

On-line breath analysis with PTR-TOF

Jens Herbig¹, Markus Müller², Simon Schallhart², Thorsten Titzmann¹,
Martin Graus² and Armin Hansel^{1,2}

¹ Ionimed Analytik GmbH, Innsbruck, Austria

² Institute of Ion Physics and Applied Physics, Leopold-Franzens University, Innsbruck, Austria

E-mail: Jens.Herbig@ionimed.com

Received 9 March 2009

Accepted for publication 15 May 2009

Published 9 June 2009

Online at stacks.iop.org/JBR/3/027004

Abstract

We report on on-line breath gas analysis with a new type of analytical instrument, which represents the next generation of proton-transfer-reaction mass spectrometers. This time-of-flight mass spectrometer in combination with the soft proton-transfer-reaction ionization (PTR-TOF) offers numerous advantages for the sensitive detection of volatile organic compounds and overcomes several limitations. First, a time-of-flight instrument allows for a measurement of a complete mass spectrum within a fraction of a second. Second, a high mass resolving power enables the separation of isobaric molecules and the identification of their chemical composition. We present the first on-line breath measurements with a PTR-TOF and demonstrate the advantages for on-line breath analysis. In combination with buffered end-tidal (BET) sampling, we obtain a complete mass spectrum up to 320 Th within one exhalation with a signal-to-noise ratio sufficient to measure down to pptv levels. We exploit the high mass resolving power to identify the main components in the breath composition of several healthy volunteers.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Breath gas analysis of volatile organic compounds (VOCs) has become a growing field of research in recent years, promising non-invasive diagnosis with many potential clinical applications such as the detection of certain diseases, therapy monitoring and drug testing [1–4]. The gas matrix of exhaled breath is a rich composition with far more than 500 VOCs identified to date [1]. In the continuing search for hitherto unknown marker compounds, many different instrumental approaches for VOC detection and characterization are being implemented. Analytical instruments that are capable of detecting a large variety of VOCs, such as mass-spectrometric (MS) techniques, are particularly well suited for this application. This is reflected by the numerous publications in which such instruments, including gas-chromatography MS (GC-MS) [5–8], ion-mobility spectrometry (IMS) [9], often used in combination with a pre-separating GC column (GC-IMS) [10], selected-ion-flow-tube MS (SIFT-MS) [11, 12] and proton-transfer-reaction MS (PTR-MS) [13, 14], have been applied to analyse breath gas samples. A gold standard for the analysis of breath VOCs has not yet been found. A

good candidate is GC-MS, which lacks, however, an important feature: the requirement of sample pre-treatment excludes the possibility of measuring samples on-line.

On-line breath gas analysis offers several advantages: first, it offers the possibility of delivering immediate results, which can be important when breath analysis is applied as a clinical test. Second, on-line sampling avoids complications arising due to the collection and storage of breath samples [15–19] and compound loss during sample pre-treatment, e.g. loss of polar compounds, is largely avoided. Also, the measurement of unstable compounds that undergo rapid degradation may only be possible by on-line analysis. And finally, the complete breathing cycle can be monitored and the end-tidal breath phase (and that of the background room-air) can be separately selected in the data processing step. Incorrect or distorted exhalations can be identified and filtered. Imperfections in the sampling setup, e.g. unwanted condensation of water vapour, can be recognized immediately.

There are several requirements for instruments to perform on-line breath analysis: a high sensitivity for VOC measurements, a fast response time and direct analysis without the need for sample pre-treatment. PTR-MS instruments

meet all these criteria. Their fast response times and the high sensitivity with limits of detection in the pptv range make them suitable tools for on-line analysis of end-tidal breath, which has been demonstrated recently [20, 21]. Furthermore, the soft ionization by proton transfer reaction with a relatively low degree of fragmentation is especially useful for the analysis of a complex gas matrix. There are several complications, however, which impose limitations on the measurement process. For quadrupole mass spectrometers, only one mass channel can be monitored at a time. In order to monitor several mass channels the detector has to switch between the m/z (mass-to-charge ratio entities) of interest. Thus the duty cycle is inversely proportional to the number of mass channels. In on-line breath analysis, a minimum sampling frequency of approximately 3 Hz is needed in order to resolve individual breath phases. There is a trade-off between the number of measured m/z , and the signal-to-noise ratio. This frame sets the limits for the number of simultaneously monitored compounds and their respective limit of detection. Using buffered end-tidal (BET) sampling the detection time and the number of simultaneously monitored compounds can be increased [20]. A general issue is that the mass resolution of quadrupole MS instruments is limited (typically around unity mass resolution) and consequently they lack discrimination between nominally isobaric molecules. Various strategies can be employed to gain additional information, such as the use of different precursor ions [22], isotopic pattern analysis or by promoting fragmentation [23]. Promising solutions are also the combination of the PTR ionization with other techniques, such as an ion trap mass spectrometer for the detection [24] or the pre-separation by a GC column [25]. All these approaches are practical for target analysis, where more information about a few specific compounds is required, but have a limited applicability for on-line breath gas analysis, due to the complex gas matrix or the need for a high time resolution in an on-line measurement.

Recent advances in PTR technology promise to resolve these drawbacks by combining the soft PTR ionization technique, for the sensitive detection of VOCs, with a time-of-flight type mass spectrometer (PTR-TOF). In a PTR-TOF, the ions are accelerated to a uniform energy by an electric field. Subsequently the ions are allowed to traverse without acceleration over a defined distance. The time of flight is directly related to the ion's mass-to-charge ratio. Inherently, the whole mass spectrum can be measured in a single shot, within a fraction of a second. Integrating several shots increases the signal-to-noise ratio. This combination has been first demonstrated by Blake *et al* [26]. The instrument had a typical mass resolution of $>1000 m/\Delta m$, exceeding that of quadrupole PTR-MS instruments by three orders of magnitude. Hence, the separation of some compounds with the same nominal molecular mass but different chemical composition—and therefore different exact mass—becomes feasible. However, the reported sensitivities of the first PTR-TOF instruments were still orders of magnitude lower [26–30] than that of commercial quadrupole PTR-MS instruments, too low for on-line breath gas analysis. As a measure, the primary ion signal reported by Blake *et al* was on the order of 10^3 counts

per second (cps). To reach limits of detection in the ppbv range approximately 1 min of integration time was necessary [26].

The prototype of a PTR-TOF system used for the measurements discussed in this paper has been developed at the University of Innsbruck, Austria [31, 32]. It has a greatly improved sensitivity, which makes it applicable for on-line breath gas analysis, where sub-ppbv limits of detection have to be achieved in comparably short integration times. Our instrument has a high mass resolution of $>5000 m/\Delta m$ (full width at half maximum; FWHM). In this paper, we present the first on-line breath measurements with PTR-TOF. We demonstrate the advantages that this technique offers especially in on-line breath analysis. We implement buffered end-tidal (BET) sampling and test its effect on signal quality. We show that we can measure all ionized volatile organic compounds up to 320 Th from one single exhalation, with a limit-of-detection in the sub-ppbv range. We have performed on-line PTR-TOF breath measurements on several healthy volunteers and report on the composition of their breath. We use the high mass resolving power of the PTR-TOF for the identification of the chemical composition of the most prominent m/z .

2. Materials and methods

2.1. Gas analysis instrumentation—PTR-TOF

For the measurements presented here, we used a prototype PTR-TOF instrument, which has been built at the University of Innsbruck, Austria. A commercial version of this instrument with similar specifications is already available from Ionicon Analytik, Innsbruck, Austria [33]. Both versions of the PTR-TOF have already been employed in several research projects [31, 32, 34–38], where the capabilities of these instruments were successfully demonstrated. Figure 1 shows a schematic drawing of the PTR-TOF instrument. The instrument consists of two parts, the ionization section where volatile organic trace gas compounds are chemically ionized by a proton-transfer reaction (PTR) and the detection section, where the ionized molecules are separated by their mass-to-charge ratio (m/z) by time-of-flight (TOF) MS and are subsequently detected.

The ionization section is identical to that of (quadrupole) PTR-MS instruments (Ionicon Analytik GmbH, Innsbruck, Austria). This ionization technique has been employed in scientific as well as industrial research and has been studied intensely for several years. We therefore omit a detailed discussion and refer the reader to publications comprehensively describing this technique [23, 39, 40]. In the present experiments, the reaction chamber has been operated at a pressure of 2.25 mbar and at a temperature of 60 °C, with an electric drift field of 600 V. This results in an E/N ratio of 132 Td. The choice of the optimal E/N ratio depends on the application (gas matrix) and the compounds of interest. A lower E/N ratio can reduce fragmentation for some compounds, such as isoprene, but leads to an increase in water cluster formation. We have chosen this E/N ratio to obtain results comparable to another PTR-MS breath gas study [21]. We have found that these conditions ensure a low amount of

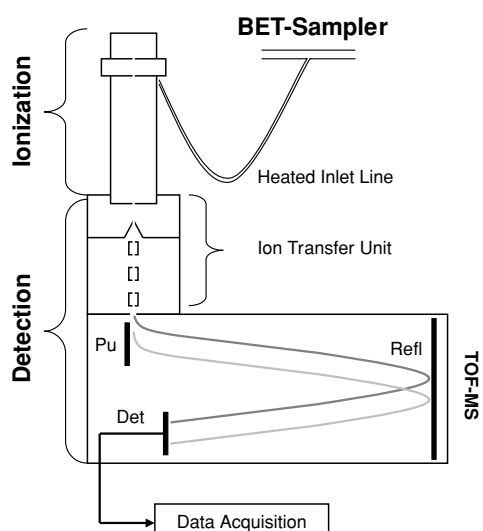


Figure 1. Schematic drawing of a PTR-TOF with a BET sampler, where breath samples are applied. In the ionization section molecules are ionized by a proton-transfer reaction. In the detection section, the ions are collimated in the ion transfer unit and accelerated to a uniform energy by the pusher (Pu). The mass separation is accomplished by a time-of-flight mass spectrometer (TOF-MS). Mid way the ions are reflected (Refl) to elongate the flight path and to re-focus the ion beam into the detector plane (Det) where they are detected using a multi-channel-plate.

clustering, which is important in the presence of high sample gas humidity, and yield moderate fragmentation.

The detection section is connected to the ionization section by the ion transfer unit (figure 1) where the ions are collimated into a parallel beam by an einzel-lens system. Moreover, this section acts as a differential pumping stage. This enables a high vacuum ($<1 \times 10^{-6}$ mbar) in the detection region, which minimizes unwanted collisions. The mass analyser used in this instrument is a high mass resolution, orthogonal acceleration, reflectron TOFMS (G-TOF platform; Tofwerk AG, Thun, Switzerland), in V-configuration, labelled TOF-MS in figure 1. A TOF pusher (figure 1, Pu) periodically extracts (every $30 \mu\text{s}$) the ions by an electric field, which is applied orthogonally to the ion beam. Between the pusher pulses, the ions are not extracted and hit a grounded plate (not shown in figure 1), where they become de-ionized. Lighter ions traverse the pusher region faster, and thus have a higher probability not to be extracted. Therefore, the duty cycle of the pusher directly leads to a mass dependent extraction efficiency.

After acceleration in the electric field of the pusher, the ions traverse through a field-free region and are reversed by a reflector (figure 1, Refl). This re-focuses the ion beam in the detector plane, where the ions are detected by a multi-channel plate (MCP; Burle Industries Inc, Lancaster, PA, USA), labelled Det in figure 1. A high performance time-to-digital converter (TDC) uses the MCP signal to measure the time-of-flight of the ions.

The resulting mass-spectral data are preprocessed by the data acquisition software TofDaqTM (Tofwerk AG, Thun, Switzerland), which controls the timing of the TOF pusher and the TDC. It initializes and starts the data acquisition and it stores series of spectra in HDF5 data format. We chose

to save spectra that were integrated for 333 ms, equivalent to 11 100 TOF pusher extractions. This integration time is an optimal trade-off between a high time resolution to resolve individual breathing cycles and sufficiently high signals for direct evaluation of the data, which can be used to monitor several compounds on-line for a visual control of the breath tests, see below.

The present PTR-TOF has a mass resolution up to 5000 $m/\Delta m$. During our experiments the device featured a typical sensitivity of, e.g., 20 cps ppbv^{-1} for acetone³, with a measured primary ion signal of 4×10^5 cps. More details on the employed instrument can be found in [31, 32, 36]. A publication covering a detailed description and recent improvements of the instrumental setup (the primary ions signal could be increased to more than 1.2×10^6 cps) is under preparation and will soon be submitted by Graus *et al* from the University of Innsbruck. This publication will also contain an updated and more detailed description of the fitting algorithms, outlined below.

2.2. Data analysis

For processing and analysis of the data, we used Matlab R2007b, 7.5 (The Mathworks Inc., MA, USA). In all experiments presented here we recorded a time series of spectra, where one spectrum represents the integrated signal over 333 ms.

A raw time-of-flight spectrum consists of a list of time bins of the TDC with the measured counts per bin. We outline how to convert these data into counts-per-second (cps) measured for a compound of interest, which then can be treated in analogy to standard PTR-MS data analysis. In a first step the time-of-flight information has to be converted into mass information. Then, integrating the counts over a selected mass range, we obtain the total counts measured in this range. Normalizing these counts to the measurement-integration time yields the signal in cps.

TOF-to-mass assignment. The time-of-flight of an ion (t_i) can be converted into mass information (m/z_i) by the simple relation $(m/z_i) = ((t_i - c)/a)^2$ [41]. Temperature variations of the setup can cause a slow change in the length of the ion's flight path, which leads to deviations in the TOF-to-mass assignment. We compensate for this effect by determining the parameters a and c for every spectrum by choosing reference peaks, which are always present. Examples are peaks of the primary ions, parasitic precursor ions (N_2H^+ , NO^+ and O_2^+), and protonated acetone, which is typically found in both exhaled air and laboratory air in sufficient concentrations. This allows for a precise and reproducible conversion of the time-of-flight information into high-resolution mass information. This important step permits the spectralwise addition of a number of spectra to further increase the signal-to-noise-ratio.

Monitoring. For monitoring signals on-line, we can use a simple way to extract information from a TOF spectrum by

³ This is a directly measured value without correction for duty cycle or normalization to primary ions.

integrating the signal measured in a selected mass range. As an example, selecting a mass range of ~ 1.0 Th around 59.0 Th, gives the total of measured ion counts for all compounds with that nominal ionic mass. We typically normalize these counts for the integration time of the measured spectrum, and give these data in counts-per-second (cps). Selecting a narrow range (one FWHM, which is approximately 0.014 Th at 59.0 Th) around the exact mass of a compound, gives the cps proportional to the concentration of this compound alone. In contrast to applying a fit to the data, see the following paragraph, this robust and fast procedure is only possible if peaks are clearly separated, but has the advantage that we can evaluate and display signals on-line during the measurement.

Exact analysis. We have implemented customized algorithms in Matlab for a more elaborate evaluation of the data. In principle the peaks can be fitted by Gaussian functions [32]. The fact that the peak width is a known function of the mass, can be used to separate even strongly overlapping peaks. The exact peak shape in a TOF spectrum has been the subject of intense study, see for example [42]. We observe peaks that slightly differ from an ideal Gaussian shape. We have therefore optimized the fit function using a measured peak with an ideal signal intensity as a reference. A superposition of that peak shape is taken to fit the number of detected isobaric peaks. The area under a fitted peak gives the measured signal in cps. The centre of the peak gives the measured exact mass with an accuracy of 15 ppm,⁴ which is sufficient for the identification of the chemical composition of the compound. As of the publication of this paper, the algorithms include the following chemical compounds to search for a compatible chemical formula: hydrogen H, nitrogen N, carbon C, oxygen O, chlorine Cl and sulfur S.

Linearity of the signal. In principle, the signal response and linearity in PTR-TOF is equal to (quadrupole) PTR-MS, i.e. the signal response is linear up to ~ 10 ppmv, because this is a limitation in the ionization process. However, the detector of a PTR-TOF imposes a further limitation: since only one count-per-time-bin can be detected in one extraction, a higher signal (above approximately 3×10^4 cps per-time-bin in our setup) leads to saturation, which is visible as a truncated peak in the spectrum. We observe such an effect for the primary ion peak (H_3O^+) and the dimer water-cluster peak ($\text{H}_2\text{O}\cdot\text{H}_3\text{O}^+$) at high humidity. Although an adapted fit to a truncated peak would still be possible, a much simpler solution is the measurement of these compounds via their isotopologues.

Correction for pusher duty cycle. As described above, the duty cycle of the pusher leads to a mass dependent extraction efficiency of the ions. This effect can simply be calculated and is corrected for in the data processing, which is also standard practice in other TOF applications. The so corrected primary

ion signal (H_3O^+ , measured via the $\text{H}_3^{18}\text{O}^+$ isotopologue on m/z 21.022 Th) amounts to 4.9×10^6 cps.

Normalization to primary ions. Once the cps measured for the compound of interest have been extracted as described above, further PTR-TOF data normalization can be performed according to standard practice in PTR-MS data analysis. The ion chemistry in the PTR drift tube has been studied for many years with PTR-MS and all knowledge can be directly applied to PTR-TOF. As an example, the normalization to the number of precursor ions could be done by adding the primary ion signal and water-cluster ion signal ($\text{H}_2\text{O}\cdot\text{H}_3\text{O}^+$ measured via its isotopologue on m/z 39.033 Th).

However, throughout this paper we will omit the normalization to primary ions, to reflect the actual sensitivity of the instrument, which would be masked by normalization. This approach is possible, since the primary ion signal was stable (variation $< 2.1\%$) over the course of the experiment.

Furthermore, also standard procedures for calibration [43] can be directly applied to PTR-TOF, which has been used to quantify those signals, which are given in units of ppbv.

2.3. On-line breath sampling setup

The central part of our on-line breath sampling setup is the buffer tube (300 mm length, Teflon tubing, 1/2" ID, inner volume 40 ml), heated to 70 °C. We attach the inlet line of the PTR-TOF instrument to the middle of the buffer tube via a heated inlet tube (1/8" Teflon tubing), see figure 1, BET sampler. By adding a capillary to the inlet line, we adjusted the inlet sampling flow of the instrument to about 50 ml min⁻¹ to optimize for time response and sampling duration. For BET on-line sampling, the test subject exhales through the buffer tube and withdraws after a complete, single exhalation, allowing inhalation and subsequent breathing of room air to proceed aside from the sampler, as described in [20]. The typical gas flow of an exhalation is much higher than the continuous sampling flow into the PTR-MS. During an exhalation, the initial breath phases (dead-space) are quickly blown through the buffer tube. At the end of an exhalation, the flow reaches zero and the last 40 ml of the exhaled breath gas—the end-tidal fraction—remain buffered in the tube. Because the tube has a small dead-space volume, this setup can also be used for direct on-line sampling, where the subject continuously exhales and inhales through the tube.

2.4. Breath measurements

We have performed breath-sampling experiments using direct on-line sampling and BET on-line sampling on three healthy, non-smoking volunteers. The setup was similar to that described in [20]. During the breath tests, the exhalate was continuously drawn from the sampling tube and analysed by the PTR-TOF. TOF spectra were stored for later analysis. We evaluated several signals on-line to monitor the exhalations. We asked all test subjects to perform approximately 3 min of direct on-line sampling, normally in- and exhaling through the sampling tube. After a 3 min break, every volunteer

⁴ This is the maximum deviation of the calculated exact mass to the center mass obtained from a measurement of a calibration gas mixture with known compounds.

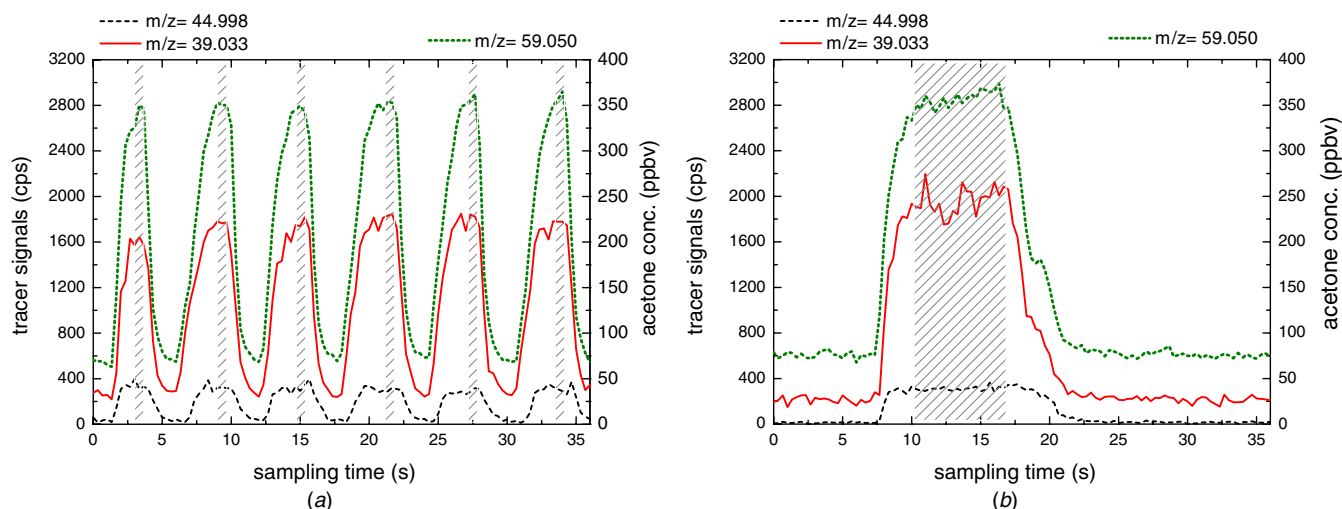


Figure 2. In the displayed measurements a complete spectrum has been obtained by integrating over 333 ms. Here we display only the signals of three exemplary ions: water cluster ion (m/z 39.033 Th) as a measure for the humidity (solid line, red), CO_2H^+ (at m/z 44.998 Th) to monitor the CO_2 content (dashed line, black) and acetone (dotted line, green, at m/z 59.050 Th). (a) These signals have been measured using direct on-line sampling, where a test person ex- and inhales through a sampling tube. The grey areas mark the sampling time for the end-tidal fraction. In direct on-line sampling we use the last 666 ms as end-tidal values. (b) Signals from a BET on-line sampling measurement: a single exhalation is applied and the end-tidal fraction is buffered to extend the sampling time. In buffered end-tidal sampling a single exhalation yields ~ 6.7 s of end-tidal measurement time, exactly 10 times longer than from one direct on-line sampling exhalation. The counts-per-second (cps) have been obtained using the monitoring method, i.e. integration of all counts over a narrow mass range, as described in the text. The conversion of the acetone signal to volume-mixing ratio (ppbv) is done by calibration with a gas standard.

performed a BET on-line sampling breath test: a single exhalation followed by approximately 30 s of analysis time until the measured signals were back at room-air levels. This procedure was repeated at least five times. Our test subjects were asked to exhale normally, but completely, into the buffer tube and then withdraw.

3. Results

3.1. Tracing breath cycles

In a first step, we identify the individual breath phases in the measured data using the signal of tracer compounds. All compounds that originate from the blood-gas exchange in the alveoli and are present in high concentration in a breath sample, such as CO_2 or acetone, can be used for the identification of the different breath phases, as well as using the sample gas humidity, which has been standard practice in PTR-MS breath gas analysis. This is also possible in PTR-TOF by monitoring the water-cluster signal ($\text{H}_2\text{O}\cdot\text{H}_3\text{O}^+$) as explained above. The ionization of CO_2 does not occur in the drift tube of a PTR-(TOF)-MS, since this is an endothermic reaction. As discussed in [21] this reaction, however, can be provoked by using one of the electric lenses (in the present setup the extraction lens, after the reaction chamber) to deliver the excess energy. In PTR-MS, this unwanted effect has to be minimized or compensated, while in PTR-TOF the signal from protonated CO_2 (CO_2H^+) is clearly distinguishable at a centre mass of 44.998 Th.

All measured data have been converted according to the procedures described in section 2.2. We use the simple method for monitoring, to extract the respective signals from each measured spectrum. In figure 2 we show a time series of the

integrated counts for the sample gas humidity and the CO_2 signal. In addition we display the signal of protonated acetone ($\text{C}_2\text{H}_7\text{O}^+$), which serves as an example of an endogenous compound originating from the alveolar blood-gas exchange.

Figure 2(a) displays several subsequent inhalation/exhalation cycles in direct on-line sampling and figure 2(b) shows one exhalation in a BET sampling experiment. We observe that the humidity signal shows the same behaviour as the signal of the alveolar compound (acetone) in both sampling methods. Thus the humidity, measured via the water-cluster ion on m/z 39.033 Th, can be used for tracing of breath cycles. The CO_2 signal shows a plateau phase, which starts earlier and ends later than that of the humidity and acetone. We attribute this to the combination of two effects. The CO_2H^+ signal is positively correlated to the CO_2 concentration but has a negative dependence on the humidity, due to a reverse reaction (de-protonation) in the presence of H_2O [21]. This means that although the CO_2 signal is clearly separated it cannot satisfactorily be applied for identifying exhalation stages.

3.2. Integrated end-tidal mass spectra

Using humidity as a tracer, we identify the end-tidal breath phases and highlight them by the shaded areas in figures 2(a) and (b). For these first experiments the end-tidal phases have been selected visually: for direct on-line sampling we select the last two data points before the sudden decrease; in buffered end-tidal sampling we select the plateau starting ~ 1 s after the onset of the exhalation until shortly before the decrease. With this information, we select the underlying complete mass spectra and perform a spectralwise integration to average the data and improve the signal-to-noise ratio. In that

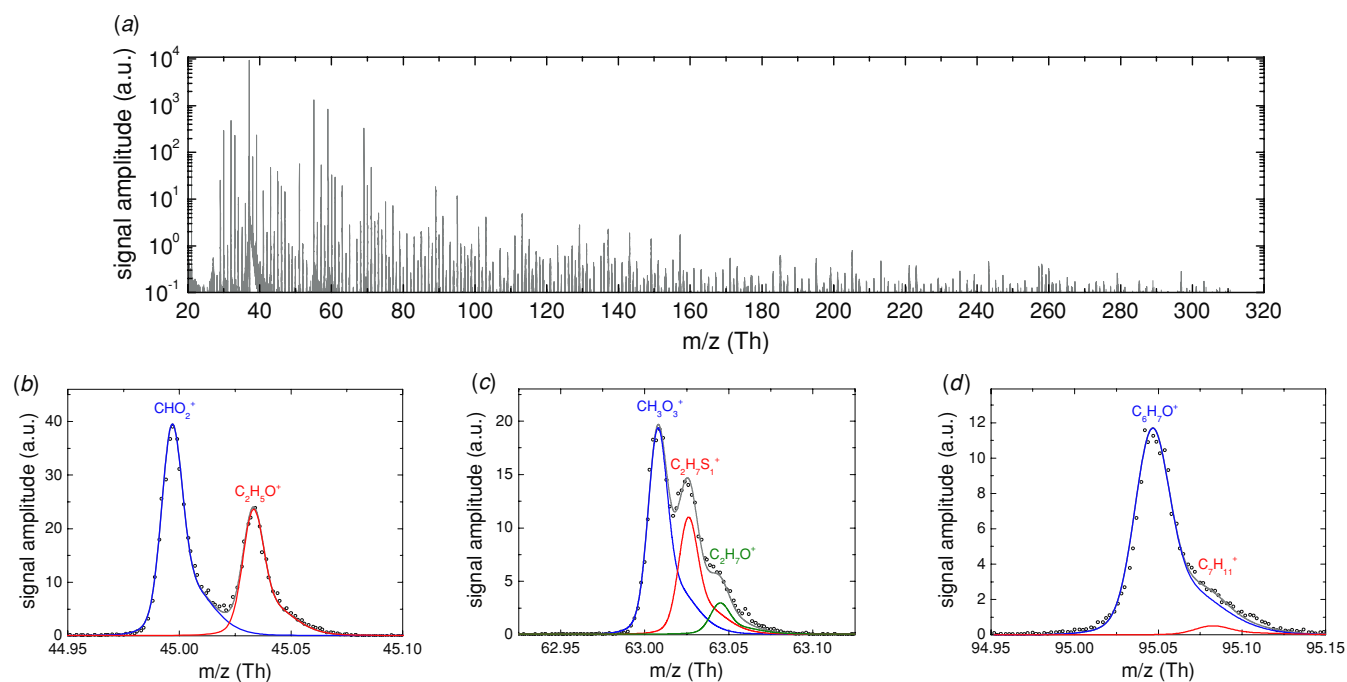


Figure 3. (a) A complete TOF spectrum (logarithmic signal scale) ranging from m/z 20 Th to 320 Th is stored every 333 ms. To improve the signal quality we have integrated approximately 100 spectra (corresponding to 33 s) measured during end-tidal breath phases. A zoom into the spectrum reveals several isobaric compounds, which can be separated with PTR-TOF. We give the mass-to-charge-ratio (m/z) and the identified sum formula of the protonated compound. (b) Around m/z 45 Th: CO_2 (CO_2H^+) and acetaldehyde ($\text{C}_2\text{H}_5\text{O}^+$), (c) m/z 63 Th: CO_2 -water cluster (CH_3O_3^+), dimethylsulfide ($\text{C}_2\text{H}_7\text{S}_1^+$), acetaldehyde-water cluster ($\text{C}_2\text{H}_7\text{O}^+$) and (d) m/z 95 Th: phenol ($\text{C}_6\text{H}_7\text{O}^+$), fragment ($\text{C}_7\text{H}_{11}^+$).

way, the total sampling time for end-tidal signals is increased and the limit-of-detection can be dramatically improved. Figure 3(a) presents a typical full range mass spectrum measured with PTR-TOF. The data have been obtained by integrating the end-tidal phases from five subsequent BET measurements of one test person. The total integration time adds up to about 33 s. The primary ion peak (H_3O^+ , at 19.018 Th) and the water cluster peak ($\text{H}_2\text{O}\cdot\text{H}_3\text{O}^+$ measured at 37.029 Th) are truncated, due to the high signal amplitude, as explained above. The primary ion peak is therefore omitted in the spectrum and the exact count rates are determined using the respective isotopologues. Furthermore, a contribution of the high back of the water cluster peak to the next higher masses (up to m/z 40 Th) is visible. This abundance sensitivity is a typical effect in TOF-MS spectra. Although it is clearly visible in the logarithmic plot in figure 3(a), it contributes only as an almost constant background to a narrow mass peak in the affected range.

In figure 3, the y-axes correspond to cps Th^{-1} . We apply the automated fit routines, outlined in section 2, for an exact analysis of all peaks in this integrated spectrum. As a result, we can clearly separate and identify the chemical composition of about 120 compounds. The data, figures 3(b), (c) and (d), show selected parts of the integrated spectrum. To demonstrate the high mass resolving power, we selected parts of the spectrum where several nominally isobaric compounds are present. Figure 3(b) shows the separation of $\text{C}_2\text{H}_4\text{O}\cdot\text{H}^+$ (protonated acetaldehyde) at an m/z of 45.02 Th and CO_2H^+ (protonated CO_2) at 44.998 Th. Around m/z 63

we observe contributions from three compounds, displayed in figure 3(c). Two compounds are water clusters of protonated CO_2 (CH_3O_3^+ at m/z 63.01 Th) and acetaldehyde ($\text{C}_2\text{H}_7\text{O}^+$ at 63.05 Th), respectively. The third contribution at m/z 63.03 Th has been identified as $\text{C}_2\text{H}_7\text{S}_1^+$, protonated dimethylsulfide. The fact that the expected peak width can be calculated as a function of the mass, can be used to distinguish real contributions from noise. One example is a tiny peak visible in figure 3(c) around 63.06 Th, which is too narrow and can thus be identified as signal noise. In figure 3(d) we plot the spectral range between 94.9 Th and 95.3 Th and observe a dominant peak (at 95.050 Th, identified as $\text{C}_6\text{H}_7\text{O}^+$) and a minor peak (at m/z 95.089 Th identified as $\text{C}_7\text{H}_{11}^+$). The origin of the latter is discussed in section 3.4.

3.3. Limit of detection

We now estimate the limits of detection (LOD) that can be achieved with PTR-TOF in on-line breath analysis. The sensitivity and LOD of the PTR-TOF have been determined in calibration experiments. In the commercial version of this instrument we achieve for 1 s integration time typical LODs below 100 pptv and for 60 s integration time LODs around 10 pptv for most compounds [33]. As can be seen from figure 2(b), we are able to extend the measurement of the end-tidal breath fraction to 7 s using a single exhalation in BET sampling. This means that a detection limit well below 100 pptv can be achieved from a single exhalation. Integrating the end-tidal fractions of nine exhalations, which would result in a total end-tidal measurement time of more than 1 min, we

Table 1. Evaluation of the 15 most prominent nominal m/z in the mass spectrum of healthy non-smoking volunteers. We analyse the individual contributions to each nominal m/z , determine the chemical formula, and give potential compounds, based on the literature for breath composition. The m/z have been classified with A, B, C and D to reflect the specificity when measured with PTR-MS, from A: highly specific to D: a clear interpretation of the signal using unity mass resolution would not be possible.

Nominal m/z (Th)/label	Measured mass (Th)	Empirical formula	Potential compound	Person 1 (cps)	Person 2 (cps)	Person 3 (cps)	Rel. contrib. (%)
45/B	45.00	CHO ₂ ⁺	CO ₂	405	380	408	61
	45.03	C ₂ H ₅ O ⁺	Acetaldehyde	248	224	277	39
47/B	47.00	CH ₃ O ₂ ⁺	Formic acid	8	5	10	2
	47.02	H ₃ N ₂ O ⁺	Instrumental background	124	141	126	26
	47.05	C ₂ H ₇ O ⁺	Ethanol	407	132	528	72
51/A	51.05	CH ₇ O ₂ ⁺	Methanol–water cluster	385	617	559	100
55/A	55.04	H ₇ O ₃ ⁺	Water cluster	12210	14095	12470	100
57/B	57.04	H ₇ ¹⁶ O ₂ ¹⁸ O ⁺	Water cluster isotopologue	139	111	143	19
	57.07	C ₄ H ₉ ⁺	Trans-2-butene	594	551	551	81
59/A	59.05	C ₃ H ₇ O ⁺	Acetone	15714	8957	11376	100
63/C	63.01	CH ₃ O ₃ ⁺	CO ₂ –water cluster	202	203	219	60
	63.03	C ₂ H ₇ S ₁ ⁺	Dimethylsulfide (DMS)	108	111	105	31
	63.05	C ₂ H ₇ O ⁺	Acetaldehyde–water cluster	34	29	31	9
65/B	65.02	C ₂ H ₇ S ₁ ⁺	DMS isotope	17	18	16	17
	65.06	C ₂ H ₉ O ₂ ⁺	Ethanol–water cluster	95	29	121	83
69/A	69.03	C ₄ H ₅ O ₁ ⁺	Furan	2	1	2	0
	69.07	C ₅ H ₉ ⁺	Isoprene	3947	3842	3867	100
71/B	71.05	C ₄ H ₇ O ⁺	MVK, MACR	42	34	75	8
	71.09	C ₅ H ₁₁ ⁺	2-methyl-1-butene	636	570	599	92
75/A	75.05	C ₃ H ₇ O ₂ ⁺	Propionic acid	174	111	198	96
	75.08	C ₄ H ₁₀ O ⁺	Butanol	10	3	8	4
77/A	77.06	C ₃ H ₉ O ₂ ⁺	Acetone–water cluster	169	87	109	100
79/C	79.04	C ₂ H ₇ O ₃ ⁺	Acetic-acid–water cluster	45	22	4	79
	79.06	C ₆ H ₇ ⁺	Benzene	6	9	4	21
89/D	89.04	C ₄ H ₉ S ⁺	Allylmethylsulfide	50	238	46	55
	89.06	C ₄ H ₉ O ₂ ⁺	Butyric acid	77	11	183	45
95/C	95.05	C ₆ H ₇ O ⁺	Phenol	65	162	44	92
	95.09	C ₇ H ₁₁ ⁺	Fragment (C ₇ H ₁₃ O ⁺ –H ₂ O)	8	11	6	8

could achieve a LOD in the lower ppt range. For direct on-line sampling, this could only be reached by integrating the end-tidal part of over 100 breath cycles, when the last 666 ms of each exhalation are taken into account. These numbers only indicate a rough estimate. For the determination of the LOD in on-line breath sampling more factors, such as breath-to-breath variability, background (room–air) concentration, etc, have to be considered, which will not be discussed here.

3.4. Breath composition

We made use of the high mass resolving power of the PTR-TOF for a detailed analysis of the VOCs present in the breath of our test subjects, three healthy, non-smoking volunteers. We give results for the 15 most prominent nominal masses (m/z) in the spectra for all test persons measured with BET sampling. For obvious reasons, we have excluded the contributions from the precursor ions. In table 1 we list the nominal molecular mass of the protonated molecule (m/z), the measured exact mass of the peak, the signal intensity together with the identified empirical formula. On several m/z a number of isobaric compounds contribute to the signal, which can all be clearly separated with PTR-TOF. We calculate the relative contribution to the respective m/z as an average over the three breath tests. We can identify the chemical formula of all contributions and give the potential compound. If there is more than one isomer, we chose the compound based on the literature on breath

VOCs. A special case is the signal on m/z 95.09 Td. There is no protonated compound corresponding to the chemical sum formula C₇H₁₁. Buhr *et al* have investigated typical fragmentation behaviour of several VOCs [44], and found that aldehydes are likely to lose H₂O during the protonation reaction. Taking this into account, we assume that C₇H₁₁ could be a fragment of protonated C₇H₁₂O, potentially heptenal, which has been observed in breath samples [45].

The aim of this experiment was not a precise determination of concentrations, but only the investigation of potential contributions of isobaric molecules to individual m/z . Since we have only analysed the breath of three non-smoking subjects, we cannot draw general conclusions. Nevertheless, there are some trends visible and we will briefly discuss the consequences for the interpretation of quadrupole PTR-MS measurements, where the extra information from the high mass resolution of PTR-TOF is not available. We have classified and labelled the m/z in four categories (A, B, C and D) reflecting the specificity when measured with quadrupole PTR-MS:

- (A) Only one major compound contributes (relative contribution >95%) to these m/z , e.g. 59 Th, 69 Th or 75 Th. The interpretation is straightforward.
- (B) We observe signals of more than one compound on the m/z labelled with B, typically background signals interfering with one VOC signal of interest. The interfering signals are considerably constant, and the

measurement is still specific. For proper quantification, the background has to be subtracted and therefore the limit of detection on these m/z can become higher than it would be without background noise. As an example, the signal of acetaldehyde interferes with CO_2H^+ , which is rather constant under end-tidal conditions. On m/z 47 Th, the instrumental background is constant and can be subtracted. On m/z 57 Th the contribution of the water-cluster isotope is rather constant and can be calculated, since it is directly linked to the signal on m/z 55 Th.

- (C) On m/z labelled with C we observe several overlapping signals. The interfering signals are not constant, i.e. vary between test subjects, and cannot be easily corrected. However, in several cases the signals can be estimated. Furthermore, we observe that in general one of the signals always dominates. An example is the signals on m/z 63 Th.
- (D) On m/z labelled with D we observe more than one signal and there is no general trend. As an example, on m/z 89 Th, two contributing compounds with inverted ratios between breath tests. An exact interpretation without the information yielded by the higher mass resolving power of PTR-TOF is hardly possible.

In general, we observe several signals from (VOC-) water-cluster ions, which are due to the high humidity of the breath gas matrix. This suggests that a higher electric drift field, or more precisely a higher E/N ratio in the reaction chamber, could be useful to further reduce the cluster formation. However, a detailed investigation of this issue is not the subject of this paper.

4. Summarizing conclusions

This paper is the first presentation of on-line breath gas measurements with a proton-transfer reaction time-of-flight mass spectrometer (PTR-TOF). These devices offer several interesting advantages, which can be optimally used for on-line breath analysis.

The sampling time resolution with a lower limit of 30 μs is more than sufficient to resolve fast changing signals, such as breathing cycles. These raw spectra have to be integrated to yield a proper signal, but it is possible to record all raw spectra and integrate them in the data processing for an optimal balance between high sampling frequency and a good signal-to-noise ratio. In our experiments, we have integrated spectra for 333 ms, equivalent to 11 100 raw spectra. These integrated spectra could be evaluated on-line and we could directly monitor the signals of the humidity, acetone and the CO_2 concentration. Using the humidity as a tracer signal, we identified the individual breath phases. In this way, it is possible to determine the data that have been measured during the end-tidal exhalation phase. By integrating these spectra, we can increase the signal-to-noise ratio.

We have also tested the suitability of CO_2 (measured via CO_2H^+) as a tracer signal. Although this signal is clearly separate from those of other VOCs, its nonlinear behaviour in the presence of varying sample gas humidity make it unsuitable as a tracer compound. However, it could be used to determine

the end-tidal CO_2 concentration, when a calibration of the CO_2 signal under different humidity conditions is performed.

We employ two different on-line breath sampling techniques with PTR-TOF. Both methods, direct on-line sampling and buffered end-tidal (BET) sampling, are suitable for breath sampling with PTR-TOF. Using the BET approach we obtain approximately 7 s integration time for sampling the end-tidal (alveolar) fraction from a single exhalation. To obtain the same signal quality in direct on-line sampling we have to evaluate at least ten exhalation cycles, considering that the last 666 ms of each exhalation are integrated. Originally, BET sampling was invented to aid the on-line breath sampling for quadrupole MS instruments yet it also offers several advantages for PTR-TOF measurements, because we can reach LODs below 100 ppt from a single exhalation and even down to single digit ppt levels when the data from several exhalations are integrated. As a consequence, the BET sampling approach is favourable for breath testing spontaneously breathing patients. But also direct on-line sampling can be employed with PTR-TOF when the BET approach is not possible, e.g. in ventilated patients, or when breath phases other than the end-tidal part are of interest.

Although the sensitivity of PTR-TOF instruments has increased dramatically since their first implementation, our PTR-TOF instrument still has a lower sensitivity (and LOD) compared to commercial high-sensitivity quadrupole PTR-MS systems. Nevertheless, it has the important advantage that the integration time does not need to be distributed between several m/z , but a full spectrum is measured simultaneously. Therefore the LOD for a given integration time will be better in PTR-TOF when a large number of compounds are to be monitored. The simultaneous measurement of a complete mass spectrum is particularly useful for clinical screening studies, where a breath test can now be performed in much shorter time, e.g. by a single (BET) exhalation. Furthermore, with its increasing detection efficiency for higher m/z the sensitivity for heavier molecules could already be superior in PTR-TOF. The simultaneous measurement has also the advantage that the measurements of the tracer and the compounds of interest always coincide, which further facilitates the data analysis.

Another significant advantage of our PTR-TOF system is its high mass resolving power, which enables the separation of nominally isobaric molecules according to their chemical composition. The separation of the different contributions greatly improves the specificity of the measurement. The high mass accuracy enables a precise determination of the exact mass and therefore we can identify the chemical composition of a measured compound. This will also improve the LOD compared to quadrupole MS systems since potential background contributions can be separated. Due to this more time-efficient measurement it now becomes feasible to include additional measurements in clinical breath tests, such as breath sampling at different E/N ratios, which can be used to explore the fragmentation behaviour to obtain further chemical information about compounds [23, 24].

We performed breath gas measurements on several healthy, non-smoking volunteers. We made use of the high

mass resolving power and report on the composition of their breath. We evaluated the most prominent m/z and resolved the individual contributions. These data can aid the interpretation of mass spectra in quadrupole PTR-MS measurements. In several cases, the measurement is specific and only one signal is observed on the respective m/z . However, we identified also other m/z where the additional information provided by PTR-TOF is helpful to separate background signals and in some cases an interpretation would not be possible without a high mass resolving power.

The presented experiments were performed with a prototype PTR-TOF system. The outstanding performance of this system, compared to other reported implementations, made it applicable for on-line breath gas analysis, which we demonstrated in this paper. The engineering expertise of this prototype has been incorporated in the construction of a commercial version of this instrument, which is already available [33]. This is an important step towards the clinical applicability and the usability of such a device. We have demonstrated the detailed analysis of the chemical composition of on-line breath samples. The implementation of the mathematical algorithms to allow for an automated and reliable analysis of these complex data is currently in progress.

Up to now, there is no other technique offering such advantages for on-line trace gas analysis and we believe that PTR-TOF will become a valuable and recognized technique for on-line breath gas analysis.

Acknowledgments

The research leading to these results has received funding from the Center of Excellence in Medicine and IT (CEMIT; formerly Kompetenzzentrum Medizin Tirol, KMT, Innsbruck, Austria) and the EU Project BAMOD (Breath-gas analysis for molecular-oriented detection of minimal diseases). The PTR-TOF instrument was developed at the Institute of Ion Physics and Applied Physics at the University of Innsbruck in collaboration with Ionicon Analytik and that project was funded by the Austrian Research Promotion Agency (FFG).

References

- [1] Miekisch W, Schubert J K and Noeldge-Schomburg G F E 2004 *Clin. Chim. Acta* **347** 25–39
- [2] Buszewski B, Keszy M, Ligor T and Amann A 2007 *Biomed. Chromatogr.* **21** 553–66
- [3] Francesco F D, Fuoco R, Trivella M and Ceccarini A 2005 *Microchem. J.* **79** 405–10
- [4] Risby T and Solga S 2006 *Appl. Phys. B* **85** 421–6
- [5] Phillips M, Cataneo R N, Cummin A R C, Gagliardi A J, Gleeson K, Greenberg J, Maxfield R A and Rom W N 2003 *Chest* **123** 2115–23
- [6] Phillips M, Gleeson K, Hughes J M, Greenberg J, Cataneo R N, Baker L and McVay W P 1999 *Lancet* **353** 1930–3
- [7] Preti G, Labows J N, Kostelc J G, Aldinger S and Daniele R 1988 *J. Chromatogr.* **432** 1–11
- [8] Pabst F, Miekisch W, Fuchs P, Kischkel S and Schubert J K 2007 *J. Cardiothorac. Surg.* **2** 37
- [9] Ruzsanyi V and Baumbach J 2005 *Int. J. IMS* **8** 5–8
- [10] Ruzsanyi V, Baumbach J I, Sielemann S, Litterst P, Westhoff M and Freitag L 2005 *J. Chromatogr. A* **1084** 145–51
- [11] Smith D and Spanel P 2005 *Mass Spectrom. Rev.* **24** 661–700
- [12] Spanel P, Davies S and Smith D 1999 *Rapid Commun. Mass Spectrom.* **13** 1733–8
- [13] Moser B, Bodrogi F, Eibl G, Lechner M, Rieder J and Lirk P 2005 *Respir. Physiol. Neurobiol.* **145** 295–300
- [14] Wehinger A, Schmid A, Mechtcheriakov S, Ledochowski M, Grabmer C, Gastl G A and Amann A 2007 *Int. J. Mass Spectrom.* **265** 49–59
- [15] Beauchamp J, Herbig J, Gutmann R and Hansel A 2008 *J. Breath Res.* **2** 046001
- [16] Steeghs M M L, Cristescu S M and Harren F J M 2007 *Physiol. Meas.* **28** 73–84
- [17] van Harreveld A P 2003 *J. Air Waste Manage. Assoc.* **53** 51–61
- [18] Petka J, Etievant P and Callement G 2000 *Analisis* **28** 330–5
- [19] McGarvey L J and Shorten C V 2000 *Am. Ind. Hyg. Assoc. J.* **61** 375–80
- [20] Herbig J, Titzmann T, Beauchamp J, Kohl I and Hansel A 2008 *J. Breath Res.* **2** 037008
- [21] Herbig J 2009 *4th Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series)* ed A Hansel and T D Märk (Innsbruck: Innsbruck University Press)
- [22] Veres P, Roberts J M, Warneke C, Welsh-Bon D, Zahniser M, Herndon S, Fall R and de Gouw J 2008 *Int. J. Mass Spectrom.* **274** 48–55 ISSN 1387–3806
- [23] Lindinger W, Hansel A and Jordan A 1998 *Int. J. Mass Spectrom. Ion. Proc.* **173** 191–241
- [24] Steeghs M, Crespo E and Harren F 2007 *Int. J. Mass Spectrom.* **263** 204–12 ISSN 1387–3806
- [25] Warneke C, Gouw J A D, Kuster W C, Goldan P D and Fall R 2003 *Environ. Sci. Technol.* **37** 2494–501
- [26] Blake R S, Whyte C, Hughes C O, Ellis A M and Monks P S 2004 *Anal. Chem.* **76** 3841–5
- [27] Ennis C, Reynolds J, Keely B and Carpenter L 2005 *Int. J. Mass Spectrom.* **247** 72–80 ISSN 1387–3806
- [28] Inomata S, Tanimoto H, Aoki N, Hirokawa J and Sadanaga Y 2006 *Rapid Commun. Mass Spectrom.* **20** 1025–9
- [29] Tanimoto H, Aoki N, Inomata S, Hirokawa J and Sadanaga Y 2007 *Int. J. Mass Spectrom.* **263** 1–11 ISSN 1387–3806
- [30] Wyche K P, Blake R S, Ellis A M, Monks P S, Brauers T, Koppmann R and Apel E C 2007 *Atmos. Chem. Phys.* **7** 609–20 ISSN 1680–7316
- [31] Graus M, Müller M, Titzmann T, Leck C, Tjernström M and Hansel A 2009 *4th Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series vol 4)* ed A Hansel and J Dunkl (Innsbruck: Innsbruck University Press) pp 103–4
- [32] Müller M, Graus M, Wisthaler A and Hansel A 2009 (*poster*) *4th Int. Conf. on PTR-MS and Its Applications* http://www.ptrms.com/downloads/Mueller_et_al_PTR-TOFMS_PerformancePoster.pdf
- [33] Ionicon Analytik 2008 Scientific results, performance data and application examples of the new ionicon ptr-tof-ms Product fact sheet Ionicon Analytik GmbH http://www.ptrms.com/downloads/facts_HRS_ptr-tof-ms.pdf
- [34] Biasioli F, Aprea E, Odorizzi G, Gasperi F and Märk T D 2009 *4th Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series vol 4)* ed A Hansel and J Dunkl (Innsbruck: Innsbruck University Press) pp 191–2
- [35] Jordan A, Haidacher S, Hanel G, Hartungen E, Märk L, Seehauser H, Schottkowsky R, Sulzer P and Märk T D 2009 *4th Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series vol 4)* ed A Hansel and J Dunkl (Innsbruck University Press) pp 239–43
- [36] Müller M, Metzger A, Graus M, Dommen J, Wisthaler A and Hansel A 2009 *4th Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series vol 4)* ed A Hansel and J Dunkl (Innsbruck University Press) pp 103–4

- [37] Yanagisawa N, Shibata K and Tashiro Y 2009 *4th Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series vol 4)* ed A Hansel and J Dunkl (Innsbruck: Innsbruck University Press) pp 103–4
- [38] Biasioli F, Aprea E, Gasperi F and Märk T D 2009 *Water Sci. Technol.* **59** 1263–9
- [39] Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W and Lindinger W 1995 *Int. J. Mass Spectrom. Ion. Proc.* **149/150** 609–19
- [40] de Gouw J, Warneke C, Karl T, Eerdekens G, Van Der Veen C and Fall R 2003 *Int. J. Mass Spectrom.* **223–4** 365–82
- [41] Brown R S and Gilfrich N L 1991 *Anal. Chim. Acta* **248** 541–52 ISSN 0003–2670
- [42] Ioanoviciu D 1995 *Rapid Commun. Mass Spectrom.* **9** 985–97
- [43] Singer W, Beauchamp J, Herbig J, Dunkl J, Kohl I and Hansel A 2007 *3rd Int. Conf. on PTR-MS and Its Applications (IUP Conf. Series)* ed A Hansel and T D Märk (Innsbruck: Innsbruck University Press) pp 232–4
- [44] Buhr K, van Ruth S and Delahunty C 2002 *Int. J. Mass Spectrom.* **221** 1–7 ISSN 1387–3806
- [45] Phillips M 1997 *Anal. Biochem.* **247** 272–8