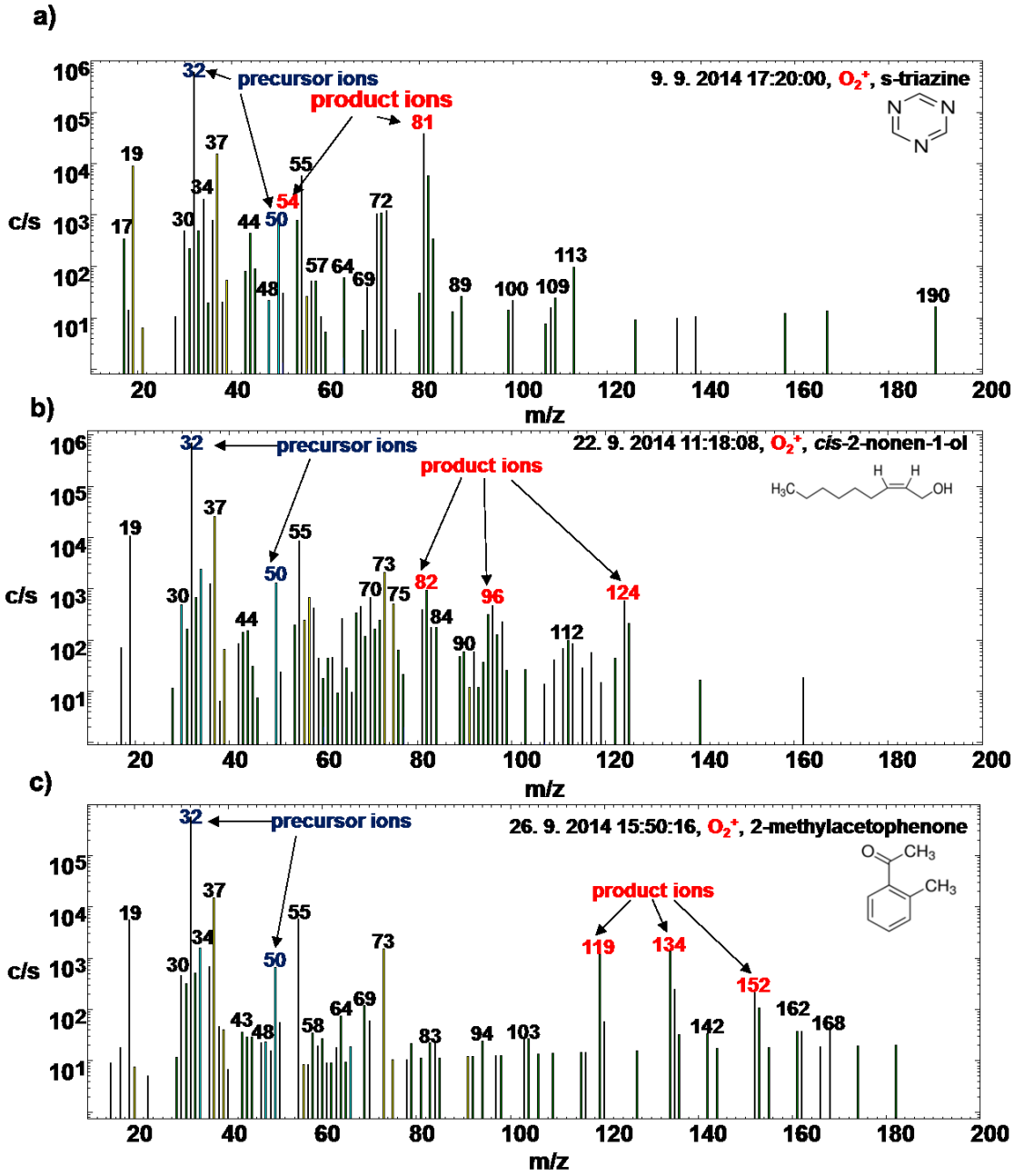


Figure 4.10 a)-c). The SIFT-MS spectra obtained when the headspace above a) *s*-triazine, b) *cis*-2-nonen-1-ol and c) 2-methylacetophenone has been sampled. The arrows indicate the ions resulting from the reactions of the O_2^+ reagent ion.

For additional details regarding the product branching ratios for the primary H_3O^+, NO^+ and $O_2^{+\bullet}$ reactions see the relevant manuscript *(*Appendix B*).*

Rate constants for the primary H_3O^+, NO^+ and $O_2^{+\bullet}$ reactions

The calculated collisional rate constants (k_c) and the experimentally derived rate constants (k) are available in the tabulated form in the relevant manuscript (Appendix B). In general, all the H_3O^+ reactions are assumed to proceed at the collisional rate for all the investigated molecules with only one exception: 2-methylpentane. This compound does not have a sufficiently high proton affinity and therefore it reacts at faster rate with both, NO^+ and $O_2^{+\bullet}$ reagent ions. Such a slow reactions have been previously observed also for other saturated hydrocarbons, including hexane and octane [295].

Regarding the NO^+ and $O_2^{+\bullet}$ reactions with IBD possible biomarkers, most of them also proceed near to collisional rate. The exception is in this case cis-2-nonen-1-ol for which only very slow reactions are observed.

Conclusions

The ion chemistry studies discussed in this section provided some new data for the kinetics of the reactions important for SIFT-MS quantification of biogenic volatile organic compounds in air, and these data can be included in the kinetics library used for automated immediate quantification. However, in case of SIFT-MS, we have to take into consideration many minor challenges, which can influence the gathered results. Although we are talking about the effect of possible overlaps of product ions from different compounds at the same *m/z*, or impurities of the utilised sampling containers. All these possible issues are discussed in the following chapters, *Section 4.3 Construction and optimization of kinetics library* and *Section 4.5 Challenges in connection with breath analysis - Nalophan sampling bags.*

4.2 Three-body association reactions – ion chemistry involving water molecules

Objectives

In the case of breath analysis, we are usually working with samples influenced by the presence of water vapour. Therefore it is necessary to take into consideration also the secondary association reactions of primary product ions with water molecules inevitably present in biological samples. The understanding of these reactions is necessary for comprehensive and full understanding of the ion chemistry, to be used for accurate quantification by SIFT-MS.

The three-body associations were again the subject of the research articles concerning carboxylic acids, Appendix A [280] and the possible IBD biomarkers, Appendix B [165]. In the case of article focused on carboxylic acids, the connection with secondary association reactions is noticeable already from the title of this study and the main topic of this work were predominantly the secondary association reactions of the primary product ions with water molecules. In case of article focused on the IBD possible biomarkers, the secondary association reactions were again investigated in order to achieve overall knowledge of the ion chemistry.

Experimental methods

The three-body association rate constants were determined for protonated molecules formed in the H_3O^+ reactions with the molecules M and for products formed in the NO^+ primary reactions.

Reactions of protonated molecules MH^+ with H_2O

As already partly outlined in the theoretical part of this thesis, in the presence of water the association reactions of proton transfer product ions MH^+ with H_2O are common, forming $MH^+(H_2O)_{1,2,3}$. Moreover, the H_3O^+ reagent ions are in part converted to hydrated hydronium ions H_3O^+ $(H_2O)_{1,2,3}$ and these cluster ions can act as additional reagent ions and produce further ion products like $MH^+(H_2O)_{1,2,3}$ via ligand switching reactions. In SIFT-MS measurements using the H_3O^+ reagent ions both, MH^+ and its hydrates $MH^+(H_2O)_{1,2,3}$ are usually formed and the ratio of the count rates of these ions is a function of the sample flow rate and the sample humidity. The efficiency of clustering as

a fundamental parameter related to the three body rate constants can be finally determined by recording the above mentioned relative count rates.

Three-body rate constants for the association reactions of MH^+ ions with H_2O molecules in helium, k_{MH+} describe the rate of hydration of the protonated molecules in the gas phase:

$$MH^+ + H_2O + He \rightarrow MH^+(H_2O) + He \quad (13)$$

In order to obtain these rate constants experimentally it is important to account for the contribution of the ligand switching reactions of the $H_3O^+(H_2O)$ ions formed in the following three-body reaction described by the rate constant k_{H_3O+}:

$$H_3O^+ + H_2O + He \rightarrow H_3O^+(H_2O) + He \quad (14)$$

The three-body rate constant for reaction (14) has been previously determined to be $k_{H_3O+} = 6 \times 10^{-28}$ cm^6 s^{-1} [261]. The cluster ions formed in reaction (14) contribute to the formation of $MH^+(H_2O)$ ions via ligand switching with M:

$$H_3O^+(H_2O) + M \rightarrow MH^+(H_2O) + H_2O \quad (15)$$

The hydrated protonated molecules $MH^+(H_2O)$ can thus be produced by two parallel routes reactions (13) and (15). The individual contribution of reaction (15) can be quantified by a parameter S_{eff} representing the contribution of the switching reactions to their formation taken relatively to the total formation of hydrated hydronium ions:

$$S_{eff} = \frac{\ln(\left([MH^+] + [MH^+(H_2O)_{1,2,3}]_s\right)/[MH^+])}{ln([H_3O^+]_0/[H_3O^+])} \quad (16)$$

Here, $[MH^+]$ and $[H_3O^+]$ are the count rates of MH^+ and H_3O^+ ions detected by the downstream mass spectrometer, assumed to be proportional to the concentrations of the ions at the end of the flow tube. $[H_3O^+]_0$ is the count rate observed in the absence of water vapour, which is also equivalent to the sum of count rates of $H_3O^+(H_2O)_{0,1,2,3}$. The subscript *s* denotes the theoretical count rates of the $MH^+(H_2O)_{1,2,3}$ ions formed in switching reactions only, as calculated from the linearised kinetic equation [261]:

$$\left[MH^+(H_2O)_{1,2,3}\right]_s = [M]t\left([37]\frac{k_{37}}{2} + [55]\frac{k_{55}}{2} + [73]\frac{k_{73}}{2}\right) \quad (17)$$

where t is reaction time, k_{37}, k_{55} and k_{73} are the rate constants for the switching reactions of the respective hydrated hydronium ions with M and *[37]*, *[55]*, and *[73]* are the actual count rates of the $H_3O^+(H_2O)_{1,2,3}$ ions as observed in the experiments. The divisors 2 in Eq. (17) describe the fact that due to their continuous formation in the flow tube [261], reaction times of the $H_3O^+(H_2O)_{1,2,3}$ ions are approximately half the reaction time of H_3O^+. The total contribution of both parallel routes of formation of the protonated hydrated ions via the association reaction (13) and via the switching reaction (15) can be described by a coefficient A_{eff}:

$$A_{eff} = \frac{2\ln(\left([MH^+] + \left[MH^+(H_2O)_{1,2,3}\right]_A\right)/([MH^+]))}{ln([H_3O^+]_0/[H_3O^+])} \quad (18)$$

The factor 2 in Eq. (18) is again related to the mean reaction time available for the continuously formed MH^+ ions, which is half of that available for the injected H_3O^+ ions. The net contribution of association without switching is then simply described as $A_{eff} - S_{eff}$ and the value of three-body association rate constant can be calculated as:

$$k_{MH^+} = \left(A_{eff} - S_{eff}\right) \cdot k_{H_3O^+} \quad (19)$$

k_{MH^+} obtained from Eq. (19) is thus a fundamental three-body association rate constant anchored to the known value of $k_{H_3O^+}$.

Reactions of the primary products of NO^+ reactions with H_2O

When using NO^+ reagent ions to analyse humid samples, a fraction of product ions also associate with H_2O molecules forming hydrated ions [75,246]. Ion products of the kind $[M+NO]^+$ can be also formed via ligand switching reactions of $NO^+(H_2O)_{1,2}$ [296]. The primary reactions of NO^+ reagent ions with carboxyl acids and group of possible IBD biomarkers, M, result in the formation of various types of product ions (for example $[M+NO]^+$, $[M-OH]^+$ or [M-H]+ ions). In the presence of water molecules some of these

product ions associate with H_2O molecules to form the adduct ions. Three-body rate constants for the association reactions of these ions with H_2O molecules in helium, k_{A^+}, describe the rate of hydration of these product ions in the gas phase. The process of formation of relevant hydrates can be described by a coefficient A_{eff} in analogy to the chemistry of the protonated molecules. A_{eff} that describes how much faster clustering of a given ion is compared to clustering of H_3O^+, can be obtained from experimental data by relating the conversion rate of primary product to their hydrates to the conversion rate of NO^+ to its hydrates:

$$A_{eff} = \frac{2\ln([A^+]_0/[A^+])}{ln([H_3O^+]_0/[H_3O^+])}$$

$$= \frac{2\ln([A^+]_0/[A^+])}{\left(k_{H_3O^+}/k_{NO^+}\right)k_{H_3O^+}ln([NO^+]_0/[NO^+])} \quad (20)$$

The ratio $\left(k_{H_3O^+}/k_{NO^+}\right)$ is used here to relate the association reaction of H_3O^+ with H_2O (rate constant, $k_{H_3O^+}$) to the significantly slower association reaction of NO^+ with H_2O (rate constant, k_{NO^+}). The subscript 0 again indicates the respective ion count rate in the absence of water vapour in the helium carrier gas. The value of the three-body association rate constant can be calculated as:

$$k_{A^+} = A_{eff}\ \frac{k_{H_3O^+}}{k_{NO^+}}k_{NO^+} = A_{eff}\ \cdot k_{H_3O^+} \quad (21)$$

k_{A^+} obtained from Eq. (21) is again a fundamental three-body association rate constant anchored to the known values of $k_{H_3O^+}$(6.0×10^{-28} cm^6 s^{-1}) [261,297] and k_{NO^+} (5.0×10^{-29} cm^6 s^{-1}) determined experimentally.

In order to investigate the secondary reactions, sample mixture with dry air and varying amounts of water has been tested for all the compounds of interest by using the Nalophan sampling bags. The humidity change has been achieved by two different ways in two separate experiments. In the first one, presence of water in the sample varied from the low humidity (approximately 0.5%), achieved by the mixture of dry air and investigated sample, over the standard laboratory value (usually 1.2%, mixture of laboratory air and investigated sample), to the concentration common for the exhaled breath (5-9%, mixture of exhaled breath and investigated sample). In the following

experiment, the humidity of the sample has been affected by the temperature change – a parameter having direct influence on the presence of water vapour in the sample. The humidity within this experiment moved around 5.5% at 37°C to 0.8% at 0°C. For both these investigations, the multi-ion monitoring (MIM) mode [47] has been utilized.

Results and Discussion

This Section presents results, which were included in the manuscripts in Appendices A and B. However, the additional details and data gathered in the scope of experiments conducted for the relevant topics were added.

1. Carboxylic acids

Formation of the hydrated protonated molecules resulting from the secondary reactions with water has been observed for all the carboxylic acids. For the entire investigated series are under experiment conditions characteristic not only primary, but also secondary hydrates. The rate constants of the three-body association reactions calculated using *Equations (16) - (19)* are given in the *Table 4.5*. The source data for these calculations have been gathered by utilisation of MIM mode.

Table 4.5. Kinetic data for formation of hydrates of primary products of NO^+ and H_3O^+ reactions with carboxylic acids.

Compound	Formula	Kinetic data for formation of hydrates of primary products of H_3O^+ reactions				Kinetic data for formation of hydrates of primary products of NO^+ reactions		
		Reaction	S_{eff}[a]	A_{eff}[b]	k_{MH^+}[c]	Reaction	A_{eff}[d]	k_{A^+}[e]
Formic acid	HCOOH	$[MH]^+ + H_2O + He$	0.43	2.76	1.40	$[M+NO]^+ + H_2O + He$	2.88	1.73
Acetic Acid	CH_3COOH	$[MH]^+ + H_2O + He$	0.43	4.50	2.44	$[M+NO]^+ + H_2O + He$	1.83	1.10
Propionic Acid	C_2H_5COOH	$[MH]^+ + H_2O + He$	0.42	6.81	3.83	$[M+NO]^+ + H_2O + He$	0.17	0.15
						$[M-OH]^+ + H_2O + He$	3.41	2.05
Butyric Acid	C_3H_7COOH	$[MH]^+ + H_2O + He$	0.41	8.64	4.93	$[M+NO]^+ + H_2O + He$	1.13	0.68

[a] Ligand switching efficiencies calculated from Eq. (16).
[b]Association efficiencies obtained from experimental data using Eq. (18).
[c]Three-body association rate constants calculated from Eq. (19). [10^{-28} cm^6 s^{-1}]
[d]Association efficiencies obtained from experimental data using Eq. (20).
[e]Three-body association rate constants calculated from Eq. (21). [$10^{-27}cm^6s^{-1}$]

The example of the time profile obtained using SIFT-MS in the MIM mode, illustrating the humidity change as a function of temperature, is available below (*Figure 4.11*). Already from this picture it is clear, that efficacy of the secondary hydrates formation is not negligible for propionic acid. This fact can be confirmed also by taking into account the three-body association rate constants for this compound, listed in the *Table 4.5*. For the purpose of this experiment, the Nalophan bag with the sample has been placed to the thermostat and the temperature has gradually decreased from 37°C to 0°C. As can be seen from gathered time profile, the ratio of the count rates of primary product ion MH^+ and its hydrates is seriously involved by the temperature and humidity, for every ion then by different way. Even though, the count rate of MH^+ is growing with the decreasing temperature and thus also decreasing humidity, the count rate of $MH^+(H_2O)_2$, secondary hydrate derived from protonated molecule, is dropping. The proportion of primary hydrate, $MH^+(H_2O)$, is (as a function of decreasing temperature and humidity) growing same as MH^+. Nevertheless, the rate of this change is divergent and at different temperatures can be dominant various products. That is the reason, why must be hydrates and secondary reactions at all always taken into consideration.

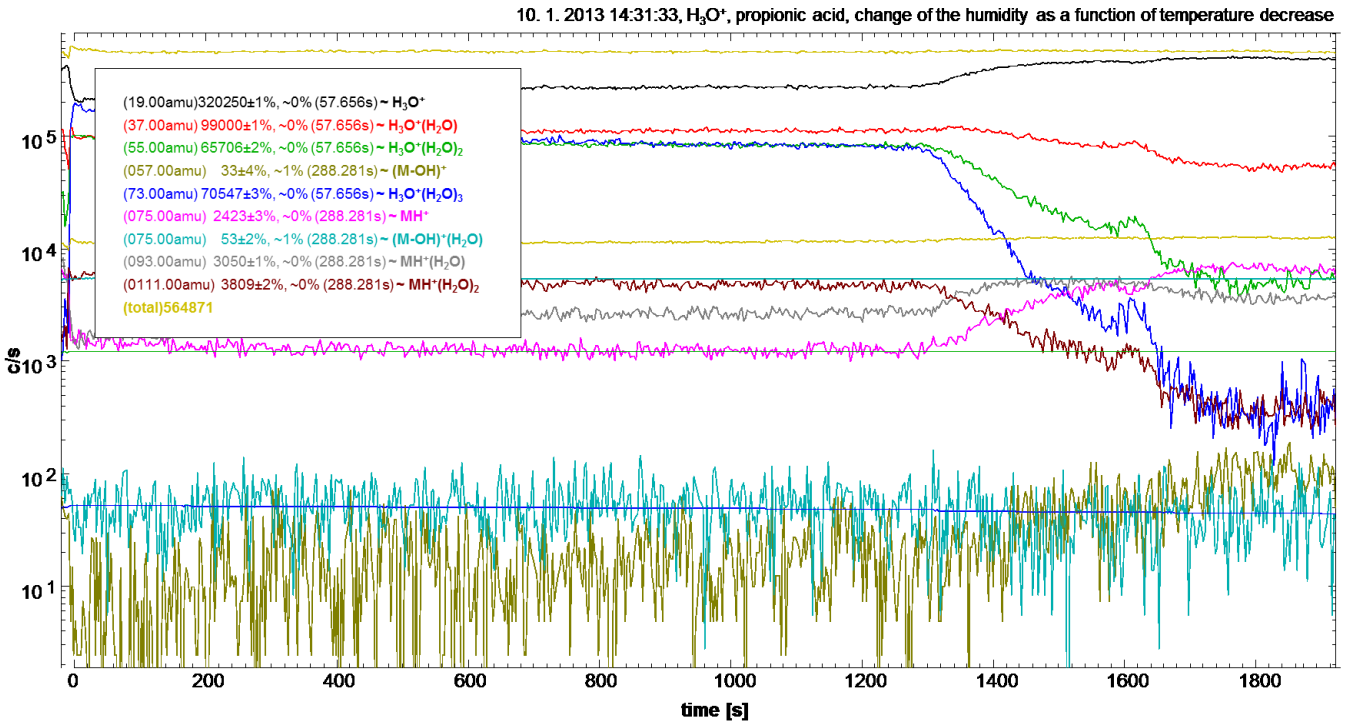


Figure 4.11. The time profile of the humidity drop based on temperature change – propionic acid.

Next figure (*Figure 4.12*) is then showing the graphical overview of individual products count rate dependence on the water concentration for acetic acid. The similar trend as in case of MIM time profile for Propionic Acid (*Figure 4.11*) is noticeable.

In general, the speed of water clusters formation for protonated molecules is relatively slow in the case of lower carboxylic acids, but grows with the number of carbons. This can be due to the number of degrees of freedom in the reaction intermediate resulting into its longer life time. Similar trend was observed previously for the reactions of protonated ketones [298].

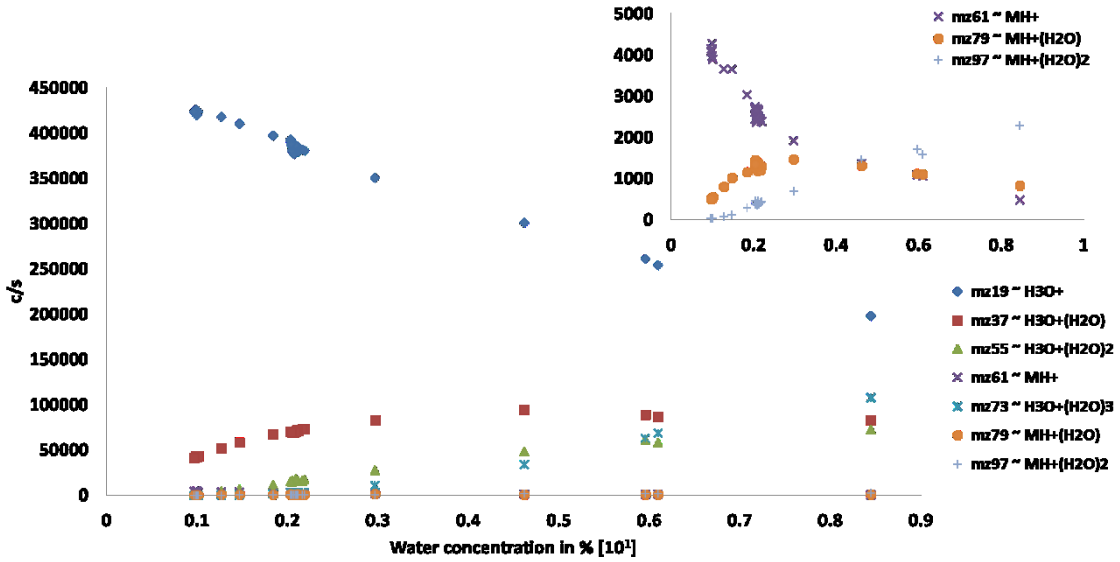


Figure 4.12. A plot of count rates of individual ions on water concentration – acetic acid.

Hydration of the primary products of NO^+ reactions with carboxylic acids was again observed and in general, only the monohydrates are created $[M+NO]^+H_2O$ and $[M-OH]^+H_2O$. The values of the three-body rate constants k_{M+NO^+}/ k_{M-OH^+} calculated using *Equations (20) and (21)* are given in the *Table 4.5*. The most efficient formation of the water clusters of the $[M+NO]^+$ product ions has been observed for the dehydroxidated propionic acid, (see the *Table 4.5*). Nevertheless, no trend was apparent in the variation of $k_{M+NO^+}$with regard to the change of the carbon atoms number.

2. Possible IBD biomarkers

In case of possible IBD biomarkers, the formation of the hydrated protonated molecules has been observed only for three compounds: 1,3,5-triazine, 2´-

methylacetophenone and 2-hexanone, whereas the three body association rate constant has a higher value only in case of 2-hexanone. In other cases we observed relatively slow association reactions. (see the *Table 4.6*)

The hydration of the NO^+ primary products by the association reactions with H_2O present in the reactant mixture was observed in case of *cis*-2-nonen-1-ol, 2-methylacetophenone and heptene. In general, only the monohydrates have been observed and very interesting in this study was 1-heptene, where the secondary products were created in case of all primary products detected for this compound of interest. As can be seen from the *Table 4.6*, the most effective formation of secondary products has been observed for *cis*-2-nonen-1-ol.

As can be seen from the *Table 4.6*, from the group of investigated IBD possible biomarkers, only 2´-methylacetophenone is forming the secondary products for both reagent ions, H_3O^+ and NO^+.

Conclusions

The three-body association reactions studies discussed in this section provided some new data for the kinetics of the reactions important for SIFT-MS quantification of carboxylic acids and IBD possible biomarkers in humid air/breath respectively. These data have been included in the kinetics library used for automated immediate quantification and will help in the future studies, where these compounds can be in the area of interest.

Table 4.6. Kinetic data for formation of hydrates of primary products of the NO^+ and H_3O^+ reactions with possible IBD biomarkers.

Compound	Linear Formula	Kinetic data for formation of hydrates of primary products of H_3O^+ reactions				Kinetic data for formation of hydrates of primary products of NO^+ reactions		
		Reaction	S_{eff}[a]	A_{eff}[b]	k_{MH^+}[c] [10^{-28} cm^6 s^{-1}]	Reaction	A_{eff}[d]	k_{A^+}[e] [10^{-28} cm^6 s^{-1}]
1,3,5-triazine		$[MH]^+ + H_2O + He$	0.41	1.62	0.73			
cis-2-nonen-1-ol	$CH_3(CH_2)_5CH=CHCH_2OH$					$[M-H]^+ + H_2O + He$	11.58	6.95
2-methylacetophenone	$CH_3C_6H_4COCH_3$	$[MH]^+ + H_2O + He$	0.41	2.29	1.13	$[M]^+ + H_2O + He$	0.08	0.05
1-heptene	$CH_3(CH_2)_4CH=CH_2$					$[M-(CH_2)_4+NO]^+ + H_2O + He$	1.43	0.86
						$[M+NO]^+ + H_2O + He$	1.05	0.63
						$[M-(CH_2)_2+NO]^+ + H_2O + He$	1.34	0.81
2-hexanone	$CH_3(CH_2)_3COCH_3$	$[MH]^+ + H_2O + He$	0.39	11.28	6.53			

[a] Ligand switching efficiencies calculated from Eq. (16).
[b]Association efficiencies obtained from experimental data using Eq. (18).
[c]Three-body association rate constants calculated from Eq. (19). [10^{-28} cm^6 s^{-1}]
[d]Association efficiencies obtained from experimental data using Eq. (20).
[e]Three-body association rate constants calculated from Eq. (21). [10^{-27}cm^{6}s^{-1}]

4.3 Construction and optimization of kinetics library

Objectives

Analysis of VOCs via SIFT-MS is not always absolutely easy. One of the today's major challenges (despite the development of this method resulted in improvements in sensitivity, accuracy and precision of quantification of known compounds present in the sample) is represented by the overlapping ion peaks in the mass spectra when isomeric, other isobaric compounds or compounds from the same chemical class are present in the mixture to be analysed (see the comparison of mass spectra for 2 isobaric compounds – ethanol and formic acid – *Figure 4.13*). In general, this method is still not as reliable for identification of unknown compounds in complex mixtures as GC-MS. However, when we consider the availability of three reagent ions, we have still (in most of the cases) the opportunity to distinguish between investigated compounds of the same molecular weight - by virtue of the different reaction processes that are for the investigated compounds characteristic [47].

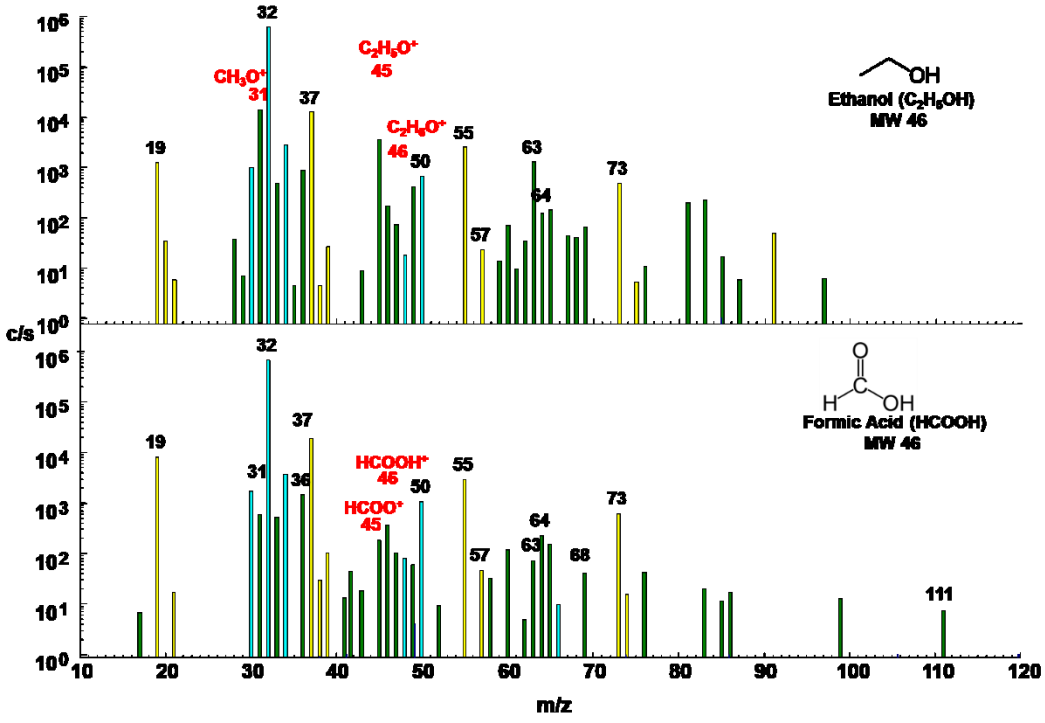


Figure 4.13. The SIFT-MS spectra obtained using the $O_2^{+\bullet}$ precursor ions when ethanol or formic acid is introduced into the helium carrier gas; ion count rates are given on semilogarithmic scale as a function of *m/z*. Note the characteristic ion products at *m/z* 45 and 46 for both products and the characteristic ion product for ethanol at *m/z* 31.

Methods

In case of SIFT-MS analysis, the determination of absolute concentrations of compounds in real time is based on obtaining the ratio of the sum of several product ion count rates to the sum of the reagent ion count rates. These values are than weighted by the reaction rate constants and additional coefficients can be also applied to account for special circumstances, such as overlaps (contributions of the different compounds with characteristic ions at the same *m/z* value). All the calculations are conducted by the SIFT-MS analytic software on-line, taking the rate constants and other coefficients from the kinetic library [259], whereas the construction of entries for the kinetic library is one of the key steps for any project. The kinetic data as the entries for kinetic library must be for the purpose of SIFT-MS analytic software in the special format. An example of kinetic data in such format is available in the *Table 4.7*. It is the kinetic library, standardly used for analysis of the carboxylic acids by using of the H_3O^+ reagent ion.

Table 4.7 SIFT-MS kinetics library in the format required by the SIFT-MS software for on-line calculations of the concentrations of the carboxylic acids.

Formic Acid (H_3O^+)	Acetic Acid (H_3O^+)	Propionic Acid (H_3O^+)	Butyric Acid (H_3O^+)
4 precursors	4 precursors	4 precursors	4 precursors
19 2.2e-9 1.0	19 2.6e-9 1.0	19 2.7e-9 1.0	19 2.9e-9 1.0
37 1.9e-9 1.0	37 1.9e-9 1.0	37 1.9e-9 1.0	37 1.9e-9 1.0
55 1.9e-9 1.0	55 1.9e-9 1.0	55 1.9e-9 1.0	55 1.9e-9 1.0
73 1.8e-9 1.0	73 1.8e-9 1.0	73 1.8e-9 1.0	73 1.8e-9 1.0
2 products	3 products	4 products	3 products
47 -0.028	61 1.0	57 1.0	89 1.0
65 0.51	79 1.0	75 1.0	107 1.0
83 0.51	97 1.0	93 1.0	125 1.0
		111 1.0	

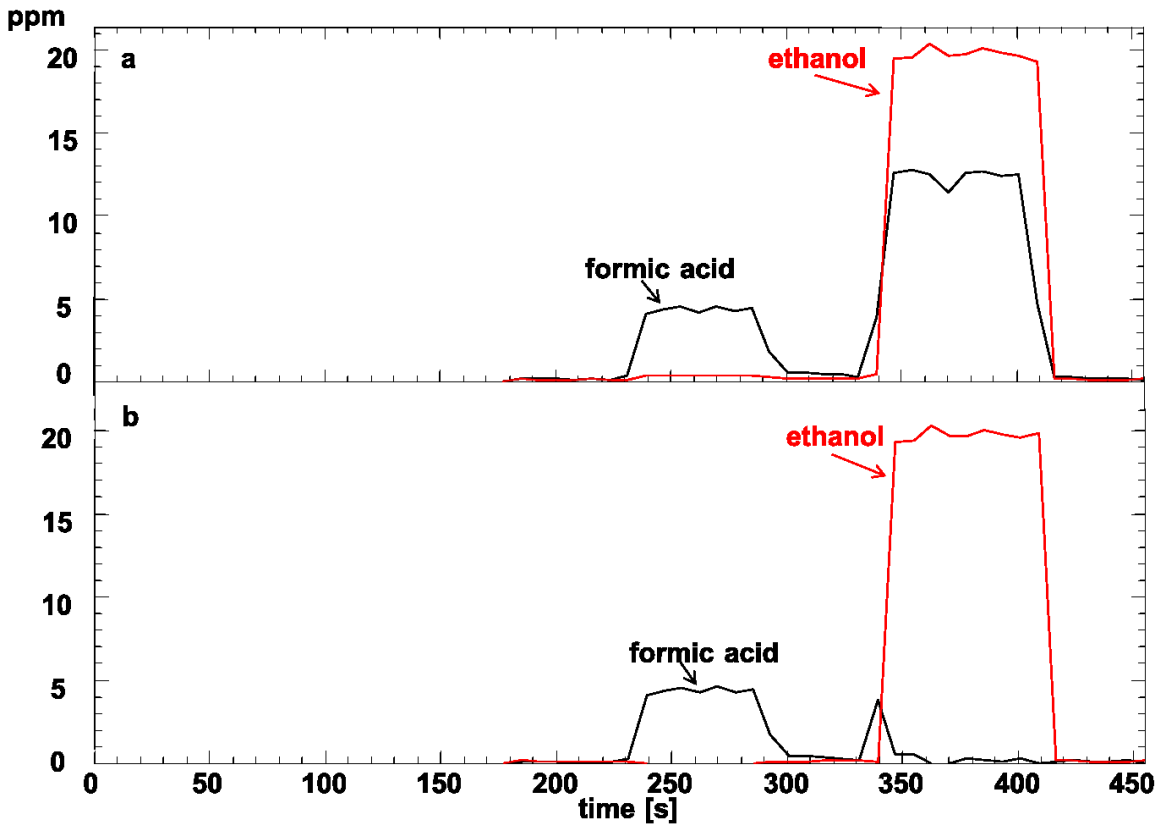
In our experiments, we have optimised the kinetic library for easy distinguishing of the formic acid from the ethanol by utilisation of the NO^+ and $O_2^{\bullet+}$ reagent ion (Appendix A). We have also used the updated kinetic library entries in case of alkenes (Appendix C). The construction of more advanced entries accounting for overlaps of the product ions is described step by step in the following text.

Results and Discussion

This subsection summarize and complement the data concerning the carboxylic acids and alkenes, thus those published in impacted peer reviewed journals attached bellow - Appendix A [280] and Appendix C [169].

The fundamentals for any kinetic library construction and the following optimisation are the kinetic data of the reactions of H_3O^+, NO^+ and $O_2^{+\bullet}$ with compounds of interest. Such a data, which were discussed previously (see the sections *4.1 Determination of rate constants and product ion branching ratios* and *4.2 Three-body association reactions – ion chemistry involving water molecules*) can be used to construct an initial simple version of the kinetics library entries as given in *Table 4.7*. For example, for acetic acid, the following details are given according to the required format:

- appropriate reagent ion (H_3O^+), which means listing all four *m/z* values including the H_3O^+ hydrates (19-37-55-73) together with the appropriate rate constant obtained theoretically or derived experimentally;
- the f_p coefficient at the default value of 1.0 (values other than 1.0 can be used to multiply the corresponding ion count rates in order to optimize kinetics entries, as it will be shown below);



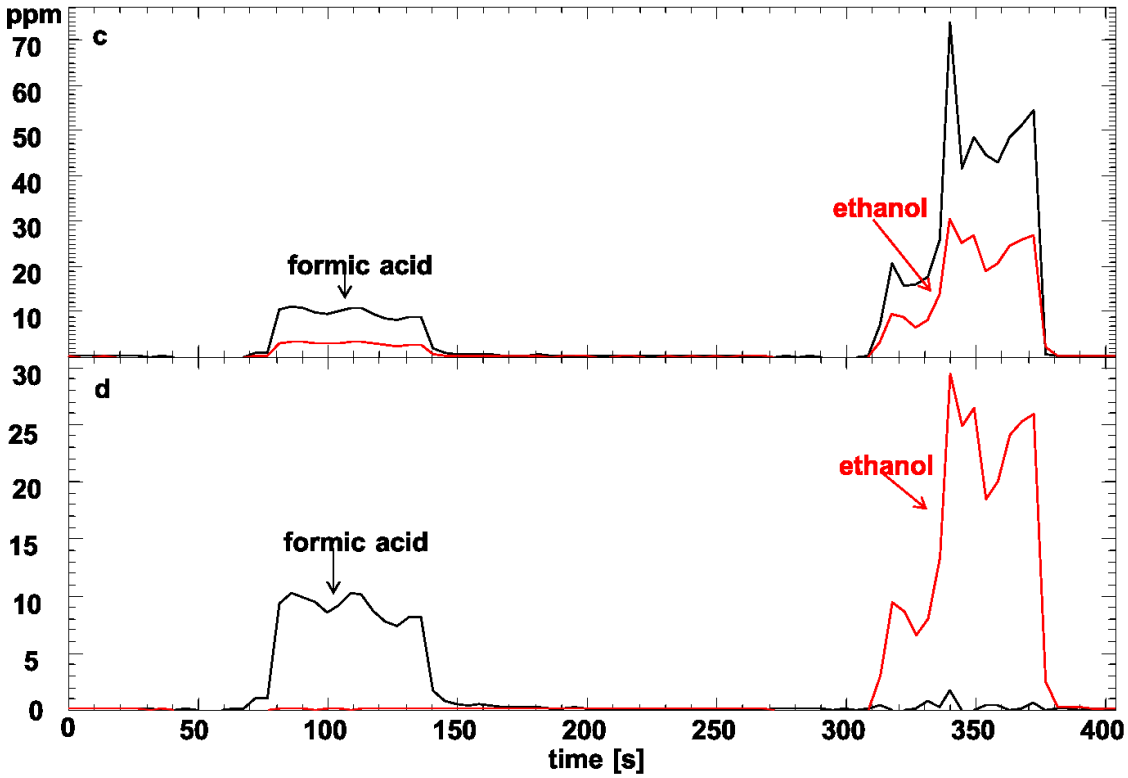


Figure 4.14. (a) Time profile of the concentration of formic acid and ethanol calculated (in ppmv) simply from the sum of the characteristic product ions in a validation experiment when diluted samples of reference compounds are introduced sequentially for NO^+ precursor and *m/z* 45 and 76 product ions. Plot (b) shows the same raw experimental data evaluated using the optimised kinetic library; note that cross sensitivity has been dramatically reduced. Plots (c and d) shows the similar data, only by using $O_2^{+\bullet}$ reagent ions and *m/z* 45 and 46 product ions for formic acid and *m/z* 31, 45 and 46 product ions for ethanol.

- the number of product ions (*m/z* 61, 79, 97) formed by primary and secondary ion-molecular reactions with each *m/z* value
- the default *fi* coefficient of 1.0.

The initial kinetic library entries compiled according to the instructions above must be subsequently validated for the effect of ion overlaps by analysing real samples. Usually, the reference mixtures (at approximately 1 ppmv concentration) of compounds of interest are analysed in the MIM mode by using the relevant reagent ions (NO^+ and $O_2^{+\bullet}$ in case of formic acid/ethanol study; H_3O^+ in case of alkenes study). Because the gathered time profiles indicated the cross sensitivity (*see Figure 4.14* as example of cross sensitivity between formic acid and ethanol), it was clear that the optimised kinetic library entries are necessary. The selection of the appropriate process in the course of cross sensitivity eradication may vary case to case. Therefore, the group of investigated compounds must be evaluated, same as signs of overlaps and their background.

In some cases can be the problem of *m/z* overlaps solved simply by exclusion of some minor product ions from the final library. This procedure is typical, when the compounds with increasing number of carbons from one chemical class are investigated. This solution has been (during my studies) used for example in case of project focused on alkenes investigation – see Appendix C).

In case of carboxylic acid study, where we tried to minimize the effect of product ion overlaps between ethanol and formic acid, the main idea in constructing kinetic library entries lied in finding the linear combinations of ion product count rates that would allow selective quantification of these compounds. It is important to highlight, that this solution requires addition of the ion count rates of the hydrated ions (in case when these are formed) to the ion count rates of the corresponding primary product ions. This must be conducted to achieve validity of the results for any concentration of water in the sample. Such linear combination can be found by the simple solution of a set of the linear equations [297] and the procedure has been used for kinetic library entries by using the NO^+ and $O_2^{+\bullet}$ reagent ions. The model *Equations 22-24* are related to measurements with utilisation of the NO^+ reagent ions:

$$F = f_F\ [45]_F + g_F\ [76]_F \qquad (a) \qquad (22)$$
$$E = f_E\ [45]_E + g_E\ [76]_E \qquad (b)$$

Here F represents the linear combination used to quantify formic acid and E represents the linear combination to quantify ethanol. The square brackets indicate the product ion count rates registered when the compound indicated by subscripts F (formic acid) and E (ethanol) is introduced into SIFT-MS. The constraints on the coefficients f and g (treated as the unknowns in the set of equations) are given by the requirement of calculating zero concentration of F when only E is introduced and oppositely:

$$0 = f_F\ [45]_E + g_F\ [76]_E \qquad (a) \qquad (23)$$
$$0 = f_E\ [45]_F + g_E\ [76]_F \qquad (b)$$

By using these equations, it is possible to calculate f and g coefficients (f_p factors used within the optimised kinetic library to multiply the acquired raw count rate of the ion, accounting so for the overlaps) from the experimental data of ion count rates using the pure compounds. The calculation procedure is described below.

First of all, factors f_E and f_F have been expressed from the *Equation 23 (a,b)*:

$$f_F = -g_F \frac{[76]_E}{[45]_E} \quad (a) \qquad (24)$$

$$f_E = -g_E \frac{[76]_F}{[45]_F} \quad (b)$$

Subsequently, these factors were substituted into *Equation 22 (a,b)*, where F and E represent the total count rate of both ions. Thanks to this, it is possible to express numerically the factors g_F and g_E, and finally also the factors f_F and f_E.

Similar experiments and calculations were used also to resolve *m/z* overlaps between formic acid and ethanol ($O_2^{+\bullet}$ reagent ions). All the results are available in the relevant manuscript (Appendix A).

Conclusions

The possible methods for optimised kinetic library construction have been discussed and described. Those are essential for accurate determination of absolute concentrations of compounds of interest. In addition, optimised kinetic data entries could be the solution for the ongoing SIFT-MS challenge - overlapping ion peaks in the mass spectra when isomeric, other isobaric compounds or compounds from the same chemical class are present in the mixture to be analysed.

4.4 Breath analysis and searching for the new biomarkers

Objectives

The main and ultimate goal of my PhD research project was (already from the onset) breath analysis for clinical diagnosis and monitoring of therapy, whereas all the investigations concerning reaction kinetics, development of new reaction schemes and methodologies in SIFT-MS have been the activities precedent and essential for reaching of this goal. Our interest in the area of breath analysis has been after the long decision process focused on inflammatory bowel diseases (IBD), the group of disorders causing inflammation of the gastrointestinal tract. The main reasons for this choice was mainly the previous success of the local investigation team with research in this area (detection of exhaled pentane concentration as a biomarker of lipid peroxidation [168]). Then also the fact, that interesting results were presented for this topic at the time of our decision-making (f.e. research patents identifying some VOCs as being present in modified concentrations in the breath of patients with IBD [179-182]). Also, the increasing incidence and prevalence of these inflammatory conditions in Europe, lack of a single gold standard, multimodal approach for the diagnostics and limited sensitivity or invasiveness of currently available methods supported our decision (see section *2.3.3 Inflammatory bowel disease* for additional details).

On the beginning of our breath analysis project, the detailed study of the ion chemistry of several possible breath biomarkers of IBD [165] (see Appendix B) has been conducted. The compounds included in this study (the group consisting of some hydrocarbons, aromatic compounds, ketones or alcohols) have been carefully selected from the above mentioned patents. Data obtained in this experiment facilitated SIFT-MS analyses of the studied compounds in the presence of water vapour in biological samples including exhaled breath.

As the following step, it was necessary to get an idea about the actual breath concentrations of these compounds in exhaled breath of health population and IBD patients (our preliminary measurements indicated the values typically below 10 ppbv and possibly below 1 ppbv). A study has been carried out of IBD possible biomarkers in the exhaled breath of patients suffering from IBD, comprising 136 with Crohn's disease (CD) and 51 with Ulcerative colitis (UC), together with a cohort of 14 healthy persons as controls. Breath samples were collected by requesting the patients to inflate Nalophan

bags which were than quantitatively analysed using SIFT-MS. The breath samples for this study were obtained in cooperation with ISCARE Clinical and Research Centre for Inflammatory Bowel Disease in Prague. The data acquired within this project shown some interesting results, specifically elevated ion signal at *m/z* 99 observed in the H_3O^+ SIFT-MS analyses of breath of IBD patients, [183] that may be characteristic for heptene. Except of this curious result, concentrations of all the remaining IBD possible biomarkers shown to be typically in the range < 5 ppbv, as can be seen from the *Figure 4.15*. Although, during the course of my PhD project, the elevated signal at *m/z* 99 has been finally explained by presence of impurities released over the time from the Nalophan sampling bags (influence of the gathered results by the false positive signal - see the Section 4.5 *Challenges in connection with breath analysis - Nalophan sampling bags*), the ion signal at *m/z* 99 observed during our pilot study has been quite significant. Taking into account the study of Patel et al. [167], which supported the hypothesis that alkenes are possible IBD biomarkers, it was clear, that further work will be necessary to establish the diagnostic value of SIFT-MS measurement of these compounds.

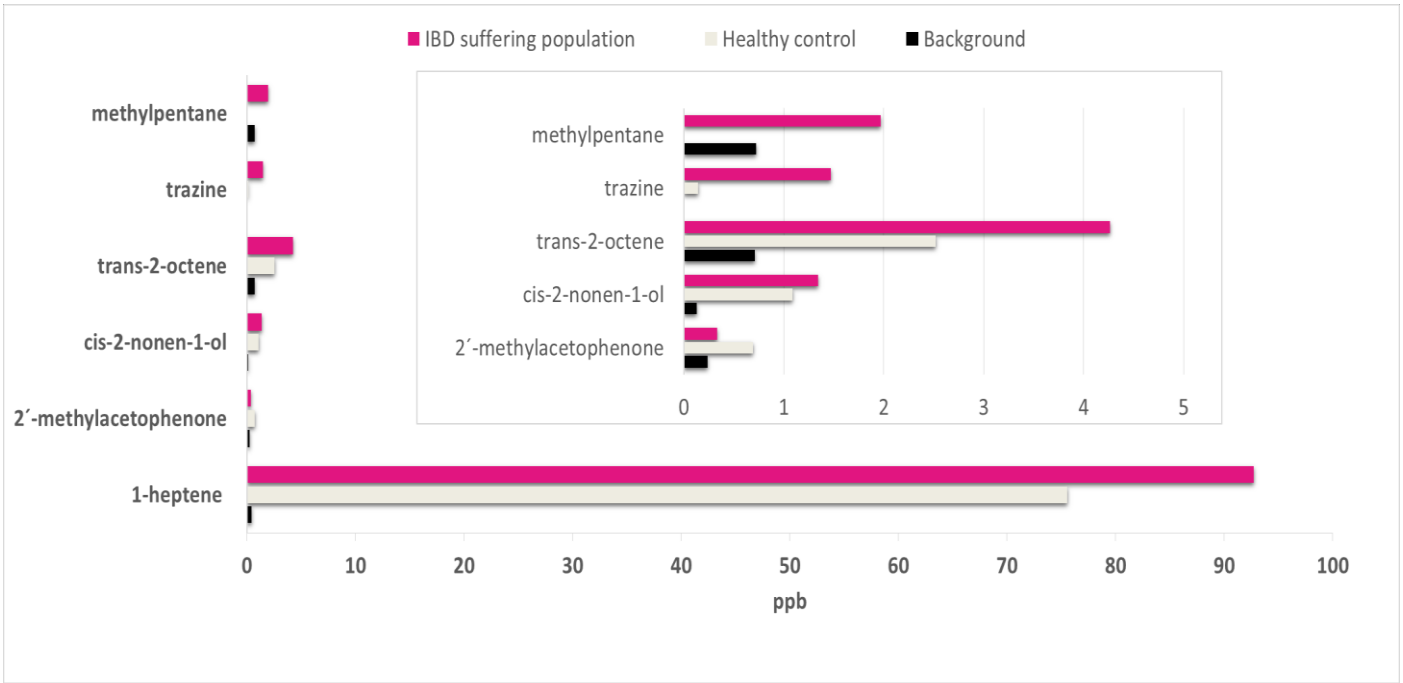


Figure 4.15. The average concentration of IBD-potential biomarkers in real samples of human breath (Cohort of subjects from the ISCARE Clinical and Research Centre for Inflammatory Bowel Disease in Prague - 136 patients with CD, 51 with UC and 14 healthy controls).

To achieve this point, it was decided to conduct the detailed study of the ion chemistry of alkenes focusing on heptenes [169] and to use the gathered results (ion-molecule kinetics data) for additional investigation in this area (in the subsequent step to confirm or exclude presence of alkenes directly in the real breath samples of healthy population and finally to compare gathered results with those obtained for IBD suffering patients). Such an effective addition of several entries to a SIFT-MS library for alkenes allowed ultimately fast SIFT-MS analyses of breath samples and the quantification of alkenes for the purpose of IBD diagnostics (see the Appendix C: *A detailed study of the ion chemistry of alkenes focusing on heptenes aimed at their SIFT-MS quantification* for additional details).

Before we have proceeded to our follow-up research project, the available literature with a focus on previous observations concerning heptene or *m/z* 99 mass peaks was reviewed, to compile as much as possible knowledge at this level. Alkenes, resp. analytes leading to the characteristic proton transfer product ion at *m/z* 99, have been observed in several areas of research. In the medical science for example in connection with chronic liver disease (CLD) in children, where 1-heptene, 1-octene and 1-decene (as analysed by SIFT-MS) were selected as breath VOCs, which can in combination with others VOCs predict CLD presence [299], or in connection with oral candidiasis, where the pattern containing nine compounds including 1-heptene was identified by GC-MS, showing characteristic changes in patients before and after antifungal therapy [300]. 1-heptene levels were investigated also in connection with carcinoma. The work of Mochalski et al. studied release and uptake of volatile organic compounds by human hepatocellular carcinoma cells and 1-heptene was one of the compounds identified by GC-MS playing role in metabolism of these harmful cells [301]. In connection with cancer, in this case lung cancer, ion signal at *m/z* 99 was also investigated, which was identified as one of the markers for bronchial adeno carcinoma. Nevertheless, the ion signal at *m/z* 99 was in this study tentatively identified as $C_6H_{10}O$ (e.g. Hexenal) or $C_5H_6O_2$ (e.g. Furanmethanol, 2-methyl-3(2H)-furanone) by proton transfer reaction time of flight mass spectrometry, PTR-TOF-MS [302]. The ion signal at *m/z* 99 was monitored also in the PTR-MS study focused on elimination characteristics of post-operative isoflurane levels in alveolar breath [303]. Another area of research where the signals of product ion at *m/z* 99 (in case of PTR-MS and H_3O^+ reactions) can be important is food industry or environmental and industrial monitoring.

To summarize all the retrieved data, heptenes and product ions at *m/z* 99 were observed in various studies of VOCs. Unfortunately, the presence of *m/z* 99 can be interpreted by individual research groups differently, because it can in the environment of PTR-MS and SIFT-MS result from many different compounds. Alkenes and heptenes can be thus possible markers not only for IBD, but they can signalize many different medical problems or body states (as can be seen from the text above).

Methods

Breath samples

The group of ostensibly healthy volunteers (no acute or chronic disease has been reported at the time of investigation) were recruited from colleagues involved in this study and from the local academic team. The study was approved by the ISCARE I.V.F. a.s. institutional ethics committee. Breath samples (on average 3-4 for any volunteer, to achieve quality and integrity of the gathered data and to exclude random false positive or negative results) were collected into 25 μm Nalophan sample bags (Kalle CZ, Žebrák, Czech Republic) of 3 L volume and after approximately 5 minutes placed into an incubator held at 37^0C. To allow correct identification of alkenes (in case that these compounds would be present in breath), we have collected also samples of breath after exposure to compound standards and breath samples spiked by compound standards. The following reagents were used for this purpose: 1-hexene, 97%, Sigma-Aldrich; 1-heptene, neat, Supelco; trans-2-heptene, 99%, and 3-heptene (cis and trans), ≥ 95%, Chromservis; 1-octene, neat, Supelco; 1-nonene, 99,5%, and 1-decene, 94%, Sigma-Aldrich.

The protocol for obtaining the samples of breath after exposure required the volunteer stay in the designated laboratory room with open flasks of alkenes standards for 1 minute and immediate collection of the breath to the Nalophan sample bag. From the other side, breath samples spiked by compound standards have been prepared by gradual addition of individual alkenes (in total 3 ml headspace of each of 7 compounds of interest) to the breath of few randomly selected volunteers. Also these “special” samples have been after 1-2 minutes transferred to the incubator held at 37°C.

Breath samples for long-term observational, comparative analysis of heptene levels in case of IBD suffering patient (CD in remission, biological treatment) and control have been collected and processed in the same way as the rest of samples.

SIFT-MS analysis of exhaled breath with a focus on presence of alkenes

Off-line SIFT-MS analyses by using H_3O^+ reagent ion were started immediately after sample collection, even before placing the bag to the incubator. This protocol has enabled to observe changes in the representation of the monitored substances in relation to the temperature, because the room temperature was much more lower (approximately 25°C). Alkenes were quantified by using the multiple ion monitoring (MIM) mode and the special kinetic library, which was created specifically for the purpose of monitoring of alkenes. The total analysis time for each bag sample, including the data storing time, was about 30 minutes. The background concentrations of all included compounds were recorded in order to ensure that the inhaled concentrations were negligible [96].

GC-MS analysis of exhaled breath with a focus on presence of alkenes

For the purpose of GC-MS analysis, the breath VOCs were extracted using solid phase microextraction (SPME) onto CAR/PDMS - coated fibres (carboxen/ polydimethylsiloxane; Supelco, Bellefonte, PA, USA) for 30 min at a temperature 37°C. To achieve the smooth course of analysis for all bag samples, the protocol of this study required the start of extraction simultaneously with the start of SIFT-MS analysis. When the extraction has been finished, the SPME fibres were directly inserted into the injector of the GC-MS instrument (FOCUS GC with instant connect Split/Splitless injector (SSL), ITQ 700 ion trap mass spectrometer using electron ionisation) held at 210 °C. The time of desorption was 5 minutes using the splitless injection mode. The GC conditions were as follows: GC oven temperature program 38°C (hold 8 min), 4°C/min ramp up to 100 °C, 30°C/min to 210°C, and a final hold 3 min (total run time 30.17 min). GC-MS capillary column TG-624 (fused 100% Cyanopropylphenyl Polisiloxane, 30 m × 0.25 mm ID × 1.0 um film) was used. Electron ionisation at 70 eV generated ions that were analysed by the ion trap operating in the scan mode (*m/z* 15–400, scan rate: 1 scan/s). The instrument setup and the method has been developed directly for the need of off-line GC-MS analysis of VOCs from breath by using CAR/PDMS-coated fibres and have been successfully used in previous studies [202,304]. The results for breath were finally compared with the results gathered for breath samples spiked by compound standards and with the results gathered for breath samples after exposure to these compounds. Thus, the identification of alkenes has been conducted with a help of standards (confirmation of retention time and mass spectra with samples obtaining standards). The results were finally verified with the help of NIST 2.0 library.

Comparison of the SIFT-MS and GC-MS results

Whereas the benefits of SIFT-MS in case of this study are mainly in its easy and fast use for quantification of alkenes, a problem which persists are the ion signal overlaps (*m/z*) of product ions and therefore uncertainty with identification of compounds present in breath - in general the non-specificity of this method. This problem was in our study solved by using a second analytical technique, in our case GC-MS, which allows easy identification of alkenes. Combination of both these methods finally allowed qualitative and also quantitative analysis, which was confirmed with a help of correlation analysis.

Results and Discussion

SIFT-MS as a method suitable for alkenes detection

The results gathered within this study confirmed the suitability of SIFT-MS method for analysis of alkenes in breath. The mean values of alkenes concentrations in breath of healthy volunteers (in ppbv) are given in *Table 4.8*, same as mean values for samples of breath after exposure to compound standards and breath samples spiked by compound standards. As can be seen from this table, in cases when alkenes are really present in the human breath, SIFT-MS can be the reliable method for their detection, even in the case of their low representation. The average concentrations of all investigated alkenes in breath are generally below 10 ppbv and in comparison with breath samples after exposure to compound standards or breath samples spiked by compound standards are negligible. The only exception in our study was the 1-heptene (sample stored in the incubator set up to 37°C), where was the concentration more than 20 ppbv (reasons for this explained below). This result is in a good agreement with the generally accepted assumption that these compounds shouldn't be presented in the breath of healthy people in higher concentrations and their low representation should be in normal situation related to exogenous factors (environment, life style, nourishment) [305]. We can also confirm, that the observed values are comparable with the above-mentioned work of Eng et al., where the levels of alkenes (series from heptene to decene) are generally also below 10 ppbv. The differences in the obtained results can be given by the fact, that in case of Eng et al., Mylar bag was used instead of Nalophan bag [299]. When we will focus on concentration of alkenes in breath after exposure to compound standards, they are in the range of hundreds for lower alkenes, such as 1-hexene (mean value: 217 ppbv) and heptene (150

ppbv) and in the range of units for higher alkenes, such as 1-nonene (3.3 ppbv) and 1-decene (5.5 ppbv). Values for 1-octene lays in the order of tens (48 ppbv). In case of breath samples spiked with alkenes standards we can observe the same trend, but in this case are the mean values in comparison to values obtained for breath exposed to alkenes even many times higher. For 1-hexene was the mean concentration 24514 ppbv and for heptene 7218 ppbv. The values for higher alkenes were than 513, 48 and 8.3 ppbv (1-octene, 1-nonene, 1-decene). Based on these learnings it is possible to say, that analysis and detection of higher alkenes may be more problematic because of their lower representation

Table 4.8. **A list of alkenes concentrations for the breath samples of healthy volunteers, breath samples after exposure to alkenes and samples of breath spiked by compound standards as measured by SIFT-MS** (MUI mode, H_3O^+ reagent ions). The median values are in ppbv, range of values is in %.

Compound of interest	Reagent	Product ions used for calc.	Breath samples (room temperature*)	Breath samples (incubator**)	Breath after exposure to alkene's standards	Breath samples spiked by alkene's standards
1-hexene	H_3O^+	*m/z* 85	0.96	0.93	217	24514
+/-			31%	20%	1%	2%
heptene	H_3O^+	*m/z* 99	4.5	24	150	7218
+/-			19 %	5%	2%	1%
1-octene	H_3O^+	*m/z* 113	2.5	2.6	48	513
+/-			21%	14%	3%	4%
1-nonene	H_3O^+	*m/z* 127	0.23	0.30	3.3	48
+/-			56%	46%	12%	7%
1-decene	H_3O^+	*m/z* 141	0.14	0.13	5.5	8.3
+/-			36%	20%	10%	10%

* air conditioner set up on 25°C
** incubator set up on 37°C

Comparison of SIFT-MS and GC-MS results, correlation analysis

As it is mentioned above, all the samples have been analysed not only by SIFT-MS technique, but also by GC-MS to achieve the correct interpretation of results in terms of identification of individual alkenes. The correlation analysis has been conducted for all compounds of interest and the harmony of results gathered by SIFT-MS and GC-MS has been tested. The best value was determined for 1-hexene, where we achieved the result R^2=0.94. Good results were achieved also in case of heptene $R^2 = 0.88$ (peak of heptene eluted at retention time (RT) =11.66), respective $R^2 = 0.91$ (peak of heptene eluted at RT=12.86), but they were not very convincing in case of 1-nonene and 1-decene. There

may be many reasons for this fact, including SPME limitations, the decreasing volatility in the series of alkenes or time delay between both analytical processes.

Is the ion signal at *m/z* 99 really heptene?

In the case of this study we have determined again interesting results for heptene. As can be seen from the *Table 4.8* above and also from the *Figure 4.16*, on the assumption that SIFT-MS characteristic ion *m/z* 99 is assigned to heptene, the result indicated higher representation of this compound for breath samples placed to the incubator in comparison with those stored at room temperature. Because the concentration of heptene in heated breath sample was approximately 4 times higher than concentration of heptene in breath sample stored at room temperature (24 ppbv respective 4,5 ppbv), the question was opened, whether we can the ion signal recorded in case of heated breath samples at *m/z* 99 really use for calculation of heptene concentration, or whether we monitor the signal of another compound. This question was opened beside the fact that the achieved results of correlation analysis were quite promising, as can be seen in the paragraph above.

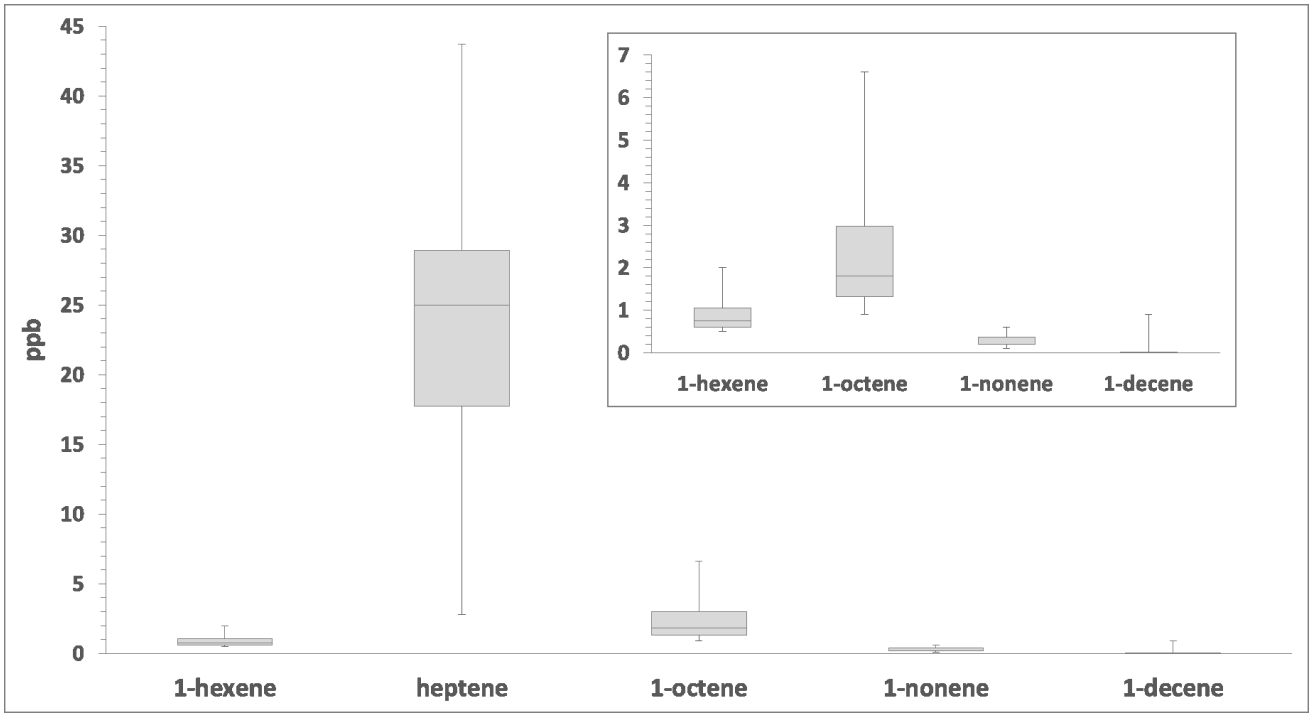


Figure 4.16. Box-and-whiskers plots of breath alkenes concentrations for the samples stored at incubator. Median values are indicated.

We have therefore tried to investigate in detail the data gathered by both methods with an ultimate goal to identify a compound, which could represent ion signal at *m/z* 99 with a higher certainty. To achieve this, it was necessary to re-investigate the results gathered by GC-MS analysis and identify all possible peaks with an occurrence of the ion signal in the *m/z* range from 98 to 100. The identified peaks have been than evaluated and obtained results later compared with the data gathered by SIFT-MS for the same samples. The correlation analysis between SIFT-MS data for *m/z* 99 and GC-MS results provided in many cases promising results and finally we have realised, that the compound giving the signal at *m/z* 99 could be any impurity released from the Nalophan sampling bag (ethylene glycol - $C_2H_6O_2$; MW 62). This challenge has been further investigated and all the circumstances are summarised in the section bellow (*4.5 Challenges in connection with breath analysis - Nalophan sampling bags*) and also in the relevant manuscript (Appendix D).

Comparison of Data for healthy volunteer and IBD suffering patients

Within this study we have conducted also long-term systematic, comparative analysis of breath of two volunteers with a focus on monitoring of heptene concentration/ion signal at *m/z* 99. This observation lasted for approximately 1 month (20 working days) and it was focused on 2 volunteers, specifically 1 healthy subject (control) and 1 IBD patient suffering by UC (biological treatment, illness in remission). The results shown slightly increased levels of heptene, respectively ion signal at *m/z* 99 for IBD patient. The value was in average 11.3 ppbv.

Conclusions

In general, this research project was targeted on analysis of breath of healthy population with a focus on alkenes (from 1-hexene to 1-decene) and determination of their mean concentration. Part of this study was also a pilot work comparing data for IBD suffering patient and control. To summarize the obtained results, SIFT-MS is a technique capable to conduct analysis of alkenes in breath (when they are really present in the sample). Nevertheless, it is very important to highlight, that only breath samples stored at the room temperature for as short time as possible are suitable for this type of analysis. In case when the samples of high humidity and temperature higher than 25°C would be analysed, there is a risk of getting the false positive signal, as the *m/z* 99 measured in case

of these breath samples can have origin in other compound (impurities released from the Nalophan sampling bag, namely ethylenglycol). This problem is also multiplied by the length of storage. All these facts played an important role also in obtaining false positive signal during our pilot study, where samples from IBD suffering patients obtained in cooperation with ISCARE Clinical and Research Centre for Inflammatory Bowel Disease in Prague have been analysed.

The mean concentrations of alkenes in the breath of healthy people, as determined by this study, are usually below 10 ppbv. The comparison of results obtained for healthy volunteer and IBD suffering patient confirmed the hypothesis, that presence of heptene in the breath of IBD suffering patient is higher.

4.5 Challenges in connection with breath analysis - Nalophan sampling bags

Objectives

Even though the analytical techniques are available for on-line real time analysis of exhaled breath (as already mentioned in the theoretical part of this thesis - *Section 2.2 Sampling and analysis*), e.g. SIFT-MS and selected ion flow-drift tube (SIFDT-MS) [306], PTR-MS, IMS, absorption spectroscopy or SESI-MS [307,308], off-line analyses are still very important and frequently utilised. The reason are in particular the following major drawbacks of on-line techniques: the cost of the instrumentation, problems with the movement of usually very large devices and the fact, that longer analyte ion integration times can be used in the case of off-line analysis to improve analytical sensitivity. The off-line exhaled breath analysis is typically carried out by collecting a sample in a suitable container prior to extraction or analysis.

The sampling procedure and the sample stability in general are the most critical steps in the entire analytical procedure. In fact, preservation of the sample integrity during sampling and sample storage is probably one of the most demanding challenges in analytical chemistry. It can be accompanied by phenomena like interaction with the sampling container (adsorption/desorption processes and release from the container material itself) or permeation through the container walls (loss of sample components and contamination of external pollutants). Chemical reactions facilitated by high sample humidity can also occur, especially at long storage times [118]. These problems are aggravated in case of breath analysis because of the very low concentrations of many volatile metabolites (from ppbv to low pptv) some of which are very reactive species like aldehydes and unsaturated organic compounds.

The standard containers for sampling have been already discussed in the theoretical part of this thesis (Section *2.2.4 Storage, stability and sampling containers for breath analysis*), where some additional details concerning this topic can be found. Just to summarize, polymer sampling bags are very often used, namely Tedlar (PVF, polyvinyl fluoride), Teflon (PTFE, polytetrafluoroethylene), Metal-coated multilayer bags (Flexfoil and polyester aluminium, PEA) and Nalophan (PET, polyethylene terephthalate).

In our study we have focused on the Nalophan sampling bags, especially because of their high popularity in Europe and extensive use in the field of breath analysis, which is probably also supported by the fact, that Nalophan bags have been evaluated in the previous studies as best choice for the collection of breath samples in terms of contaminants released during storage, good sample stability (up to 24 h for both dry and humid samples), and very limited costs. However, previous studies have reported some impurity compounds in Nalophan bags including 2-methyl-1,3-dioxolane [115, 309]. Because this compound can react with hydrogen sulphide [310], it is not recommended to use Nalophan sampling bags for analysis of compounds containing sulphur [112].

Over the years, the product ions at *m/z* 45, 63 and 81 were commonly observed in SIFT-MS analyses of breath stored in Nalophan bags using the H_3O^+ reagent ions (not only by our research group, but also in different laboratories and for separately acquired Nalophan material). This signal was (in retrospect incorrectly) attributed to acetaldehyde [130]. Nevertheless, the study of alkenes (see the chapter above - *4.4 Breath analysis and searching for the new biomarkers*) encouraged the idea, that another compound could be responsible for this signal. Based on some pilot studies of Nalophan bag samples using GC-MS we noted that this compound could be attributed to 1,2-ethanediol (ethylene glycol) released from the Nalophan material. Therefore, we have decided to revisit this important topic. It was done by investigating the release of VOC impurities into Nalophan bags using three analytical methods, viz. gas chromatography mass spectrometry, GC-MS, SESI-MS and SIFT-MS. The objective of this study was to identify main VOCs released by Nalophan, to examine the ion-molecule reaction kinetics and also to evaluate the influence of storage time, temperature and sample humidity on the release rate of the identified VOCs.

Methods

Nalophan bag construction

The bags used for this study were fabricated from a roll of a Nalophan tube with a diameter of 15 cm and a thickness of 25 μm (Kalle, Germany). The method of Nalophan bags fabrication previously described by Ghimenti [115] was slightly modified. 60 cm long pieces of Nalophan tube were sealed at one end by z-folding and tightened by a nylon cable tie. Similarly, the other end was also z-folded and a 1 mL disposable syringe

was inserted and tightened by a cable tie. The volume of these approximately cylindrical bags was 8 L and their inner surface area was 2400 cm^2. The bags were first flushed and then refilled with dry cylinder nitrogen (Messer) and after a suitable lapse period (see later) the nitrogen gas was analysed, as described later. To investigate the influence of water vapour, 100 μL of liquid water was injected into the nitrogen. Experiments were carried out for both the "dry" and "humid" bags at room temperature (about 21°C) and at 37°C using a thermostat. All analyses for trace VOCs using GC-MS, SESI-MS and SIFT-MS were repeated in triplicate. To support our gathered results, the further checks have been conducted with a new Nalophan roll, in order to check for material contamination. These additional results only confirmed the previous ones.

Gas chromatography mass spectrometry (GC-MS)

The VOCs were extracted from the nitrogen contained in the Nalophan bag using the conventional solid phase microextraction (SPME). Thus, a carboxen/ polydimethylsiloxane coated fibre (CAR/PDMS; 75 μm diameter, 1 cm length, Supelco) was exposed for 30 min in the nitrogen. To achieve consistent analyses for all bag samples, the SIFT-MS and SESI-MS analyses began at the start of the SPME extraction. The SPME fibres were introduced into the injector of the GC-MS instrument (FOCUS GC with SSL, ITQ 700 ion trap mass spectrometer using electron ionisation) held at 210°C. The time of desorption was 5 minutes using the splitless injection mode. An intermediate polarity VOCOL Capillary column (Supelco, 30 m × 0.25 mm, 1.50 μm) was used for the analyses and the GC temperature program was as previously used (section *4.4 Breath analysis and searching for the new biomarkers*) viz. GC oven temperature program 38°C (hold 8 min), 4°C/min ramp up to 100°C, 30°C/min to 210°C, and a final hold 3 min (total run time 30.17 min). Electron ionisation at 70 eV generated ions that were analysed by the ion trap operating in the scan mode (*m/z* 15 – 400, scan rate 1 scan/s).

Secondary electrospray ionisation mass spectrometry (SESI-MS)

In secondary electrospray ionization – mass spectrometry (SESI-MS), trace VOCs in a sample gas are ionized at atmospheric pressure and detected by a mass spectrometer. It has shown promise in a number of applications calling for fast and sensitive analysis of

VOCs. For the present study, a home-built SESI source [311] was coupled to a commercial ion trap mass spectrometer LCQ Deca XP (Thermo Finnigan). The SESI source consisted of a cylindrical stainless steel reaction chamber with two observation windows (glass), two inlets (electrospray and sample inlet) and one outlet (connected to an MS transfer capillary). Coaxial with the inlet of the mass spectrometer, an uncoated fused silica capillary (internal diameter 20 μm, TaperTip Emitters) was fixed in the chamber wall to establish an electrospray. The spray was pneumatically supplied (approx. 500 mbar overpressure of air) with 0.1% formic acid (98%; Sigma-Aldrich) solution in pure water from a reservoir to which high voltage (+ 3.5 kV) was applied by a platinum wire. Gas samples were introduced into the reaction chamber where gas phase ionization by the electrospray generated ions took place. The mass spectrometer was set to acquire mass spectra in the low mass range (*m/z* 15–200, low mass cut-off was *m/z* 15) in the positive ionization mode.

Selected ion flow tube mass spectrometry (SIFT-MS)

Data for the present study were obtained using the full scan (FS) mode of the downstream analytical mass spectrometer(*m/z* range 10-200, scan rate 100 s/scan) with all three available reagent ions (H_3O^+, NO^+, and $O_2^{+\bullet}$) and the multiple ion monitoring (MIM) mode involving rapid switching between selected analyte ions. The total analysis time for each bag sample, including the data saving time, was about 30 minutes.

Results and Discussion

The following text summarizes and complements the data concerning the challenges in connection with Nalophan sampling bags, as published in the relevant article (Appendix D) [312]. In summary, basic features of the GC-MS, SESI-MS and SIFT-MS data obtained for the analysis of the nitrogen contained in the Nalophan bags are presented.

Gas chromatography mass spectrometry (GC-MS)

Sample chromatograms resulting from an unexposed SPME fibre and one exposed to the nitrogen bag gas are shown in *Figure 4.17*. These chromatograms are remarkably clean showing in *Figure 4.17a* only the silanediol and siloxane emitted from the SPME fibre and two additional peaks in *Figure 4.17b*. These two additional peaks are attributed

to the ethylene glycol and 2-methyl-1,3-dioxolane. The elution times for these two compounds were confirmed using 99% pure compounds standards (Sigma-Aldrich).

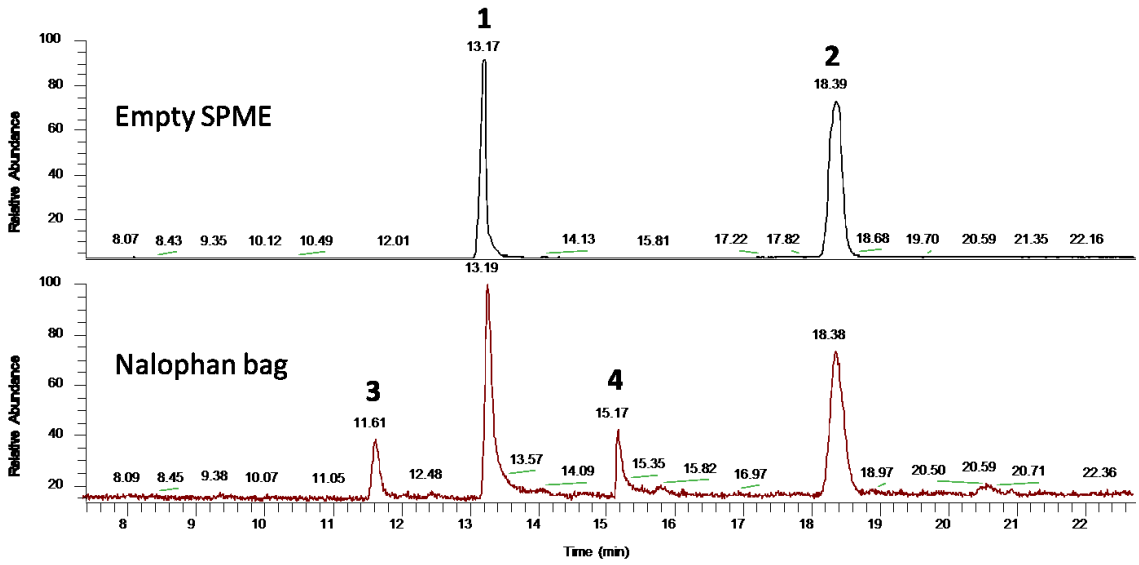


Figure 4.17. Total ion current gas chromatograms obtained for a) blank SPME fibre and b) nitrogen gas stored in a Nalophan bag for 1 hour at 37˚C. 1. dimethylsilanediol; 2. hexamethylcyclotrisiloxane (both from the SPME fiber); 3. 2-methyl-1,3-dioxolane and 4.1,2-ethanediol (ethylene glycol) (both released by the Nalpohan bag material).

Ion chemistry relating to the analysis of selected VOCs by SIFT-MS and SESI-MS

Detection and quantification of specific VOCs by SIFT-MS and SESI-MS requires a detailed knowledge of the ion-molecule chemistry and the reaction kinetics by which analyte ions are formed. Although these data were at the time of our study available for ethylene glycol (1,2-ethanediol), they were not available for 2-methyl-1,3-dioxolane. So, as a part of this study we had to obtain the kinetics data for the reactions of SIFT-MS reagent ions with 2-methyl-1,3-dioxolane using the Profile 3 instrument (see sections *4.1 Determination of rate constants and product ion branching ratios*, *4.2 Three-body association reactions – ion chemistry involving water molecules* and 4.3 *Construction and optimization of kinetics library* for additional details about the general process for gathering of these data). To achieve the up-to-date data under the same experimental conditions, those for ethylene glycol were also obtained under the same conditions. The obtained kinetics data for the H_3O^+ and NO^+ reactions are given in *Table 4.9*. The more complicated $O_2^{+\bullet}$ data are not provided, as they are not relevant for the present study.

Table 4.9. Kinetics data for the reactions of H_3O^+ and NO^+ ions with ethylene glycol and 2-methyl-1,3-dioxolane. The branching percentages for the primary reactions only are given together with their observed major hydrates.

Compound	**H_3O^+**			**NO^+**		
MW, formula	k^a	product ions		k^a	product ions	
ethylene glycol (MW 62) $C_2H_6O_2$	3.2	*m/z* 45 $(M\text{-}OH)^+$ *m/z* 63 MH^+ *m/z* 81 MH^+H_2O	40% 60%	2.0	*m/z* 61 $(M\text{-}H)^+$ *m/z* 92 MNO^+	60% 40%
2-methyl-1,3-dioxolane (MW 88) $C_4H_8O_2$	2.3	*m/z* 45 $(MH^+\text{-}C_2H_4O)^+$ *m/z* 63 $(MH^+\text{-}C_2H_4O)^+ H_2O$ *m/z* 89 MH^+ *m/z* 107 MH^+H_2O	15% 85%	1.8	*m/z* 87 $(M\text{-}H)^+$	100%

[a] Rate constants for the reactions of H_3O^+, NO^+ and $O_2^{+\bullet}$ with ethylene glycol and 2-methyl-1,3-dioxolane (at 300K) are given in the units of 10^{-9} cm^3 s^{-1}.

To summarize the gathered results, the H_3O^+ reaction of 2-methyl-1,3-dioxolane proceed via the following way:

$$H_3O^+ + C_4H_8O_2 \rightarrow C_4H_9O_2^+ + H_2O \quad (25a)$$

$$\rightarrow C_2H_5O^+ + C_2H_4O + H_2O \quad (25b)$$

Whilst the reaction mechanism is interesting, a detailed discussion is not warranted in this paper. Just to say that the reaction proceeds via exothermic proton transfer that results in partial dissociation of the nascent protonated methyl dioxolane. The rate coefficient for this reaction is calculated to be at the collisional limiting value, as given in *Table 4.9*. It is worthy of note that the protonated molecule at *m/z* 89 becomes partially hydrated as MH^+H_2O at *m/z* 107. This hydration process, well recognised and important in the analysis of many VOCs by SIFT-MS, is crucial to the understanding of acetaldehyde and ethylene glycol analyses (see below).

The NO^+ reaction is simple and proceeds thus:

$$NO^+ + C_4H_8O_2 \rightarrow C_4H_7O_2^+ + HNO \quad (26)$$

This reaction proceeds via hydride ion transfer, which is very typical of NO^+ reactions at thermal energies (300K) at which these SIFT measurements were carried out [47, 313]. The rate coefficient is also close to the collisional value and is given in *Table 4.9*.

Using these kinetics data for either H_3O^+ or NO^+ reagent ion, the concentration of the 2-methyl-1,3-dioxolane released into the nitrogen gas in the Nalophan bag can be determined (see later).

The kinetics data for the reactions of H_3O^+ and NO^+ with ethylene glycol have been determined previously [313,314], but present data are again summarised in *Table 4.9*. Note that the product ions for the H_3O^+ reaction are unfortunately just the same as those for acetaldehyde reaction [315], thus it is important to discuss them side-by-side. The primary reactions of acetaldehyde with H_3O^+ proceed thus:

$$H_3O^+ + C_2H_4O \rightarrow C_2H_5O^+ + H_2O \quad (27)$$

Whereas, the primary reactions of acetaldehyde with H_3O^+ proceed by the following channel:

$$H_3O^+ + C_2H_6O_2 \rightarrow C_2H_7O_2{}^+ + H_2O \quad (28a)$$

$$\rightarrow C_2H_5O^+ + 2H_2O \quad (28a)$$

Thus, there is a common product ion at *m/z* 45 for these two reactions. Further to this complication, in the inevitable presence of water vapour, the latter ion hydrates producing $C_2H_5O^+(H_2O)_2$ ions at *m/z* 63 and *m/z* 81, as shown in *Table 4.9*. This complicates the analysis of these VOCs when they are both present in a sample. Fortunately, in SIFT-MS, NO^+ can be used to analyse a sample, as indicated below.

The reaction of NO+ with acetaldehyde proceeds thus:

$$NO^+ + C_2H_4O \rightarrow C_2H_3O^+ + HNO \quad (29a)$$

$$+ (He) \rightarrow NO^+.C_2H_4O + (He) \quad (29b)$$

Reaction (29a) is hydride ion transfer producing the closed shell ion $C_2H_3O^+$ which does not undergo the secondary reactions of primary products with water [47, 313]. Reaction (29b) is a ternary association reaction mediated by carrier gas helium atoms (He) producing the adduct ion.

The ethylene glycol reaction with NO^+ proceeds thus:

$$NO^+ + C_2H_6O_2 \rightarrow C_2H_5O_2{}^+ + HNO \quad (30a)$$

$$+ (He) \rightarrow NO^+.C_2H_6O_2 + (He) \quad (30b)$$

The reaction processes are as for acetaldehyde, but different product ions are formed in these reaction. Thus, NO^+ reactions can be used to distinguish between acetaldehyde and ethylene glycol and NO^+ reagent ions can be used to quantify concentrations of these compounds by using SIFT-MS analytical method.

Secondary electrospray ionisation mass spectrometry (SESI-MS)

As can be seen from the Figure 4.18, nitrogen gas contained in the Nalophan bags is giving rather simple SESI-MS spectrum. The major ion peaks are the hydrated hydronium ions $H_3O^+(H_2O)_{1,2,3}$ at *m/z* 37, 55 and 73, which are always present in SESI mass spectra. At *m/z* 63, 81 and 99 we can see the ion peaks related to the protonated ethylene glycol (reaction 28a) and its hydrates, $C_2H_7O_2^+(H_2O)_{0,1,2}$. The peak at *m/z* 45 corresponds to loss of H_2O from $C_2H_7O_2^+$ (reaction 28b). 2-Methyl-1,3-dioxolane corresponds to protonated molecule at *m/z* 89 (reaction 25a).

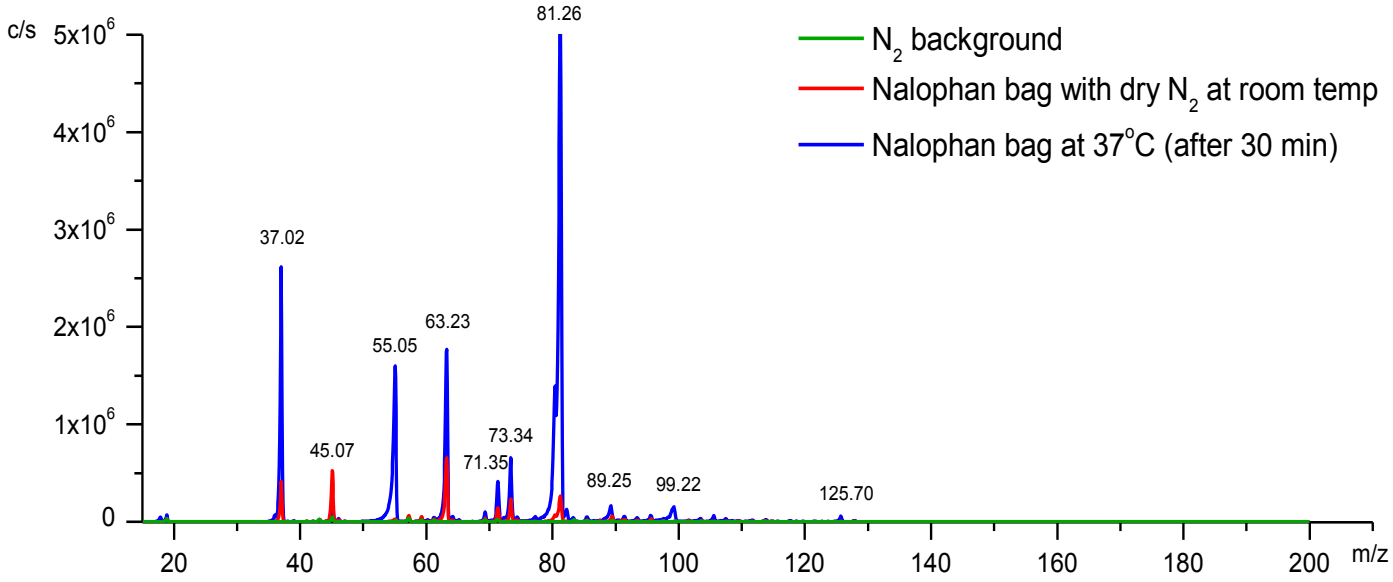


Figure 4.18. SESI-MS spectra of Nalophan bags at different temperatures and N_2 background (analysed by continuously flushing dry nitrogen into the SESI source - labeled as "N2 background" to avoid the influence of any sampling bags or other materials).

Selected ion flow tube mass spectrometry (SIFT-MS) and time profiles (MIM)

SIFT-MS quantification of both methyl dioxolane and ethylene glycol can be achieved using the ion-molecule reaction kinetics data given in *Table 4.9*.

With the knowledge of the analyte ion *m/z* values for the methyl dioxolane and the ethylene glycol, the MIM mode of SIFT-MS can be used to record their concentrations in the Nalophan bags as a function of time. Thus, four Naplophan bags were constructed and each was fully expanded with dry cylinder nitrogen. A drop of liquid water was injected into two of the bags, which were then labelled as "dry" and "humid". The nitrogen in one "dry" and one "humid" bag were analysed at 20˚C by SIFT-MS. This procedure was repeated for a "dry" bag and a "humid" bag placed in a thermostat held at 37˚C (simulating exhaled breath). The concentrations, in parts-per-billion by volume, ppbv, in the bags immediately after inflation, 0 mins, and after times durations of 15, 30, 45, 100 and 160 minutes at the two temperatures are shown by the vertical bar charts for ethylene glycol in *Figure 4.19a* and for the dioxolane in *Figure 4.19b*. These graphic data are essentially self-explanatory. It is seen that release of both compounds from the Nalophan is promoted by increased temperature and storage time.

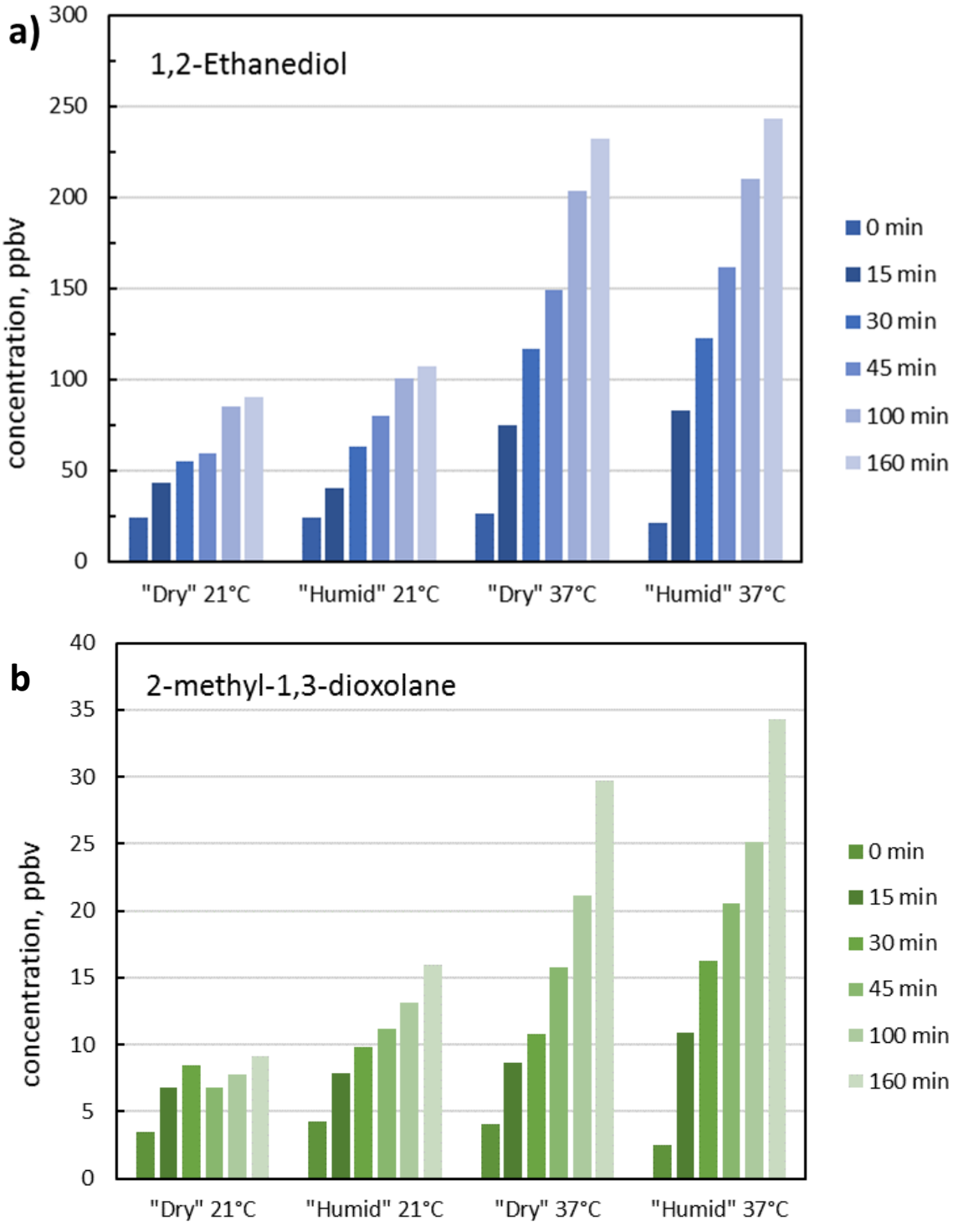


Figure 4.19. Concentrations in ppbv a) ethylene glycol and b) 2-methyl-1,3-dioxolane released by Nalophan bags into dry and humid nitrogen at atmospheric pressure stored for different times at different temperatures.

The influence of water vapour is only small but, perhaps, somewhat greater for 2-methyl-1,3-dioxolane release. The increase in the concentrations of both compounds is not linear with time, but appears to asymptotically approach a steady concentration at which compound release ceases or the compounds in the headspace are in equilibrium with those in the Nalophan material. The approximate release of ethylene glycol from the 25 μm Nalophan foil is about 10 pmol per cm^2 and that for the methyl dioxolane is about 1 pmol per cm^2.

Conclusions

The release of contaminants from the sampling bag material is obviously of concern and the present work, focused on Nalophan bags commonly used for exhaled breath sample collection and analysis, has shown that two VOCs are released, resulting in gas phase concentrations of 20 to 250 ppbv for ethylene glycol and 2 to 35 ppbv for 2-methyl-1,3-dioxolane, as measured in sample bags of typical diameter. These concentrations increased with storage time and temperature and to a lesser degree with the stored sample humidity. In addition to the above determined impurities connected with Nalophan bags, other compounds may be present at much lower concentration they are below the limit of detection of the current SIFT-MS instrumentation.

The main issue connected with this topic is that metabolites in exhaled breath of clinical significance are often at sub-ppbv levels and bag material released impurities must not be wrongly interpreted as breath metabolites. The particular danger is connected to impurities that change with storage time and could thus lead to artefacts in multivariate statistical analyses of mass spectral data (as was also the case of breath samples obtained in cooperation with ISCARE, which were used in the pilot study focused on IBD biomarkers).

In SIFT-MS breath analyses the following analyte ions are affected by Nalophan bag impurities: for H_3O^+ *m/z* 45, 63, 81, 89 and 99 and for NO^+ *m/z* 61, 87 and 92. The compounds affected are thus acetaldehyde for H_3O^+ (noting that it can be analysed using NO^+ at *m/z* 74), obviously ethylene glycol itself, any of the heptene isomers or hexanal (overlapping at *m/z* 99), many terpenes (fragment at *m/z* 81), acetoin (m/z 89), butyric acid (also *m/z* 89). Any of these compound producing analyte ions overlapping with Nalophan impurities should be analysed using NO^+ when possible. The NO^+ situation is

less severe with no notable overlaps with identified breath metabolites other than ethylene glycol itself. Review of the literature does not reveal any impact of these findings on previously published breath analyses results obtained using SIFT-MS, as breath acetaldehyde reported by [316] was measured from direct exhalations without using Nalophan bags and none of the metabolites listed above were reported in any previous Nalophan bag breath studies.

5 Summary and concluding remarks

This thesis summarises my research in the area of breath analysis for clinical diagnosis and selected ion flow tube mass spectrometry, SIFT-MS. It covers all the steps of my work, which were performed to achieve the aim of my PhD research project as formulated at the onset of my postgraduate study. Thus, variety of subjects was covered from the fundamental ion chemistry, via determination of new possible breath biomarkers in connection with inflammatory bowel disease, IBD, to characterisation of impurities presented in the Nalophan bags - the sampling material commonly used for exhaled breath sample collection and analysis.

There are several outcomes of my work:

1. The kinetic data for the ion-molecule reaction of group of carboxylic acids, IBD possible biomarkers and the compounds released from the Nalophan bags as impurities have been obtained. The rate constants and product ion branching ratios were published in peer reviewed journals for more than 50 reactions (Appendices A, B, C and D).
2. The secondary association reactions of primary product ions with water molecules have been investigated and the relevant kinetic data obtained. The understanding of these reactions is necessary for comprehensive and full understanding of the ion chemistry, to be used for accurate quantification by SIFT-MS (Appendices A, B).
3. The methodology for optimization of kinetics library entries avoiding cross sensitivity was formulated for compounds of interest and in detail described (Appendices A, C).
4. Selected VOCs have been investigated in order to confirm their significance as IBD possible biomarkers. SIFT-MS has been confirmed as a technique capable to conduct analysis of IBD possible biomarkers, including alkenes, in breath (when they are really present in the sample), but only under certain conditions (on-line analysis/defined storage time of breath samples in Nalophan bags, choice of suitable reagent ions). The reason is the possible cross sensitivity with the compounds released by Nalophan bag (ethylene glycol) (so far not published).
5. The mean concentrations of alkenes in the breath of healthy people were determined (usually below 10 ppbv). The comparison of results obtained for

healthy volunteer and IBD suffering patient confirmed the hypothesis, that presence of heptene in the breath of IBD suffering patient is higher (so far not published).

6. We have investigated in detail the release of contaminants from the Nalophan bags, whereas two VOCs released in concentrations detectable by SIFT-MS have been confirmed in sample bags of typical diameter, namely ethylene glycol (gas phase concentrations of 20 to 250 ppbv) and 2-methyl-1,3-dioxolane (2 to 35 ppbv). It was also confirmed, that concentrations of these compounds increase depending on storage time, temperature and to a lesser degree on sample humidity. This study has again indicated, that qualitative GC-MS analysis is important in order to facilitate reliable SIFT-MS targeted quantification. The following analyte ions were identified as those affected by Nalophan bag impurities: for H_3O^+ *m/z* 45, 63, 81, 89 and 99 (thus acetaldehyde, ethylene glycol itself, heptene isomers, hexanal, many terpenes, acetoin and butyric acid) and for NO^+ *m/z* 61, 87 and 92. The solution could be analysis by using of NO^+ reagent ions. (Appendix D).

I really hope, that the above mentioned results will be useful for other researchers working in areas of science related to breath analyses and SIFT-MS and will facilitate further progress and developments.

References

[1] P.S. Dhami; G.Chopra; H.N. Shrivastava, A Textbook of Biology. Pradeep Publications, Jalandhar, Punjab, 2015, pp. V/101.
[2] P. Španěl, D. Smith, On-line measurement of the absolute humidity of air, breath and liquid headspace samples by selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 15(8), 2001, 563–569.
[3] K.A. Cope, M.T. Watson, W.M. Foster, S.S. Sehnert, T.H. Risby, Effects of ventilation on the collection of exhaled breath in humans. Journal of Applied Physiology, 96(4), 2004, 1371–1379.
[4] A. Amann, D. Smith, Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring, World Scientific Publishing Co. Pte. Ltd.,Singapore, 2005.
[5] A. Amann, D. Smith, Volatile Biomarkers: Non-Invasive Diagnosis in Physiology and Medicine, Elsevier, Oxford UK, 2013.
[6] P. Španěl, K. Dryahina, A. Rejšková, T.W.E. Chippendale, D. Smith, Breath acetone concentration; biological variability and the influence of diet, Physiological Measurement, 32(8), 2011,23–31.
[7] C. Wang, A. Mbi, M. Shepherd, A Study on Breath Acetone in Diabetic Patients Using a Cavity Ringdown Breath Analyzer: Exploring Correlations of Breath Acetone With Blood Glucose and Glycohemoglobin A1C, IEEE Sensors Journal, 10(1), 2010, 54–63.
[8] P. Španěl, C. Turner, T.S. Wang, R. Bloor, D. Smith, Generation of volatile compounds on mouth exposure to urea and sucrose: implications for exhaled breath analysis, Physiological Measurement, 27(2), 2005, N7–N17.
[9] D. Smith, P. Španěl, The challenge of breath analysis for clinical diagnosis and therapeutic monitoring, The Analyst, 132(5),2007, 390-396.
[10] L.R. Narasimhan, W. Goodman, C.K.N. Patel, Correlation of breath ammonia with blood urea nitrogen and creatinine during hemodialysis, Proceedings of the National Academy of Sciences of the United States of America, 98(8), 2001, 4617-4621.
[11] S. Pendharkar, S. Mehta, The Clinical Significance of Exhaled Nitric Oxide in Asthma. Canadian Respiratory Journal, 15(2), 2008, 99–106.
[12] D.H Yates, Role of exhaled nitric oxide in asthma. Immunology and Cell Biology, 79(2), 2001, 178–190.
[13] B. Ross, I. Glen, Breath Ethane Concentrations in Healthy Volunteers Correlate with a Systemic Marker of Lipid Peroxidation but Not with Omega-3 Fatty Acid Availability,. Metabolites, 4(3), 2014, 572–579.
[14] R. Brian, S. Sandeep, P. Malcolm, Increased breath ethane and pentane concentrations in currently unmedicated patients with schizophrenia. Open Journal of Psychiatry. 1, 2011, 1-7.
[15] K.A. Gorham, M.P. Sulbaek Andersen, S. Meinardi, R.J. Delfino, N. Staimer, T. Tjoa, F. Sherwood Rowland, D.R. Blake, Ethane and n-pentane in exhaled breath are biomarkers of exposure not effect. Biomarkers, 14(1), 2009, 17–25.
[16] L. Pauling, A.B. Robinson, R. Teranishi, P. Cary, Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. Proc. Nat. Acad. Sci. USA, 68, 1971, 2374–2376.
[17] A.S. Modak, Single time point diagnostic breath tests: a review, Journal of Breath Research, 4(1), 2010, 017002.
[18] R. Greger, U. Windhorst, Pulmonary Gas Exchange. In Comprehensive Human Physiology; Springer-Verlag: Berlin, Germany, 1996, Volume 2., pp. 2037–2049.

[19] R. Schubert, H. Schwoebel, A. Mau-Moeller, M. Behrens, P. Fuchs, M. Sklorz, J.K. Schubert, S. Bruhn, W. Miekisch, Metabolic monitoring and assessment of anaerobic threshold by means of breath biomarkers. Metabolomics, 8(6), 2012, 1069–1080.
[20] L.E. Farhi, Elimination of inert gas by the lung, Respiration Physiology 3(1), 1967, 1-11.
[21] J. Anderson, A. Babb, M.P. Hlastala, Modeling Soluble Gas Exchange in the Airways and Alveoli. Annals of Biomedical Engineering, 31(11), 2003, 1402–1422.
[22] J. Anderson, M. Hlastala, Breath tests and airway gas exchange. Pulmonary Pharmacology & Therapeutics, 20(2), 2007, 112–117.
[23] J. King, K. Unterkofler, S. Teschl, P. Mochalski, H. Koc, H. Hinterhuber, A. Amann, Isoprene and acetone concentration profiles during exercise on an ergometer. Journal of Breath Research, 3(2), 2009, 027006.
[24] T. Karl, P. Prazeller, D. Mayr, A. Jordan, J. Rieder, R. Fall, W. Lindinger, Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling. Journal of Applied Physiology, 91(2), 2001, 762–770.
[25] D. Smith, W.E.T. Chippendale, K. Dryahina, P. Španěl, SIFT-MS analysis of nose-exhaled breath; mouth contamination and the influence of exercise. Current Analytical Chemistry 9, 2013, 565–575.
[26] J. King, H. Koc, K. Unterkofler, P. Mochalski, A. Kupferthaler, G. Teschl, S. Teschl, H. Hinterhuber, A. Amann, Physiological modeling of isoprene dynamics in exhaled breath. Journal of Theoretical Biology, 267(4), 2010, 626–637.
[27] A.M. Diskin, P.Španěl, D. Smith, Time variation of ammonia, acetone, isoprene and ethanol in breath: A quantitative SIFT-MS study over 30 days. Physiological Measurement 24(1), 2003, 107–119.
[28] D. Smith, C. Turner, P. Španěl, Volatile metabolites in the exhaled breath of healthy volunteers: their levels and distributions. Journal of Breath Research, 1(1), 2007, 014004.
[29] C. Turner, P. Španěl, D. Smith, A longitudinal study of methanol in the exhaled breath of 30 healthy volunteers using selected ion flow tube mass spectrometry, SIFT-MS. Physiological Measurement, 27(7), 2006, 637–648.
[30] C. Turner, P. Španěl, D. Smith, A longitudinal study of ammonia, acetone and propanol in the exhaled breath of 30 subjects using selected ion flow tube mass spectrometry, SIFT-MS. Physiological Measurement, 27(4), 2006, 321–337.
[31] C. Turner, P. Španěl, D. Smith, A longitudinal study of breath isoprene in healthy volunteers using selected ion flow tube mass spectrometry (SIFT-MS). Physiological Measurement, 27(1), 2005, 13–22.
[32] C. Turner, P. Španěl, D. Smith, A longitudinal study of ethanol and acetaldehyde in the exhaled breath of healthy volunteers using selected-ion flow-tube mass spectrometry. Rapid Communications in Mass Spectrometry, 20(1), 2005, 61–68.
[33] D. Smith, T. Wang, A. Pysanenko, P. Španěl, A selected ion flow tube mass spectrometry study of ammonia in mouth- and nose-exhaled breath and in the oral cavity. Rapid Communications in Mass Spectrometry, 22(6), 2008, 783–789.
[34] C. Turner, B. Parekh, C. Walton, P. Španěl, D. Smith, M. Evans, An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 22(4), 2008, 526–532.
[35] M.P. Kalapos, On the mammalian acetone metabolism: from chemistry to clinical implications. Biochimica et Biophysica Acta (BBA) - General Subjects, 1621(2), 2003, 122–139.

[36] W. Lindinger, J. Taucher, A. Jordan, A. Hansel, W. Vogel, Endogenous Production of Methanol after the Consumption of Fruit. Alcoholism: Clinical and Experimental Research, 21(5), 1997, 939–943.
[37] D. Smith, T. Wang, P. Spanel, On-line, simultaneous quantification of ethanol, some metabolites and water vapour in breath following the ingestion of alcohol. Physiological Measurement, 23(3), 2002, 477–489.
[38] B. Enderby, W. Lenney, M. Brady, C. Emmett, P. Španěl, D. Smith, Concentrations of some metabolites in the breath of healthy children aged 7–18 years measured using selected ion flow tube mass spectrometry (SIFT-MS). Journal of Breath Research, 3(3), 2009, 036001.
[39] R. Hyšpler, Š. Crhová, J. Gasparič, Z. Zadák, M. Čížková, V. Balasová, Determination of isoprene in human expired breath using solid-phase microextraction and gas chromatography–mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications, 739(1), 2000, 183–190.
[40] P. Lirk, F. Bodrogi, H. Raifer, K. Greiner, H. Ulmer, J. Rieder, Elective haemodialysis increases exhaled isoprene. Nephrology Dialysis Transplantation, 18(5), 2003, 937–941.
[41] J. Taucher, A. Hansel, A. Jordan, R. Fall, J.H. Futrell, W. Lindinger, Detection of isoprene in expired air from human subjects using proton-transfer-reaction mass spectrometry. Rapid Communications in Mass Spectrometry, 11(11), 1997, 1230–1234.
[42] D. Smith, P. Španěl, B. Enderby, W. Lenney, C. Turner, S.J. Davies, Isoprene levels in the exhaled breath of 200 healthy pupils within the age range 7–18 years studied using SIFT-MS. Journal of Breath Research, 4(1), 2009, 017101.
[43] M. Lechner, B. Moser, D. Niederseer, A. Karlseder, B. Holzknecht, M. Fuchs, S. Colvin, H. Tilg, J. Rieder, Gender and age specific differences in exhaled isoprene levels. Respiratory Physiology & Neurobiology, 154(3), 2006, 478–483.
[44] P. Španěl, K. Dryahina, D. Smith, Acetone, ammonia and hydrogen cyanide in exhaled breath of several volunteers aged 4–83 years. Journal of Breath Research, 1(1), 2007, 011001.
[45] A.M. Diskin, P. Španěl, D. Smith, Increase of acetone and ammonia in urine headspace and breath during ovulation quantified using selected ion flow tube mass spectrometry. Physiological Measurement, 24(1), 2003, 191–199.
[46] D. Smith, K.M.K. Ismail, A.M. Diskin, G. Chapman, J.L. Magnay, P. Španěl, S. O'Brien, Increase of acetone emitted by urine in relation to ovulation. Acta Obstetricia et Gynecologica Scandinavica, 85(8), 2006, 1008–1011.
[47] D. Smith, P. Španěl, Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. Mass Spectrometry Reviews, 24(5), 2005, 661–700.
[48] D. Smith, P. Španěl, S. Davis, Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. Journal of Applied Physiology, 87(5), 1999, 1584–1588.
[49] P. Španěl, S. Davis, D. Smith, Breath ammonia dips after feeding independently of the protein content of the meal. The FASEB Journal 14:A490.
[50] N.V. Dahl, Measuring total body water in peritoneal dialysis patients using an ethanol dilution technique. Kidney International, 56(6), 1999, 2297.
[51] K. Winkler, J. Herbig, I. Kohl, Real-time metabolic monitoring with proton transfer reaction mass spectrometry. Journal of Breath Research, 7(3), 2013, 036006.
[52] P. Španěl, D. Smith, Selected ion flow tube - mass spectrometry: detection and real-time monitoring of flavours released by food products. Rapid Communications in Mass Spectrometry, 13(7), 1999, 585–596.

[53] J. Taucher, A. Hansel, A. Jordan, W. Lindinger, Analysis of Compounds in Human Breath after Ingestion of Garlic Using Proton-Transfer-Reaction Mass Spectrometry. Journal of Agricultural and Food Chemistry, 44(12), 1996, 3778–3782.
[54] A. Jordan, A. Hansel, R. Holzinger, W. Lindinger, Acetonitrile and benzene in the breath of smokers and non-smokers investigated by proton transfer reaction mass spectrometry (PTR-MS). International Journal of Mass Spectrometry and Ion Processes, 148(1-2), 1995, L1–L3.
[55] P. Španěl, P. Rolfe, B. Rajant, D. Smith, The selected ion flow tube (SIFT)—A novel technique for biological monitoring. The Annals of Occupational Hygiene 40, 1996, 615–626.
[56] S.M. Abbott, J.B. Elder, P. Španěl, D. Smith, Quantification of acetonitrile in exhaled breath and urinary headspace using selected ion flow tube mass spectrometry. International Journal of Mass Spectrometry, 228(2-3), 2003, 655–665.
[57] A. Hansel, A. Jordan, R. Holzinger, P. Prazeller, W. Vogel, W. Lindinger, Proton transfer reaction mass spectrometry: on-line trace gas analysis at the ppb level. International Journal of Mass Spectrometry and Ion Processes, 149-150, 1995, 609–619.
[58] B. Buszewski, A. Ulanowska, T. Ligor, N. Denderz, A. Amann, Analysis of exhaled breath from smokers, passive smokers and non-smokers by solid-phase microextraction gas chromatography/mass spectrometry. Biomedical Chromatography, 23(5), 2009, 551–556.
[59] P. Prazeller, T. Karl, A. Jordan, R. Holzinger, A. Hansel, W. Lindinger, Quantification of passive smoking using proton-transfer-reaction mass spectrometry. International Journal of Mass Spectrometry, 178(3), 1998, L1–L4.
[60] M.P. Habib, N.C. Clements, H.S. Garewal, Cigarette smoking and ethane exhalation in humans. American Journal of Respiratory and Critical Care Medicine, 151(5), 1995, 1368–1372.
[61] I. Kushch, K. Schwarz, L. Schwentner, B. Baumann, A. Dzien, A. Schmid, K.Unterkofler ,G. Gastl , P. Španěl , D. Smith, A. Amann, Compounds enhanced in a mass spectrometric profile of smokers' exhaled breath versus non-smokers as determined in a pilot study using PTR-MS. Journal of Breath Research, 2(2), 2008, 026002.
[62] A. Sandberg, C.M. Sköld, J. Grunewald, A. Eklund, Å. M Wheelock, Assessing Recent Smoking Status by Measuring Exhaled Carbon Monoxide Levels. PLoS ONE, 6(12), 2011, e28864.
[63] S.T. Senthilmohan, M.J. McEwan, P.F. Wilson, D.B. Milligan, C.G. Freeman, Real time analysis of breath volatiles using SIFT-MS in cigarette smoking. Redox Report, 6(3), 2001, 185–187.
[64] J. Beauchamp, Inhaled today, not gone tomorrow: pharmacokinetics and environmental exposure of volatiles in exhaled breath. Journal of Breath Research, 5(3), 2011, 037103.
[65] P. Španěl, J. Cocker, B. Rajan, D. Smith, Validation of the SIFT technique for trace gas analysis of breath using the syringe injection technique. The Annals of Occupational Hygiene, 41(3), 1997, 373–382.
[66] P.F. Wilson, C.G. Freeman, M.J. McEwan, D.B. Milligan, R.A. Allardyce, G.M. Shaw, In situ analysis of solvents on breath and blood: a selected ion flow tube mass spectrometric study. Rapid Communications in Mass Spectrometry, 16(5), 2002, 427–432.
[67] T. Wang, D. Smith, P. Španěl, Selected ion flow tube studies of the reactions of H3O+, NO+ and O2+ with the anaesthetic gases halothane, isoflurane and sevoflurane. Rapid Communications in Mass Spectrometry, 16(19), 2002, 1860–1870.

[68] J. King, A. Kupferthaler, B. Frauscher, H. Hackner, K. Unterkofler, G. Teschl, H. Hinterhuber, A. Amann, B. Hogl, Measurement of endogenous acetone and isoprene in exhaled breath during sleep. Physiological Measurement, 33(3), 2012, 413–428.
[69] J. King, P. Mochalski, K. Unterkofler, G. Teschl, M. Klieber, M. Stein, A. Amann, M. Baumann, Breath isoprene: Muscle dystrophy patients support the concept of a pool of isoprene in the periphery of the human body. Biochemical and Biophysical Research Communications, 423(3), 2012, 526–530.
[70] H. Koc, J. King, G. Teschl, K. Unterkofler, S. Teschl, P. Mochalski, H. Hinterhuber, A. Amann, The role of mathematical modeling in VOC analysis using isoprene as a prototypic example. Journal of Breath Research, 5(3), 2011, 037102.
[71] J. King, K. Unterkofler, G. Teschl, S. Teschl, H. Koc, H. Hinterhuber, A. Amann, A mathematical model for breath gas analysis of volatile organic compounds with special emphasis on acetone, Journal of mathematical biology 63 (5), 2011, 959-999.
[72] - J. King, P. Mochalski, A. Kupferthaler, K. Unterkofler, H. Koc, W. Filipiak, S. Teschl, H. Hinterhuber, A. Amann, Dynamic profiles of volatile organic compounds in exhaled breath as determined by a coupled PTR-MS/GC-MS study. Physiological Measurement, 31(9), 2010, 1169–1184.
[73] J. King, K. Unterkofler, G. Teschl, S. Teschl, P. Mochalski, H. Koc, H. Hinterhuber, A. Amann, A modeling-based evaluation of isothermal rebreathing for breath gas analyses of highly soluble volatile organic compounds. Journal of Breath Research, 6(1), 016005.
[74] - Z. H. Endre, J. W. Pickering, M. K. Storer, W. P. Hu, K. T. Moorhead, R. Allardyce, D. O. McGregor, J. M. Scotter, Breath ammonia and trimethylamine allow real-time monitoring of haemodialysis efficacy. Physiological Measurement, 32(1), 2010, 115–130.
[75] P. Španěl, D. Smith, Progress in SIFT-MS: Breath analysis and other applications. Mass Spectrometry Reviews, 30(2), 2010, 236–267.
[76] B. Thekedar, W. Szymczak, V. Höllriegl, C. Hoeschen, U. Oeh, Investigations on the variability of breath gas sampling using PTR-MS. Journal of Breath Research, 3(2), 2009, 027007.
[77] W. Vautz, J. Nolte, R. Fobbe, J.I. Baumbach, Breath analysis—performance and potential of ion mobility spectrometry. Journal of Breath Research, 3(3), 2009, 036004.
[78] M.R. McCurdy, Y. Bakhirkin, G. Wysocki, R. Lewicki, F.K. Tittel, Recent advances of laser-spectroscopy-based techniques for applications in breath analysis. Journal of Breath Research, 1(1), 2007, 014001.
[79] C. Deng, J. Zhang, X. Yu, W. Zhang, X. Zhang, Determination of acetone in human breath by gas chromatography–mass spectrometry and solid-phase microextraction with on-fiber derivatization. Journal of Chromatography B, 810(2), 2004, 269–275.
[80] K.A. Cope, M.T. Watson, W.M. Foster, S.S. Sehnert, T.H. Risby, Effects of ventilation on the collection of exhaled breath in humans. Journal of Applied Physiology, 96(4), 2004, 1371–1379.
[81] A. Hryniuk, B.M. Ross, Detection of acetone and isoprene in human breath using a combination of thermal desorption and selected ion flow tube mass spectrometry. International Journal of Mass Spectrometry, 285(1-2), 2009, 26–30.
[82] A. Amann, M. Corradi, P. Mazzone, A. Mutti, Lung cancer biomarkers in exhaled breath. Expert Review of Molecular Diagnostics, 11(2), 2011, 207–217.
[83] A. Bajtarevic, C. Ager, M. Pienz, M. Klieber, K. Schwarz, M. Ligor, T. Ligor , W. Filipiak, H. Denz , M. Fiegl , W. Hilbe , W. Weiss , P. Lukas , H. Jamnig , M. Hackl, A. Haidenberger , B. Buszewski, W. Miekisch , J. Schubert, A. Amann, Noninvasive detection of lung cancer by analysis of exhaled breath. BMC Cancer, 9(1),2009, 348.

[84] M. Phillips, N. Altorki, J.H.M. Austin, R.B. Cameron, R.N. Cataneo, R. Kloss, R.A. Maxfield , M. I. Munawar , H. I. Pass, A. Rashid , W.N. Rom , P. Schmitt , J. Wai, Detection of lung cancer using weighted digital analysis of breath biomarkers. Clinica Chimica Acta, 393(2), 2008, 76–84.
[85] M. Ligor, T. Ligor, A. Bajtarevic, C. Ager, M. Pienz, M. Klieber, H. Denz, M. Fiegl, W. Hilbe, W. Weiss, P. Lukas, H. Jamnig, M. Hackl, B. Buszewski, W. Miekisch, J. Schubert, A. Amann, Determination of volatile organic compounds in exhaled breath of patients with lung cancer using solid phase microextraction and gas chromatography mass spectrometry. Clinical Chemistry and Laboratory Medicine, 47(5), 2009, 550-560.
[86] J.D. Beauchamp, J.D. Pleil, Simply breath-taking? Developing a strategy for consistent breath sampling. Journal of Breath Research, 7(4), 2013, 042001.
[87] A. Amann W. Miekisch J.D. Pleil, T. Risby J.K. Schubert in European Respiratory Society Monograph, ed. I. Horvath and J.C. de Jongste, vol 49 (Leeds: Maney Publishing) p 96
[88] W. Miekisch, J.K. Schubert, G.F. Noeldge-Schomburg, Diagnostic potential of breath analysis—focus on volatile organic compounds. Clinica Chimica Acta, 347(1-2), 2004, 25–39.
[89] D. Poli, P. Carbognani, M. Corradi, M. Goldoni, O. Acampa, B. Balbi, L. Bianchi, M. Rusca, A. Mutti, Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term follow-up study. Respiratory Research, 6(1), 2005, 71.
[90] Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide in Adults and Children—1999. American Journal of Respiratory and Critical Care Medicine, 160(6), 1999, 2104–2117.
[91] ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. American Journal of Respiratory and Critical Care Medicine, 171(8), 2005, 912–930.
[92] I. Horváth, J. Hunt, P.J. Barnes, Exhaled breath condensate: methodological recommendations and unresolved questions. European Respiratory Journal, 26(3), 2005, 523–548.
[93] F.J. Gilchrist, C. Razavi, A.K. Webb, A.M. Jones, P. Španěl, D. Smith, W. Lenney, An investigation of suitable bag materials for the collection and storage of breath samples containing hydrogen cyanide. Journal of Breath Research, 6(3), 2012, 036004.
[94] H. Le, E.C. Sivret, G. Parcsi, R.M. Stuetz, Stability of Volatile Sulfur Compounds (VSCs) in sampling bags – impact of temperature. Water Science and Technology, 68(8), 2013, 1880–1887.
[95] M. Levitt, C. Ellis, J. Furne, Influence of method of alveolar air collection on results of breath tests. Digestive Diseases and Sciences, 43(8), 1998, 1938–1945.
[96] P. Španěl, K. Dryahina, D. Smith, A quantitative study of the influence of inhaled compounds on their concentrations in exhaled breath. Journal of Breath Research, 7(1), 2013, 017106.
[97] W.S. Fowler, LUNG FUNCTION STUDIES. II. THE RESPIRATORY DEAD SPACE. American Journal of Physiology-Legacy Content, 154(3), 1948, 405–416.
[98] J. Herbig, T. Titzmann, J. Bauchamp, I. Kohl, A. Hansel, Buffered end-tidal (BET) sampling—a novel method for real-time breath-gas analysis. Journal of Breath Research, 2(3), 2008, 037008.
[99] F. Di Francesco, C. Loccioni, M. Fioravanti, A. Russo, G. Pioggia, M. Ferro, I. Roehrer, S. Tabuchi, M. Onor, Implementation of Fowler's method for end-tidal air sampling. Journal of Breath Research, 2(3), 2008, 037009.

[100] K.M. Dubowski, Biological aspects of breath-alcohol analysis. Clinical Chemistry, 20, 1974, 294–299.
[101] J.C. Anderson, W.J.E. Lamm, M.P. Hlastala, Measuring airway exchange of endogenous acetone using a single-exhalation breathing maneuver. Journal of Applied Physiology, 100(3), 2006, 880–889.
[102] W. Filipiak, A. Filipiak, C. Ager, H. Wiesenhofer, A. Amann, Optimization of sampling parameters for collection and preconcentration of alveolar air by needle traps. Journal of Breath Research, 6(2), 2012, 027107.
[103] M.E. O'Hara, S. O'Hehir, S. Green, C.A. Mayhew, Development of a protocol to measure volatile organic compounds in human breath: a comparison of rebreathing and on-line single exhalations using proton transfer reaction mass spectrometry. Physiological Measurement, 29(3), 2008, 309–330.
[104] M.M.L. Steeghs, S.M.Cristescu, P. Munnik, P. Zanen, F.J.M. Harren, An off-line breath sampling and analysis method suitable for large screening studies. Physiological Measurement, 28(5), 2007, 503–514.
[105] C.M. Salome, A.M. Roberts, N.J. Brown, J. Dermand, G.B. Marks, A.J. Woolcock, Exhaled Nitric Oxide Measurements in a Population Sample of Young Adults. American Journal of Respiratory and Critical Care Medicine, 159(3), 1999, 911–916.
[106] B. Buszewski, A. Ulanowska, T. Ligor, N. Denderz, A. Amann, Analysis of exhaled breath from smokers, passive smokers and non-smokers by solid-phase microextraction gas chromatography/mass spectrometry. Biomedical Chromatography, 23(5), 2009, 551–556.
[107] T. Ligor, M. Ligor, A. Amann, C. Ager, M. Bachler, A. Dzien, B. Buszewski, The analysis of healthy volunteers' exhaled breath by the use of solid-phase microextraction and GC-MS. Journal of Breath Research, 2(4), 2008, 046006.
[108] I. Kushch, B. Arendacká, S. Štolc, P. Mochalski, W. Filipiak, K. Schwarz, L. Schwentner, A. Schmid, A. Dzien, M. Lechleitner, V. Witkovsky, W. Miekisch, J. Schubert, K. Unterkofler, A. Amann, Breath isoprene – aspects of normal physiology related to age, gender and cholesterol profile as determined in a proton transfer reaction mass spectrometry study. Clinical Chemistry and Laboratory Medicine, 46(7), 2008, 1011-1018.
[109] K. Schwarz, A. Pizzini, B. Arendacka, K. Zerlauth, W. Filipiak, A. Schmid, A. Dzien, S. Neuner, M. Lechleitner, S. Scholl-Burgi, W. Miekisch, J. Schubert, K. Unterkofler, V. Witkovsky, G. Gastl, A. Amann, Breath acetone—aspects of normal physiology related to age and gender as determined in a PTR-MS study. Journal of Breath Research, 3(2), 2009, 027003.
[110] K. Schwarz, W. Filipiak, A. Amann, Determining concentration patterns of volatile compounds in exhaled breath by PTR-MS. Journal of Breath Research, 3(2), 2009, 027002.
[111] S. Erhart, A. Amann, E. Haberlandt, G. Edlinger, A. Schmid, W. Filipiak, K. Schwarz, P. Mochalski, K. Rostasy, D. Karall, S. Scholl-Bürgi, 3-Heptanone as a potential new marker for valproic acid therapy. Journal of Breath Research, 3(1), 2009, 016004.
[112] P. Mochalski, B. Wzorek, I. Śliwka, A. Amann, Suitability of different polymer bags for storage of volatile sulphur compounds relevant to breath analysis. Journal of Chromatography B, 877(3), 2009, 189–196.
[113] J. Beauchamp, J. Herbig, R. Gutmann, A. Hansel, On the use of Tedlar® bags for breath-gas sampling and analysis. Journal of Breath Research, 2(4), 2008, 046001.
[114] A.Ph. (Ton) van Harreveld, Odor Concentration Decay and Stability in Gas Sampling Bags, Journal of the Air & Waste Management Association, 53:1, 2003, 51-60.

[115] S. Ghimenti, T. Lomonaco, F.G. Bellagambi, S. Tabucchi, M. Onor, M.G. Trivella, A. Ceccarini , R. Fuoco F.Di Francesco, Comparison of sampling bags for the analysis of volatile organic compounds in breath. Journal of Breath Research, 9(4), 2015, 047110.
[116] Levine, S.P.; Hebel, K.G.; Bolton, J.; Kugel, R.E, Industrial Analytical Chemists and OSHA Regulations for Vinyl Chloride. (1975). Analytical Chemistry, 47(12), 1075A–1080A.
[117] Z. Fan, J. Zhang, C.-W., Fan, D.M. Pennise, The MMT Bag for Emission Source Sampling: Design and Evaluation. Journal of the Air & Waste Management Association, 51(1), 2001, 60–68.
[118] P. Mochalski, J. King, K. Unterkofler, A. Amann, Stability of selected volatile breath constituents in Tedlar, Kynar and Flexfilm sampling bags. Analyst, 138, 2013, 1405–1418.
[119] W.A. Groves, E.T. Zellers, Investigation of Organic Vapor Losses to Condensed Water Vapor in Tedlar® Bags Used for Exhaled-Breath Sampling. American Industrial Hygiene Association Journal, 57(3), 1996, 257–263.
[120] M. P. Hlastala, The alcohol breath test—a review. Journal of Applied Physiology, 84(2), 1998, 401–408.
[121] P. Kerlin, L. Wong, Breath hydrogen testing in bacterial overgrowth of the small intestine. Gastroenterology, 95(4), 1988, 982–988.
[122] V. De Francesco, A. Zullo, F. Perna, F. Giorgio, C. Hassan, L. Vannella, F. Cristofari, C. Panella, D. Vaira and E. Ierardi, Helicobacter pylori antibiotic resistance and [13C] urea breath test values. Journal of Medical Microbiology, 59(5), 2010, 588–591.
[123] M. Phillips, K. Gleeson, J.M.B. Hughes, J. Greenberg, R.N. Cataneo, L. Baker, W.P. McVay, Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. The Lancet, 353(9168), 1999, 1930–1933.
[124] M. Phillips, R.N. Cataneo, A.R.C. Cummin, A.J. Gagliardi, K. Gleeson, J.Greenberg, R.A. Maxfield, W. N. Rom, Detection of Lung Cancer With Volatile Markers in the Breatha. Chest, 123(6), 2003, 2115–2123.
[125] M Phillips, N. Altorki, J.H.M.Austin, R.B. Cameron, R.N. Cataneo, R. Kloss, R.A. Maxfield), M.I. Munawar , H.I. Pass , A. Rashid, W.N. Rom, P. Schmitt, J. Wa. Detection of lung cancer using weighted digital analysis of breath biomarkers. Clinica Chimica Acta, 393(2), 2008, 76–84.
[126] T.H. Risby, Current status of clinical breath analysis. In Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring; World Scientific: Tuck Link, Singapore, 2005; pp. 251–265.
[127] D. Smith, T. Wang, J. Sulé-Suso, P. Španěl, A.E. Haj, Quantification of acetaldehyde released by lung cancer cells in vitro using selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 17(8), 2003, 845–850.
[128] W. Filipiak, A. Sponring, T. Mikoviny, C. Ager, J. Schubert, W. Miekisch, A. Amann, J. Troppmair, Release of volatile organic compounds (VOCs) from the lung cancer cell line CALU-1 in vitro. Cancer Cell International, 8(1), 2008, 17.
[129] J. Sulé-Suso, A. Pysanenko, P. Španěl, D. Smith, Quantification of acetaldehyde and carbon dioxide in the headspace of malignant and non-malignant lung cells in vitro by SIFT-MS. The Analyst, 134(12), 2009, 2419.
[130] D. Smith, P. Španěl, J. Sulé-Suso, Advantages of breath testing for the early diagnosis of lung cancer. Expert Review of Molecular Diagnostics, 10(3), 2010, 255–257.

[131] A.V. Rutter, T.W.E. Chippendale, Y. Yang, P. Španěl, D. Smith, J. Sulé-Suso, Quantification by SIFT-MS of acetaldehyde released by lung cells in a 3D model. The Analyst, 138(1), 2013, 91–95.
[132] V. Shestivska, A.V. Rutter, J. Sulé-Suso, D. Smith, P. Španěl, Evaluation of peroxidative stress of cancer cells in vitro by real-time quantification of volatile aldehydes in culture headspace. Rapid Communications in Mass Spectrometry, 31(16), 2017, 1344–1352.
[133] I.P.V. Munster, F.M. Nagengast, The Role of Carbohydrate Fermentation in Colon Cancer Prevention. Scandinavian Journal of Gastroenterology, 28(sup200), 1993, 80–86.
[134] K. Windey, V. De Preter, K. Verbeke, Relevance of protein fermentation to gut health. Molecular Nutrition & Food Research, 56(1), 2011, 184–196.
[135] S.C. Larsson, A. Wolk, Meat consumption and risk of colorectal cancer: A meta-analysis of prospective studies. International Journal of Cancer, 119(11), 2006, 2657–2664.
[136] A. Chao, M.J. Thun, C.J. Connell, M.L. McCullough, E.J. Jacobs, W.D. Flanders, C. Rodriguez, R. Sinha, E.E. Calle, Meat Consumption and Risk of Colorectal Cancer. JAMA, 293(2), 2005, 172–182.
[137] D.S.M. Chan, R. Lau, D. Aune, R. Vieira, D.C. Greenwood, E. Kampman, T. Norat, Red and Processed Meat and Colorectal Cancer Incidence: Meta-Analysis of Prospective Studies. PLoS ONE, 6(6), 2011, e20456.
[138] M. Rossi, M.J. Anwar, A. Usman, A. Keshavarzian F. Bishehsari, Colorectal Cancer and Alcohol Consumption—Populations to Molecules. Cancers , 10(2), 2018, 38.
[139] S.D. Shukla, R.W. Lim, Epigenetic effects of ethanol on the liver and gastrointestinal system. Alcohol Research Current Reviews, 35, 2013, 47–55.
[140] O.A. Ajibola, D. Smith, P. Španěl, G.A.A. Ferns, Effects of dietary nutrients on volatile breath metabolites. Journal of Nutritional Science, 2, 2013, 1-15.
[141] D.F. Altomare, M. Di Lena, F. Porcelli, L. Trizio, E. Travaglio, M. Tutino, S. Dragonieri, G. de Gennaro, Exhaled volatile organic compounds identify patients with colorectal cancer. British Journal of Surgery, 100(1), 2012, 144–150.
[142] S. Kumar, J. Huang, J.R. Cushnir, P. Španěl, D. Smith, G.B. Hanna, Selected Ion Flow Tube-MS Analysis of Headspace Vapor from Gastric Content for the Diagnosis of Gastro-Esophageal Cancer. Analytical Chemistry, 84(21), 2012, 9550–9557.
[143] J. Huang, S. Kumar, N. Abbassi-Ghadi, P. Španěl, D. Smith, G.B. Hanna, Selected Ion Flow Tube Mass Spectrometry Analysis of Volatile Metabolites in Urine Headspace for the Profiling of Gastro-Esophageal Cancer. Analytical Chemistry, 85(6), 2013, 3409–3416.
[144] S. Kumar, J. Huang, N. Abbassi-Ghadi, P. Španěl, D. Smith, G.B. Hanna, Selected Ion Flow Tube Mass Spectrometry Analysis of Exhaled Breath for Volatile Organic Compound Profiling of Esophago-Gastric Cancer. Analytical Chemistry, 85(12), 2013, 6121–6128.
[145] S. Kumar, J. Huang, N. Abbassi-Ghadi, H.A. Mackenzie, K.A. Veselkov, J.M. Hoare, L.B. Lovat, P. Španěl, D. Smith, G.B. Hanna, Mass Spectrometric Analysis of Exhaled Breath for the Identification of Volatile Organic Compound Biomarkers in Esophageal and Gastric Adenocarcinoma. Annals of Surgery, 262(6), 2015, 981–990.
[146] M. Phillips, R.N. Cataneo, B.A. Ditkoff, P. Fisher, J. Greenberg, R. Gunawardena, C.S. Kwon, F. Rahbari-Oskoui, C. Wong, Volatile Markers of Breast Cancer in the Breath. The Breast Journal, 9(3), 2003, 184–191.
[147] M. Phillips, R.N. Cataneo, C. Saunders, P. Hope, P. Schmitt, J. Wai, Volatile biomarkers in the breath of women with breast cancer. Journal of Breath Research, 4(2), 2010, 026003.

[148] E. Hietanen, H. Bartsch, J.C. Bériziat, A.M. Camus, S. McClinton, O. Eremin, L. Davidson, P. Boyle, Diet and oxidative stress in breast, colon and prostate cancer patients: A case-control study. European Journal of Clinical Nutrition 48(8), 1994, 575-86.
[149] P. Španěl, D. Smith, Quantification of trace levels of the potential cancer biomarkers formaldehyde, acetaldehyde and propanol in breath by SIFT-MS. Journal of Breath Research, 2(4), 2008, 046003.
[150] P. Španěl, D. Smith, T.A. Holland, W.A. Singary, J.B. Elder, Analysis of formaldehyde in the headspace of urine from bladder and prostate cancer patients using selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 13(14), 1999, 1354–1359.
[151] C.G. Waltman, T.A.T. Marcelissen, J.G.H. van Roermund, Exhaled-breath Testing for Prostate Cancer Based on Volatile Organic Compound Profiling Using an Electronic Nose Device (Aeonose™): A Preliminary Report. European Urology Focus. E U R O P E A N U R O L O G Y F O C U S X X X (2 0 18) X X X – X X X.
[152] S. Chen, L. Zieve, V. Mahadevan, Mercaptans and dimethyl sulfide in the breath of patients with cirrhosis of the liver. Effect of feeding methionine. The Journal of Laboratory and Clinical Medicine, 75(4), 1970, 628-635.
[153] P. W. D. Scislowski, K. Pickard, The Regulation of Transaminative Flux of Methionine in Rat Liver Mitochondria. Archives of Biochemistry and Biophysics, 314(2), 1994, 412–416.
[154] S.S. Sehnert, L. Jiang, J.F. Burdick, T.H. Risby, Breath biomarkers for detection of human liver diseases: preliminary study. Biomarkers, 7(2), 2002, 174–187.
[155] M.L. Simenhoff, J.F. Burke, J.J. Saukkonen, A.T. Ordinario, R. Doty, S. Dunn, Biochemical Profile of Uremic Breath. New England Journal of Medicine, 297(3), 1977, 132–135.
[156] S. Van den Velde, S. Nevens, P. van Hee, D. van Steenberghe, M. Quirynen, GC–MS analysis of breath odor compounds in liver patients. Journal of Chromatography B, 875(2), 2008, 344–348.
[157] F. Morisco, E. Aprea, V. Lembo, V. Fogliano, P. Vitaglione, G. Mazzone, L. Cappellin, F. Gasperi, S. Masone, G. Domenico de Palma, R. Marmo, N. Caporaso, F. Biasioli, Rapid “Breath-Print” of Liver Cirrhosis by Proton Transfer Reaction Time-of-Flight Mass Spectrometry. A Pilot Study. PLoS ONE, 8(4), 2013, e59658.
[158] R. Fernández del Río, M.E. O’Hara, A. Holt, P. Pemberton, T. Shah, T. Whitehouse, C.A. Mayhew, Volatile Biomarkers in Breath Associated With Liver Cirrhosis — Comparisons of Pre- and Post-liver Transplant Breath Samples. EBioMedicine, 2(9), 2015, 1243–1250.
[159] T.Y. Khalid, B.D.L. Costello, R. Ewen, P. White, S. Stevens, F. Gordon, P. Collins, A. McCune, A. Shenoy, S. Shetty, N.M. Ratcliffe, CH.S. Probert, Breath volatile analysis from patients diagnosed with harmful drinking, cirrhosis and hepatic encephalopathy: a pilot study. Metabolomics, 9(5), 2013, 938–948.
[160] F. Gomollon, A. Dignass, V. Annese, H. Tilg, G. Van Assche, J.O. Lindsay, L. Peyrin-Biroulet, G.J. Cullen, M. Daperno, T. Kucharzik, F. Rieder, S. Almer, A. Armuzzi, M. Harbord, J. Langhorst, M. Sans, Y. Chowers, G. Fiorino, P. Juillerat, G.J. Mantzaris, F. Rizzello, S. Vavricka, P. Gionchetti, 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and Medical Management, Journal of Crohn’s and Colitis, 11(1), 2017, 3–25.
[161] S.P.L.Travis, E.F. Stange, M. Lémann, T. Øresland, W.A. Bemelman, Y. Chowers, J.F.Colombel, G. D'Haens, S.Ghosh, P. Marteau, W. Kruis, N.J.McC. Mortensen, F. Penninckx, M. Gassull for the European Crohn's and Colitis Organisation (ECCO),

European evidence-based Consensus on the management of ulcerative colitis: Current management, Journal of Crohn's and Colitis, 2(1), 2008, 24–62.
[162] M. Lukas, M. Bortlik, Z. Maratka, What is the origin of ulcerative colitis? Still more questions than answers. Postgraduate Medical Journal, 82(972), 2006, 620–625.
[163] L. Peyrin-Biroulet, J. Panés, W.J. Sandborn, S. Vermeire, S. Danese, B.G. Feagan, J.F. Colombel, S.B. Hanauer S, B. Rycroft, Defining Disease Severity in Inflammatory Bowel Diseases: Current and Future Directions. Clinical Gastroenterology and Hepatology, 14(3), 2016, 348–354.e17.
[164] S. Ardizzone, G.B. Porro, Inflammatory bowel disease: new insights into pathogenesis and treatment. Journal of Internal Medicine, 252(6), 2002, 475–496.
[165] R. Brůhová Michalčíková, K. Dryahina, P. Španěl, SIFT-MS quantification of several breath biomarkers of inflammatory bowel disease, IBD: A detailed study of the ion chemistry, International Journal of Mass Spectrometry, 396, 2016, 35–41.
[166] L.C. Hicks, J. Huang, S. Kumar, S.T. Powles, T.R. Orchard, G.B. Hanna, H.R.T. Williamsa, Analysis of exhaled breath volatile organic compounds in inflammatory bowel disease: a pilot study, Journal of Crohn's and Colitis, 9(9), 2015, 731–737.
[167] N. Patel, N. Alkhouri, K. Eng, F. Cikach, L. Mahajan, C. Yan, D. Grove, E.S. Rome, R. Lopez, R.A. Dweik, Metabolomic analysis of breath volatile organic compounds reveals unique breathprints in children with inflammatory bowel disease: a pilot study, Alimentary Pharmacology & Therapeutics, 40, 2014, 498–507.
[168] K. Dryahina, P. Španěl, V. Pospíšilová, K. Sovová, L. Hrdlička, N. Machková, M. Lukáš, D. Smith, Quantification of pentane in exhaled breath a potential biomarker of bowel disease, using selected ion flow tube mass spectrometry, Rapid Communications in Mass Spectrometry, 27(17), 2013, 1983–1992.
[169] R. Brůhová Michalčíková, K. Dryahina, P. Španěl, A detailed study of the ion chemistry of alkenes focusing on heptenes aimed at their SIFT-MS quantification, International Journal of Mass Spectrometry 425, 2018, 16–21.
[170] J. Burisch, T. Jess, M. Martinato, P.L. Lakatos, The burden of inflammatory bowel disease in Europe. Journal of Crohn's and Colitis, 7(4), 2013, 322–337.
[171] B.E. Sands, From symptom to diagnosis: clinical distinctions among various forms of intestinal inflammation. Gastroenterology, 126(6), 2004, 1518–1532.
[172] W.J. Sandborn, B.G. Feagan, S.B. Hanauer, H. Lochs, R. Löfberg, R. Modigliani, D.H. Present, P. Rutgeerts, J. Schölmerich, E.F. Stange, L.R. Sutherland, A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. Gastroenterology, 122(2), 2002, 512–530.
[173] S.C.Truelove, L.J. Witts, Cortisone in Ulcerative Colitis. BMJ, 2(4947), 1955, 1041–1048.
[174] R.S. Walmsley, R.C.S. Ayres, R.E. Pounder, R.N. Allan, A simple clinical colitis activity index. Gut, 43(1), 1998, 29–32.
[175] P. Munkholm, E. Langholz, M. Davidsen, V. Binder, Disease Activity Courses in a Regional Cohort of Crohn's Disease Patients. Scandinavian Journal of Gastroenterology, 30(7), 1995, 699–706.
[176] E. Langholz, P. Munkholm, M. Davidsen, V. Binder, Course of ulcerative colitis: Analysis of changes in disease activity over years. Gastroenterology, 107(1), 1994, 3–11.
[177] K.V. Winther, T. Jess, E. Langholz, P. Munkholm, V. Binder, Survival and cause-specific mortality in ulcerative colitis: follow-up of a population-based cohort in Copenhagen County. Gastroenterology, 125(6), 2003, 1576–1582.
[178] T. Card, R. Hubbard, R.F. Logan, Mortality in inflammatory bowel disease: a population-based cohort study. Gastroenterology, 125(6), 2003, 1583–1590.

[179] Patent: Universiteit Maastricht, Academish Ziekenhuis Maastricht. Method for the diagnosis of Inflammatory Bowel Disease by detecting volatile organic compounds in exhaled air. Inventors: Jan Willem Dallinga et al. EP 2 315 025 A1. Appl. no. 09173486.3. Int. Cl.: G01N 33/497 (2006.01). Life Science Patents.
[180] Patent: Universiteit Maastricht, Academish Ziekenhuis Maastricht. Method for the differentiation between Crohn's disease and ulcerative colitis by detecting volatile organic compounds in exhaled air. Inventors: Jan Willem Dallinga et al. EP 2 315 029 A1. Appl. no. 09173487.7. Int. Cl.: G01N 33/68 (2006.01). Life Science Patents.
[181] Patent: Universiteit Maastricht, Academish Ziekenhuis Maastricht. Method for the differentiation between patients with active Crohn's disease and patients in remission by detecting volatile organic compounds in exhaled air. Inventors: Jan Willem Dallinga et al. EP 2 315 030 A1. Appl. no. 09173488.9. Int. Cl.: G01N 33/68 (2006.01). Life Science Patents.
[182] Patent: Universiteit Maastricht, Academish Ziekenhuis Maastricht. Method for the differentiation between patients with active ulcerative colitis and patients in remission by detecting volatile organic compounds in exhaled air. Inventors: Jan Willem Dallinga et al. EP 2 315 031 A1. Appl. no. 09173489.7. Int. Cl.: G01N 33/68 (2006.01). Life Science Patents.
[183] R.B. Michalcikova, K. Dryahina, P. Španěl, SIFT-MS quantification of several breath biomarkers of bowel disease, IBD: a detailed study of the ion chemistry. Poster session presented at: IABR summit 2015-IABR's 10th anniversary conference, 2015 September 15-16, Vienna
[184] M. Phillips, R.N. Cataneo, R. Condos, G.A. Ring Erickson, J. Greenberg, V. La Bombardi, M.I. Munawar, O. Tietje, Volatile biomarkers of pulmonary tuberculosis in the breath. Tuberculosis, 87(1), 2007, 44–52.
[185] M. Syhre, L. Manning, S. Phuanukoonnon, P. Harino, S.T. Chambers, The scent of Mycobacterium tuberculosis – Part II breath. Tuberculosis, 89(4), 2009, 263–266.
[186] J. Julák, E. Stránská, V. Rosová, H. Geppert, P. Španěl, D. Smith, Bronchoalveolar lavage examined by solid phase microextraction, gas chromatography–mass spectrometry and selected ion flow tube mass spectrometry. Journal of Microbiological Methods, 65(1), 2006, 76–86.
[187] J.R. Riordan, J.M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J.L. Chou, Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA, Science, 245(4922), 1989, 1066-1073.
[188] P.M. Quinton, Cystic fibrosis: lessons from the sweat gland, Physiology, 22(3), 2007, 212-225.
[189] B.W. Ramsey, S. Banks-Schlegel, F.J. Accurso, R.C. Boucher, G.R. Cutting, J.F. Engelhardt, W.B. Guggino, C.L. Karp, M.R. Knowles, J.K. Kolls, Future Directions in Early Cystic Fibrosis Lung Disease Research, American Journal of Respiratory and Critical Care Medicine, 185(8), 2012, 887–892.
[190] J.R.W. Govan, V. Deretic, Microbial pathogenesis in cystic fibrosis: Mucoid Pseudomonas aeruginosa and Burkholderia cepacia, Microbiological Reviews, 60 (3), 1996, 539-574.
[191] D. Tubbs, W. Lenney, P. Alcock, C.A. Campbell, J. Gray, C. Pantin, Pseudomonas aeruginosa in cystic fibrosis: cross-infection and the need for segregation, Respiratory Medicine, 95(2), 2001,147-152.
[192] W. Carroll, W. Lenney, T. Wang, P. Španěl, A. Alcock, D. Smith, Detection of volatile compounds emitted by Pseudomonas aeruginosa using selected ion flow tube mass spectrometry, Pediatric Pulmonology, 39(5), 2005, 452-456.

[193] F.J. Gilchrist, A. Alcock, J. Belcher, M. Brady, A. Jones, D. Smith, P. Španěl, K. Webb, W. Lenney, Variation in hydrogen cyanide production between different strains of Pseudomonas aeruginosa, European Respiratory Journal, 38(2), 2011, 409-414.
[194] B. Enderby, D. Smith, W. Carroll, W. Lenney, Hydrogen Cyanide as a Biomarker for Pseudomonas Aeruginosa in the Breath of Children With Cystic Fibrosis, Pediatric Pulmonology, 44(2), 2009, 142-147.
[195] C.M. Robroeks, J.J.B.N. Van Berkel, J.W. Dallinga, Q. Jöbsis, L.J.I. Zimmermann, H.J.E. Hendriks, M.F.M. Wouters, C.P.M. Van Der Grinten, K.D.G. Van De Kant, F.J. Van Schooten, Metabolomics of volatile organic compounds in cystic fibrosis patients and controls, Pediatric Research, 68(1), 2010, 75-80.
[196] P. Španel, D. Smith, Volatile compounds in health and disease, Current Opinion in Clinical Nutrition & Metabolic Care, 14(5), 2011, 455-460.
[197] V. Shestivska, A. Nemec, P. Dřevínek, K. Sovová, K. Dryahina, P. Španěl, Quantification of methyl thiocyanate in the headspace of Pseudomonas aeruginosa cultures and in the breath of cystic fibrosis patients by selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 25(17), 2011, 2459–2467.
[198] F.J. Gilchrist, R.J. Bright-Thomas, A.M. Jones, D. Smith, P.Španěl, A.K. Webb, W. Lenney, 137 Hydrogen cyanide concentrations in the breath of adult cystic fibrosis patients with and without Pseudomonas aeruginosa infection. Journal of Cystic Fibrosis, 12, 2013, S83.
[199] F.J. Gilchrist, J. Belcher, A.M. Jones, D. Smith, A.R. Smyth, K.W. Southern, P. Španěl, A.K. Webb, W. Lenney on behalf of the SPACE Study Team, Exhaled breath hydrogen cyanide as a marker of earlyPseudomonas aeruginosa infection in children with cystic fibrosis. ERJ Open Research, 1(2), 2015, 00044–2015.
[200] V. Shestivska, K. Dryahina, J. Nunvář, K. Sovová, D. Elhottová, A. Nemec, D. Smith, P. Španěl, Quantitative analysis of volatile metabolites releasedin vitroby bacteria of the genus Stenotrophomonasfor identification of breath biomarkers of respiratory infection in cystic fibrosis. Journal of Breath Research, 9(2), 2015, 027104.
[201] K. Dryahina, K. Sovová, A. Nemec, P. Španěl, Differentiation of pulmonary bacterial pathogens in cystic fibrosis by volatile metabolites emitted by the in vitro cultures: Pseudomonas aeruginosa, Staphylococcus aureus, Stenotrophomonas maltophiliaand theBurkholderia cepaciacomplex. Journal of Breath Research, 10(3), 2016, 037102.
[202] D. Smith, K. Sovová, K. Dryahina, T. Doušová, P. Dřevínek, P. Španěl, Breath concentration of acetic acid vapour is elevated in patients with cystic fibrosis. Journal of Breath Research, 10(2), 2016, 021002.
[203] P. Španěl, K. Sovová, K. Dryahina, T. Doušová, P. Dřevínek, D. Smith, Acetic acid is elevated in the exhaled breath of cystic fibrosis patients. Journal of Cystic Fibrosis, 16(5),2017, e17–e18.
[204] F. Argnani, M.D. Camillo, V. Marinaro, T. Foglietta, V. Avallone, C. Cannella, P. Vernia, Hydrogen breath test for the diagnosis of lactose intolerance, is the routine sugar load the best one? World Journal of Gastroenterology, 14(40), 2008, 6204.
[205] U.C. Ghoshal, How to Interpret Hydrogen Breath Tests. Journal of Neurogastroenterology and Motility, 17(3), 2011, 312–317.
[206] D.A. Lindberg, Hydrogen Breath Testing in Adults. Gastroenterology Nursing, 33(Supplement), 2010, 8–13.
[207] E. Aprea, L. Cappellin, F. Gasperi, F. Morisco, V. Lembo, A. Rispo, R. Tortora, P.Vitaglione, N. Caporaso, F. Biasioli, Application of PTR-TOF-MS to investigate metabolites in exhaled breath of patients affected by coeliac disease under gluten free diet. Journal of Chromatography B, 966, 2014, 208–213.

[208] P.A. Sobotka, D.K. Gupta, D.M. Lansky, M.R. Costanzo, E.J. Zarling, Breath pentane is a marker of acute cardiac allograft rejection. Breath pentane is a marker of acute cardiac allograft rejection. The Journal of heart and lung transplantation: the official publication of the International Society for Heart Transplantation, 13(2), 1994, 224-229.
[209] D.W. Holt, A. Johnston, J.D. Ramsey, Breath pentane and heart rejection. The Journal of heart and lung transplantation, 13(6), 1994, 1147-1148.
[210] S.M. Studer, J.B. Orens, I. Rosas, J.A. Krishnan, K.A. Cope, S. Yang, J.V. Conte, P.B. Becker, T.H. Risby, Patterns and significance of exhaled-breath biomarkers in lung transplant recipients with acute allograft rejection. The Journal of Heart and Lung Transplantation, 20(11), 2001, 1158–1166.
[211] M. Phillips, J.P. Boehmer, R.N. Cataneo, T. Cheema, H.J. Eisen, J.T. Fallon, P.E. Fisher, A. Gass, J. Greenberg, J. Kobashigawa, D. Mancini, B. Rayburn, M.J. Zucker, Heart allograft rejection: detection with breath alkanes in low levels (the HARDBALL study). The Journal of Heart and Lung Transplantation, 23(6), 2004, 701–708.
[212] M. Phillips, J.P. Boehmer, R.N. Cataneo, T. Cheema, H.J. Eisen, J.T. Fallon, P.E. Fisher, A. Gass, J. Greenberg, J. Kobashigawa, D. Mancini, B. Rayburn, M.J. Zucker, Prediction of heart transplant rejection with a breath test for markers of oxidative stress. The American Journal of Cardiology, 94(12), 2004, 1593–1594.
[213] D. Smith, P. Španěl, J. Herbig, J. Beauchamp, Mass spectrometry for real-time quantitative breath analysis. Journal of Breath Research 8(2), 2014, 027101.
[214] H. Borsdorf, G.A. Eiceman, Ion Mobility Spectrometry: Principles and Applications. Applied Spectroscopy Reviews, 41(4), 2006, 323–375.
[215] V. Ruzsanyi, P. Mochalski, A. Schmid, H. Wiesenhofer, M. Klieber, H. Hinterhuber, A. Amann, Ion mobility spectrometry for detection of skin volatiles. Journal of Chromatography B, 911, 2012, 84–92.
[216] M. Westhoff, P. Litterst, L. Freitag, W. Urfer, S. Bader, J.-I. Baumbach, Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. Thorax, 64(9), 2009, 744–748.
[217] T. Perl, E. Carstens, A. Hirn, M. Quintel, W. Vautz, J. Nolte, M. Jünger, Determination of serum propofol concentrations by breath analysis using ion mobility spectrometry. British Journal of Anaesthesia, 103(6), 2009, 822–827.
[218] M.R. McCurdy, Y. Bakhirkin, G. Wysocki, R. Lewicki, F.K. Tittel, Recent advances of laser-spectroscopy-based techniques for applications in breath analysis. Journal of Breath Research, 1(1), 2007, 014001.
[219] O. Vaittinen, F. Manfred Schmidt, M. Metsala, L. Halonen, Exhaled Breath Biomonitoring Using Laser Spectroscopy. Current Analytical Chemistry, 9(3), 2013, 463–475.
[220] H. Shurmer, A. Fard, J. Barker, P. Bartlett, G. Dodd, U. Hayat, Development of an electronic nose. Physics in Technology, 18(4), 1987, 170–176.
[221] B. Li, G. Sauvé, M.C. Iovu, M. Jeffries-EL, R. Zhang, J. Cooper, S. Santhanam, L. Schultz, J.C. Revelli, A.G. Kusne, T. Kowalewski, J.L. Snyder, L.E. Weiss, G.K. Fedder, R.D. McCullough, D.N. Lambeth, Volatile Organic Compound Detection Using Nanostructured Copolymers. Nano Letters, 6(8), 2006, 1598–1602.
[222] M. Righettoni, A. Tricoli, S. Gass, A. Schmid, A Amann, S.E. Pratsinis, Breath acetone monitoring by portable Si:WO3 gas sensors. Analytica Chimica Acta, 738, 2012, 69–75.
[223] Franzblau, A., Levine, S. P., Burgess, L. A., Qu, Q., Schreck, R. M., & D'Arcy, J. B. (1992). The use of a transportable fourier transform infrared (FTIR) spectrometer for the direct measurement of solvents in breath and ambient air – I: Methanol. American Industrial Hygiene Association Journal, 53(4), 221–227.

[224] J. Griffiths, A brief history of mass spectrometry, Analytical Chemistry, 80(15), 2008, 5678-5683.
[225] K.D. Bartle, P. Myers, History of gas chromatography, TrAC Trends in Analytical Chemistry, 21 (2002) 547-557.
[226] E. Matisova, M. Dömötörová, Fast gas chromatography and its use in trace analysis, Journal of Chromatography A, 1000(1-2), 2003, 199-221.
[227] M.S. Klee, L.M. Blumberg, Theoretical and practical aspects of fast gas chromatography and method translation, Journal of Chromatographic Science, 40(5), 2002, 234-247.
[228] W. Filipiak, A. Sponring, A. Filipiak, C. Ager, J. Schubert, W. Miekisch, A. Amann, J. Troppmair, TD-GC-MS analysis of volatile metabolites of human lung cancer and normal cells in vitro, Cancer Epidemiology Biomarkers & Prevention, 19(1), 2010, 182-195.
[229] V.T. Virkki, R.A. Ketola, M. Ojala, T. Kotiaho, V. Komppa, A. Grove, S. Facchetti, On-site environmental analysis by membrane inlet mass spectrometry, Anal. Chem., 67 (1995) 1421-1425.
[230] A. Taylor, R. Linforth, B. Harvey, A. Blake, Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release, Food Chemistry, 71 (2000) 327-338.
[231] F.M. Benoit, W. Davidson, A. Lovett, S. Nacson, A. Ngo, Breath analysis by atmospheric pressure ionization mass spectrometry, Anal. Chem., 55 (1983) 805-807.
[232] B. Thomson, W. Davidson, A. Lovett, Applications of a versatile technique for trace analysis: atmospheric pressure negative chemical ionization, Environmental Health Perspectives, 36, 1980, 77.
[233] I. Déléris, A. Saint‐Eve, E. Sémon, H. Guillemin, E. Guichard, I. Souchon, J.L. Le Quéré, Comparison of direct mass spectrometry methods for the on-line analysis of volatile compounds in foods. Journal of Mass Spectrometry, 48(5), 2013, 594–607.
[234] L. Zhu, G. Gamez, H.W. Chen, H.X. Huang, K. Chingin, R. Zenobi, Real-time, on-line monitoring of organic chemical reactions using extractive electrospray ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 22(19), 2008, 2993–2998.
[235] J.J. Zhu, H.D. Bean, Y.M. Kuo, J.E. Hill, Fast Detection of Volatile Organic Compounds from Bacterial Cultures by Secondary Electrospray Ionization-Mass Spectrometry. Journal of Clinical Microbiology, 48(12), 2010, 4426–4431.
[236] W. Lindinger, A. Hansel, A Jordan, On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) medical applications, food control and environmental research. International Journal of Mass Spectrometry and Ion Processes, 173(3), 1998, 191–241.
[237] A. Amann, The State of Breath Analysis: Achievements and Challenges, Consequences for Applications, in: CONFERENCE SERIES, 2013, pp. 20.
[238] E.E. Ferguson, F.C. Fehsenfeld, A.L. Schmeltekopf Ion-Molecule Reaction Rates Measured in a Discharge Afterglow. Chemical Reactions in Electrical Discharges, 1969, 83–91.
[239] A. Jordan, S. Haidacher, G. Hanel, E. Hartungen, J. Herbig, L. Märk, R. Schottkowsky, H. Seehauser, P. Sulzer, T.D. Märk, An online ultra-high sensitivity Proton-transfer-reaction mass-spectrometer combined with switchable reagent ion capability (PTR+SRI−MS). International Journal of Mass Spectrometry, 286(1), 2009, 32–38.
[240] J. Herbig, M. Müller, S. Schallhart, T. Titzmann, M. Graus, A. Hansel, On-line breath analysis with PTR-TOF. Journal of Breath Research, 3(2), 2009, 027004.

[241] A. Jordan, S. Haidacher, G. Hanel, E. Hartungen, L. Märk, H. Seehauser, R. Schottkowsky, P. Sulzer, T.D. Märk, A high resolution and high sensitivity proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS). International Journal of Mass Spectrometry, 286(2-3), 2009, 122–128.
[242] J. Beauchamp, J. Herbig, J. Dunkl, W. Singer, A. Hansel, On the performance of proton-transfer-reaction mass spectrometry for breath-relevant gas matrices. Measurement Science and Technology, 24(12), 2013, 125003.
[243] C.N. Hewitt, S. Hayward, A. Tani, The application of proton transfer reaction-mass spectrometry (PTR-MS) to the monitoring and analysis of volatile organic compounds in the atmosphere. Journal of Environmental Monitoring, 5(1), 2002, 1–7.
[244] P. Španěl, D. Smith, Advances in On-line Absolute Trace Gas Analysis by SIFT-MS. Current Analytical Chemistry, 9(4), 2013, 525–539.
[245] J. Herbig, A. Amann, Proton Transfer Reaction-Mass Spectrometry Applications in Medical Research. Journal of Breath Research, 3(2), 2009, 020201.
[246] N.G. Adams, D. Smith, The selected ion flow tube (SIFT); A technique for studying ion-neutral reactions. International Journal of Mass Spectrometry and Ion Physics, 21(3-4), 1976, 349–359.
[247] D. Smith, N.G. Adams, Recent advances in flow tubes: Measurement of ion–molecule rate coefficients and product distributions. Gas Phase Ion Chemistry, 1, 1979, 1–44.
[248] D. Smith, N.G. Adams, The Selected Ion Flow Tube (Sift): Studies of Ion-Neutral Reactions. Advances in Atomic and Molecular Physics Volume 24, 1988, 1–49.
[249] D. Smith, N.G. Adams, W. Lindinger, Reactions of the HnS+ ions (n = 0 to 3) with several molecular gases at thermal energies. The Journal of Chemical Physics, 75(7), 1981, 3365–3370.
[250] D. Smith, P. Španěl, Application of ion chemistry and the SIFT technique to the quantitative analysis of trace gases in air and on breath. International Reviews in Physical Chemistry, 15(1), 1996, 231–271.
[251] P. Španěl, D. Smith, Selected ion flow tube: a technique for quantitative trace gas analysis of air and breath. Medical & Biological Engineering & Computing, 34(6), 1996, 409–419.
[252] D. Smith, P. Španěl, The Novel Selected-ion Flow Tube Approach to Trace Gas Analysis of Air and Breath. Rapid Communications in Mass Spectrometry, 10(10), 1996, 1183–1198.
[253] P. Španěl, P. Rolfe, B. Rajan, D. Smith, The selected ion flow tube (SIFT) - A novel technique for biological monitoring. The Annals of Occupational Hygiene, 40(6), 1996, 615–626.
[254] P. Španěl, D. Smith, Selected Ion Flow Tube Mass Spectrometry for On-Line Trace Gas Analysis in Biology and Medicine. European Journal of Mass Spectrometry, 13(1), 2007, 77–82.
[255] P. Španěl, D. Smith, A selected ion flow tube study of the reactions of NO+ and O+2 ions with some organic molecules: The potential for trace gas analysis of air. The Journal of Chemical Physics, 104(5), 1996, 1893–1899.
[256] D. Smith, P. Španěl, Ions in the terrestrial atmosphere and in interstellar clouds. Mass Spectrometry Reviews, 14(4-5), 1995, 255–278.
[257] D. Smith, A. Pysanenko, P. Španěl, Ionic diffusion and mass discrimination effects in the new generation of short flow tube SIFT-MS instruments. International Journal of Mass Spectrometry, 281(1-2), 2009, 15–23.

[258] D. Smith, P. Španěl, SIFT applications in mass spectrometry. In: J.C. Lindon, G.E. Tranter, J.L.Holmes, editors. Encyclopaedia of spectroscopy and spectrometry. London: Academic Press, 1999. pp 2092 – 2105
[259] P. Španěl, K. Dryahina, D. Smith, A general method for the calculation of absolute trace gas concentrations in air and breath from selected ion flow tube mass spectrometry data. International Journal of Mass Spectrometry, 249-250, 2006, 230–239.
[260] P. Španěl, D. Smith, Reactions of Hydrated Hydronium Ions and Hydrated Hydroxide Ions with Some Hydrocarbons and Oxygen-Bearing Organic Molecules. The Journal of Physical Chemistry, 99(42), 1995, 15551–15556.
[261] P. Španěl, D. Smith, Influence of water vapour on selected ion flow tube mass spectrometric analyses of trace gases in humid air and breath. Rapid Communications in Mass Spectrometry, 14(20), 2000, 1898–1906.
[262] P. Španěl, S. Davies, D. Smith, Quantification of ammonia in human breath by the selected ion flow tube analytical method using H3O+ and O2+ precursor ions. Rapid Communications in Mass Spectrometry, 12(12), 1998, 763–766.
[263] K. Dryahina, D. Smith, P. Španěl, Quantification of methane in humid air and exhaled breath using selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 24(9), 2010, 1296–1304.
[264] S. Davies, P. Španel, D. Smith, A new “online” method to measure increased exhaled isoprene in end-stage renal failure. Nephrology Dialysis Transplantation, 16(4), 2001, 836–839.
[265] C.L. Arthur, J. Pawliszyn, Solid phase microextraction with thermal desorption using fused silica optical fibers. Analytical Chemistry, 62(19), 1990, 2145–2148.
[266] S. Ann Scheppers Wercinski, J. Pawliszyn: Solid Phase Microextraction Theory. Marcel Dekker Inc., NY, 1999.
[267] J. Chen, J.B. Pawliszyn, Solid Phase Microextraction Coupled to High-Performance Liquid Chromatography. Analytical Chemistry, 67(15), 1995, 2530–2533.
[268] Z. Zhang, M.J. Yang, J. Pawliszyn, Solid-Phase Microextraction. A Solvent-Free Alternative for Sample Preparation. Analytical Chemistry, 66(17), 1994, 844A–853A.
[269] A.A. Boyd-Boland, J.B. Pawliszyn, Solid-Phase Microextraction Coupled with High-Performance Liquid Chromatography for the Determination of Alkylphenol Ethoxylate Surfactants in Water. Analytical Chemistry, 68(9), 1996, 1521–1529.
[270] C. Grote, J. Pawliszyn, Solid-Phase Microextraction for the Analysis of Human Breath. Analytical Chemistry, 69(4), 1997, 587–596.
[271] M. Chai, J. Pawliszyn, Analysis of Environmental Air Samples by Solid-Phase Microextraction and Gas Chromatography/Ion Trap Mass Spectrometry. Environmental Science & Technology, 29(3), 1995, 693–701.
[272] H. Kataoka, H.L. Lord, J. Pawliszyn, Applications of solid-phase microextraction in food analysis. Journal of Chromatography A, 880(1-2), 2000, 35–62.
[273] E. Woolfenden, Thermal desorption for gas chromatography, in: Gas Chromatography, ed. C.F. Poole, Elsevier, Oxford UK, 2012.
[274] W.J. Lautenberger, E.V. Kring, J.A. Morello, A new personal badge monitor for organic vapors". American Industrial Hygiene Association Journal, 41(10), 1980, 737–747.
[275] E.D. Palmes, A.F. Gunnison, Personal monitoring device for gaseous contaminants. American Industrial Hygiene Association Journal, 34(2), 1973, 78–81.
[276] H.T. Badings, C. de Jong, R.P.M. Dooper, Automatic system for rapid analysis of volatile compounds by purge-and-cold-trapping/capillary gas chromatography. Journal of High Resolution Chromatography, 8(11), 1985, 755–763.

[277] E. Woolfenden, Sorbent-based sampling methods for volatile and semi-volatile organic compounds in air. Journal of Chromatography A, 1217(16), 2010, 2674–2684.
[278] E. Woolfenden, Sorbent-based sampling methods for volatile and semi-volatile organic compounds in air. Part 2. Sorbent selection and other aspects of optimizing air monitoring methods. Journal of Chromatography A, 1217(16), 2010, 2685–2694.
[279] A. Zlatkis, H.A. Lichtenstein, A. Tishbee, Concentration and analysis of trace volatile organics in gases and biological fluids with a new solid adsorbent. Chromatographia, 6(2),1973, 67–70.
[280] R. Brůhová Michalčíková, P. Španěl, A selected ion flow tube study of the ion molecule association reactions of protonated (MH+), nitrosonated (MNO+) and dehydroxidated (M-OH)+ carboxylic acids (M) with H2O, International Journal of Mass Spectrometry 368 (2014) 15-22.
[281] B.M. Ross, Changes in oral trace gas concentrations following orthognathic surgery and intermaxillary fixation: a case study using selected ion flow tube mass spectrometry, International Journal of Oral Science, 3 (3), 2011, 160-164.
[282] B.M. Ross, N. Dadgostar, M. Bloom, L. McKeown, The analysis of oral air using selected ion flow tube mass spectrometry in persons with and without a history of oral malodour, International Journal of Dental Hygiene, 7 (2), 2009, 136-143.
[283] R. Greenwald, A.M. Fitzpatrick, B. Gaston, N.V. Marozkina, S. Erzurum, W.G. Teague, Breath Formate Is a Marker of Airway S-Nitrosothiol Depletion in Severe Asthma, PLoS ONE, 5 (7), 2010, e11919.
[284] N. Sumonsiri, S. A Barringer, Application of SIFT-MS in Monitoring Volatile Compounds in Fruits and Vegetables, Current Analytical Chemistry, 9(4), 2013, 631-641.
[285] X.S. Huang, N. Peng, Effects of Isolated Soy Protein and Ascorbic Acid on Formation of Furan, Acetic and Formic Acids from Fructose, in: Mechanical Properties of Materials and Information Technology, H.H. Tan (Ed.), Springer, Netherlands, 2012.
[286] A. Olivares, K. Dryahina, J.L. Navarro, M. Flores, D. Smith, P. Španěl, Selected Ion Flow Tube-Mass Spectrometry for Absolute Quantification of Aroma Compounds in the Headspace of Dry Fermented Sausages, Analytical Chemistry, 82 (13), 2010, 5819-5829.
[287] P. Španěl, D. Smith, Selected ion flow tube - Mass spectrometry: Detection and real-time monitoring of flavours released by food products, Rapid Communication in Mass Spectrometry, 13 (7), 1999, 585-596.
[288] V. Anicich, An Index of the Literature for Bimolecular Gas Phase Cation-Molecule Reaction Kinetics, JPL Publication 03-19, National Aeronautics and Space Administration, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, 2003.
[289] G. Bouchoux, J.Y. Salpin, D. Leblanc, A relationship between the kinetics and thermochemistry of proton transfer reactions in the gas phase, International Journal of Mass Spectrometry and Ion Processes, 153(1), 1996, 37-48.
[290] T. Su, W.J. Chesnavich, Parametization of the ion-polar molecule collision rate-constant by trajectory calculations, The Journal of Chemical Physics, 76(10), 1982, 5183-5185.
[291] K.J. Miller, J. Savchik, J. A new empirical method to calculate average molecular polarizabilities. Journal of the American Chemical Society, 101(24), 1979, 7206–7213.
[292] P. Ŝpaněl, D. Smith, SIFT studies of the reactions of H_3O^+, NO^+ and O_2^+ with a series of volatile carboxylic acids and esters. International Journal of Mass Spectrometry and Ion Processes, 172(1-2), 1998, 137–147.
[293] A. Pysanenko, T. Wang, P. Španěl, D. Smith, Acetone, butanone, pentanone, hexanone and heptanone in the headspace of aqueous solution and urine studied by

selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 23(8), 2009, 1097–1104.
[294] S. G. Lias, J. E. Bartmess, J. F. Liebman, J. L. Holmes, R. D. Levin, W. G. Mallard: Journal of Physical and Chemical Reference Data, *17*(1988), 1-861.
[295] P. Španěl, D. Smith, Selected ion flow tube studies of the reactions of H_3O^+, NO^+, and O_2^+ with several aromatic and aliphatic hydrocarbons. International Journal of Mass Spectrometry, 181(1-3), 1998, 1–10.
[296] D. Smith, A.M. Diskin, Y.F. Ji, P. Španěl, Concurrent use of H_3O^+, NO^+, and O_2^+ precursor ions for the detection and quantification of diverse trace gases in the presence of air and breath by selected ion-flow tube mass spectrometry, Int. J. Mass Spectrom., 209 (1), 2001, 81-97.
[297] K. Sovová, K. Dryahina, P. Španěl, Selected ion flow tube (SIFT) studies of the reactions of H_3O^+, NO^+ and O_2^+ with six volatile phytogenic esters, International Journal of Mass Spectrometry, 300 (1), 2011, 31-38.
[298] D. Smith, T.S. Wang, P. Španěl, Analysis of ketones by selected ion flow tube mass spectrometry, Rapid Commun. Mass Spectrom., 17 (23), 2003, 2655-2660.
[299] K. Eng, N. Alkhouri, F. Cikach, N. Patel, C. Yan, D. Grove, R. Lopez, E. Rome, R.A. Dweik, Analysis of breath volatile organic compounds in children with chronic liver disease compared to healthy controls. Journal of Breath Research, 9(2), 2015, 026002.
[300] M. Hertel, E. Schuette, I. Kastner, S. Hartwig, A.M. Schmidt-Westhausen, R. Preissner, S. Paris, S. Preissner, Volatile organic compounds in the breath of oral candidiasis patients: a pilot study. Clinical Oral Investigations, 22(2), 2017, 721–731.
[301] P. Mochalski, A. Sponring, J. King, K. Unterkofler, J.Troppmair, A. Amann, Release and uptake of volatile organic compounds by human hepatocellular carcinoma cells (HepG2) in vitro. Cancer Cell International, 13(1), 2013, 72.
[302] J. Herbig, M. Seger, I. Kohl, K. Winkler, H.Jamnig, A. Zabernigg, Ch. Baumgartner, A.Hansel: Breath Analysis with PTR-MS: More breath markers for lung cancer. In A. Hansel, J. Dunkl (Eds.), Contributions. 5th International Conference on Proton Transfer Reaction Mass Spectrometry and its Applications (pp. 31-33), 2011, Innsbruck: Universität Innsbruck.
[303] R. Fernández del Río, M.E. O'Hara, P. Pemberton, T. Whitehouse, C.A. Mayhew, Elimination characteristics of post-operative isoflurane levels in alveolar exhaled breath via PTR-MS analysis. Journal of Breath Research, 10(4), 2016, 046006.
[304] K. Dryahina, D. Smith, P. Španěl, Quantification of volatile compounds released by roasted coffee by selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry, 32(9), 2018, 739–750.
[305] W. Filipiak, V. Ruzsanyi, P. Mochalski, A. Filipiak, A. Bajtarevic, C. Ager, H. Denz, W. Hilbe, H. Jamnig, M. Hackl, A. Dzien, A. Amann, Dependence of exhaled breath composition on exogenous factors, smoking habits and exposure to air pollutants. Journal of Breath Research, 6(3), 2012, 036008.
[306] A. Spesyvyi, D. Smith, P. Španěl, Selected Ion Flow-Drift Tube Mass Spectrometry: Quantification of Volatile Compounds in Air and Breath. Analytical Chemistry, 87(24), 2015, 12151–12160.
[307] A. T. Rioseras, K.D. Singh, N.Nowak, M.T.Gaugg, T. Bruderer, R. Zenobi, P. M.-L. Sinues, Real-Time Monitoring of Tricarboxylic Acid Metabolites in Exhaled Breath. Analytical Chemistry, 90(11), 2018, 6453-6460.
[308] J. F. de la Mora, Ionization of vapor molecules by an electrospray cloud. International Journal of Mass Spectrometry, 300(2-3), 2011, 182–193.

[309] P. Salvo, C. Ferrari, R. Persia, S. Ghimenti, T. Lomonaco, F. Bellagambi, F. Di Francesco, A dual mode breath sampler for the collection of the end-tidal and dead space fractions. Medical Engineering & Physics, 37(6), 2015, 539–544.
[310] M. F. Shostakovskii, A. S. Atabin, B. A. Trofimov, A. V. Gusarov, Reaction of 2-methyl-1,3-dioxolane with hydrogen sulfide. Bulletin of the Academy of Sciences of the USSR 14 (6), 1965, 1032–1033.
[311] L. Bregy, A.R. Müggler, P. Martinez-Lozano Sinues, D. García-Gómez, Y. Suter, G.N. Belibasakis, M. Kohler, P.R. Schmidlin, R. Zenobi, Differentiation of oral bacteria in in vitro cultures and human saliva by secondary electrospray ionization – mass spectrometry. Scientific Reports, 5(1), 2015, 15163.
[312] R. Brůhová Michalčíková, K. Dryahina, D.Smith, P. Španěl, Volatile compounds released by Nalophan; implications to SIFT-MS and other chemical ionisation mass spectrometry analytical methods. Rapid Communications in Mass Spectrometry, In press, 2019, Accepted 13th September 2019.
[313] D. Smith, T. W. E. Chippendale, P. Španěl, Reactions of the selected ion flow tube mass spectrometry reagent ions H3O+ and NO+ with a series of volatile aldehydes of biogenic significance. Rapid Communications in Mass Spectrometry, 28(17), 2014, 1917–1928.
[314] P. Španěl, T. S. Wang, D. Smith, A selected ion flow tube, SIFT, study of the reactions of H3O+, NO+ and O2+ ions with a series of diols. International Journal of Mass Spectrometry, 218(3), 2002, 227–236.
[315] P. Španěl, J. M. Van Doren, D. Smith, A selected ion flow tube study of the reactions of H_3O^+, NO^+, and O_2^+ with saturated and unsaturated aldehydes and subsequent hydration of the product ions. *International Journal of Mass Spectrometry, 213(2-3),* 2002, *163–176.*
[316] C. Turner, P. Španěl, D. Smith, A longitudinal study of ethanol and acetaldehyde in theexhaled breath of healthy volunteers using selected-ion flow-tube mass spectrometry. Rapid Communications in Mass Spectrometry, 20(1), 2006, 61–68.