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ORIGINAL PAPER

Phase-resolved real-time breath analysis during exercise by means of smart processing of PTR-MS data

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Abstract Separation of inspiratory, mixed expired and alveolar air is indispensable for reliable analysis of VOC breath biomarkers. Time resolution of direct mass spectrometers often is not sufficient to reliably resolve the phases of a breathing cycle. To realise fast on-line breath monitoring by means of direct MS utilising low-fragmentation soft ionisation, a data processing algorithm was developed to identify inspiratory and alveolar phases from MS data without any additional equipment. To test the algorithm selected breath biomarkers (acetone, isoprene, acetaldehyde and hexanal) were determined by means of quadrupole proton transfer reaction mass spectrometry (PTR-MS) in seven healthy volunteers during exercise on a stationary bicycle. The results were compared to an off-line reference method consisting of controlled alveolar breath sampling in Tedlar® bags, preconcentration by solid-phase micro extraction (SPME), separation and identification by GC-MS. Based on the data processing method, quantitative attribution of biomarkers to inspiratory, alveolar and mixed expiratory phases was possible at any time during the experiment, even under respiratory rates up to 60/min. Alveolar concentrations of the breath markers, measured by PTR-MS ranged from 130 to 2,600 ppb (acetone), 10 to 540 ppb (isoprene), 2 to 31 ppb (acetaldehyde), whereas the concentrations of hexanal were always below the limit of detection (LOD) of 3 ppb. There was good correlation between on-line PTR-MS and SPME-GC-MS measurements during phases with stable physiological parameters but results diverged during rapid changes of heart rate and minute ventilation. This clearly demonstrates the benefits of breath-resolved MS for fast on-line monitoring of exhaled VOCs.

Keywords PTR-MS · Data processing algorithm · Stationary bicycle · Breath analysis · SPME-GC-MS

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Introduction

Blood-borne volatile organic compounds (VOCs) are of particular interest in breath analysis, as they may reflect physiological and pathological biochemical processes [1]. However, if concentrations of these substances are to be determined quantitatively, a reliable and reproducible analysis of alveolar air, which has been in direct contact with the blood in the pulmonary capillaries, is necessary [2]. For off-line breath analysis, different methods of controlled alveolar sampling have been described [3–8]. Common parameters used to identify the alveolar phase of exhalation are gas temperature, flow or partial pressure of carbon dioxide (PCO_2).

For continuous monitoring or recognition of fast-changing substance concentrations in breath, direct real-time measurements would be preferable. Direct soft ionisation mass-spectrometric methods such as chemical ionisation (CI) or photo ionisation (PI) [9] mass spectrometry can principally be used for this purpose. In the case of CI-MS-based techniques, the application of proton transfer reaction mass spectrometry technology (PTR-MS) [10] or the selected ion flow tube mass spectrometry approach [11] have been reported. An important aspect for real-time on-line monitoring of human breath is the achievable time resolution of the mass analyser. This is particularly true if the inspiratory and the alveolar expiratory phases shall be resolved. The common quadrupole mass analyser acts as a mass filter and detects analyte ions of one mass-to-charge ratio (*m*/*z*) at one time, and thus need to be scanned for multi-analyte detection, causing a limited time resolution [12, 13]. Therefore, it is often not possible to resolve a full breath cycle (alveolar and mixed expiratory as well as the inspiratory phases) for all compounds under investigation. The detection limit in the real-time mode is typically down to a few ppb, depending on the dwell time of the selected masses. Therefore, reliable breath-to-breath determination of exhaled and inspired substance concentrations becomes difficult when several masses of interest are to be measured at the same time with a sequentially working mass spectrometer. In order to optimise the number of simultaneously detectable substances, this study was intended to develop a data processing algorithm for separation of inspiratory and alveolar phases. For testing and evaluation, this algorithm was applied to determine the blood-borne volatile substances isoprene (cholesterol biosynthesis) [3, 14, 15], acetone (dextrose metabolism, lipolysis) [16], hexanal (oxidative stress) [17] and acetaldehyde (metabolic product of ethanol) [3] in the breath of seven healthy volunteers under physical exercise on a stationary bicycle. In the course of the investigation the following issues were addressed in detail:

(a) Is it possible to develop an algorithm that provides unequivocal identification of both inspiratory and alveolar expiratory phases of the respiratory cycle?
(b) Is it possible to determine blood-borne volatile substances by means of the algorithm under high respiratory rates and rapid physiological changes?
(c) Are the results in agreement with other already validated methods and under which conditions are there any differences?

Experimental

Test design

Seven healthy volunteers (seven males, five non-smokers and two smokers, age 23±3 years) participated in this study. Informed consent was obtained from all volunteers. Breath analyses were performed before and during exercise on a stationary bicycle. The experiment was done in a large hall (>200 m^2, height>8 m) vented with fresh air before the experiment. There was no person traffic during the experiments. Temperature was between 21 and 23 °C.

Measurements

Continuous on-line breath gas sampling by means of PTR-MS and alveolar off-line breath gas sampling in Tedlar® bags for SPME-GC-MS analyses were applied in parallel. A schematic diagram of the experimental setup and the sampling concept is shown in Fig. 1. The test persons wore a head mask during the whole experiment. A Teflon® Y-piece was incorporated into the mask. One opening of the Y-piece was connected to the PTR-MS inlet, the other to the CO_2-controlled SPME sampling system. A small part of the breath sample (50 ml/min) was continuously transferred directly into the PTR-MS inlet by a heated transfer-line (2 m deactivated fused-silica capillary; ID 0.53 mm, MXT Guard Column, BGB-Analytik, Schlossboeckelheim, Germany). The transfer-line was connected to the PTR-MS by a home-built interface and was completely enclosed by a 1/8-in. copper tubing to prevent condensation and cold spots. Both, the inlet and the transfer-line, were heated to a temperature of 60°C, using a heating hose and a heating jacket (Horst GmbH, Lorsch, Germany). Application of the PTR-MS technique for breath analysis has been described before [12, 13]. The primary ions (H_3O^+) are produced in the ion source by a hollow cathode discharge, using water vapour as reactant gas. The proton transfer reaction between the formed H_3O^+ and neutral analyte molecules (M) occurs in the drift tube ($M+H_3O^+ \rightarrow MH^+ + H_2O$). The reaction only occurs if the analyte has a higher proton affinity than water. Therefore, N_2, CO_2, CO, O_2 and Ar as the main components of breath gas are not protonated, because their proton affinities are lower. For many organic analytes soft or medium soft ionisation is achieved.

A high-sensitivity PTR-Quadrupole-MS system (Ionicon Analytik GmbH, Innsbruck, Austria) equipped with two turbo pumps was applied for this study. The drift chamber was operated at a drift voltage of 600 V, a pressure of 2.1 mbar and a temperature of 60°C. The pressure in the mass analyser was about 5×10^{-5} mbar. Three mass-to-charge ratios were measured for the unequivocal identification of inspiratory and alveolar phases (Table 1: 2, 3, 8). The remaining four *m*/*z* (Table 1: 4–7) represent the target analytes.

One complete cycle took 0.19 s whereas the actual measurement time was 0.12 s plus an overhead time of about 0.07 s for mass filter settling, data transfer and processing. The water cluster dimer (H_2O (H_3O^+)) at *m*/*z* 37 was detected

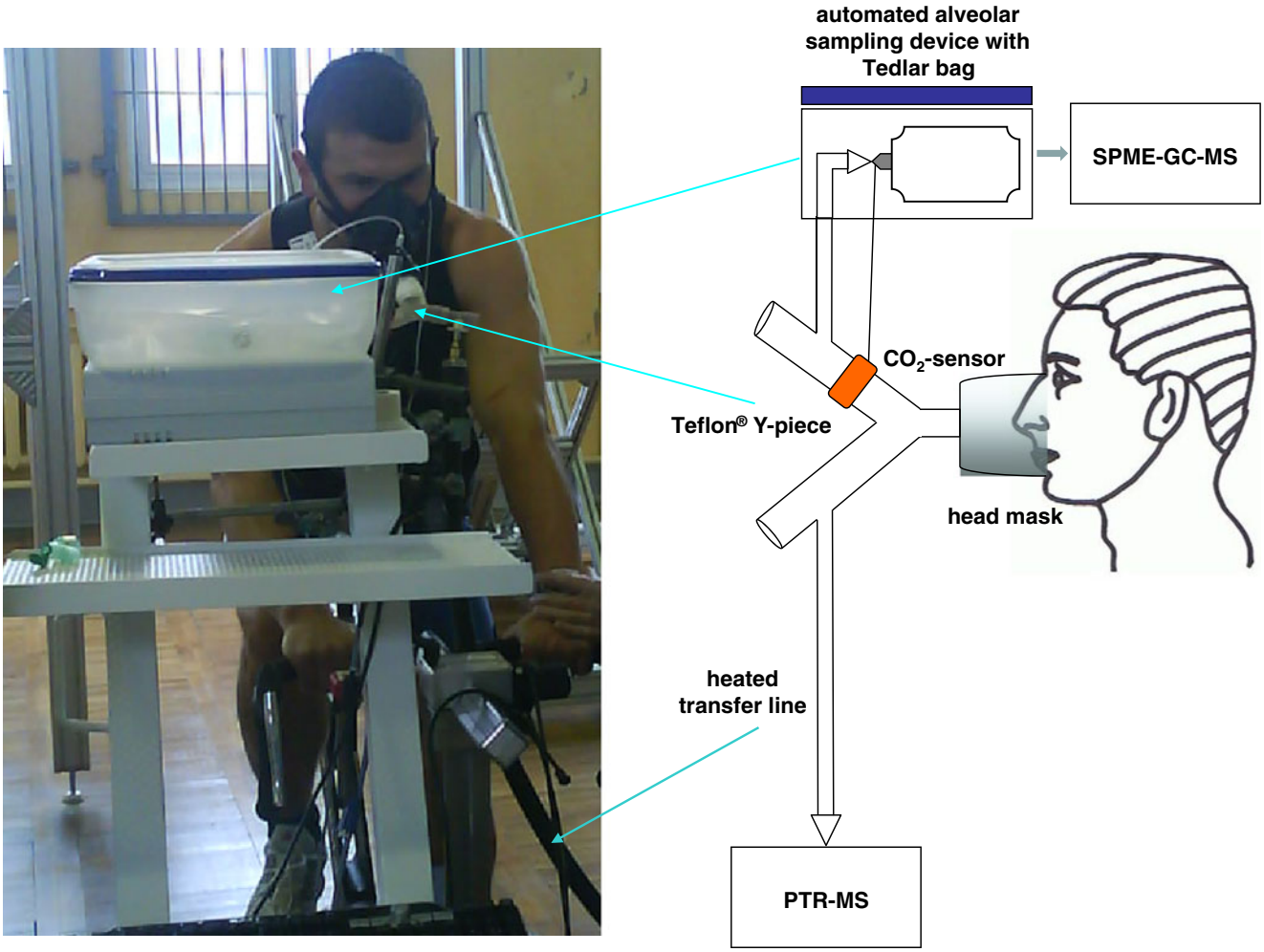

Fig. 1 Experimental and schematic setup of breath sampling procedures

twice: at the beginning (first=sequence 2), and at the end (last=sequence 8) of each cycle. As oxygen is taken up into the organism concentrations are minimal during alveolar phases and maximal during inspiratory phases. Therefore, ionised oxygen signals were used to control the assignment of respiratory phases. ^{18}O isotope of H_3O^+ was measured at m/z=21 for quantification and to check that the primary ion concentration was constant during the analysis. The duration of a single cycle is limited by the duration of the alveolar phase and the number of ions monitored. To ensure sufficient detection limits for all compounds under investigation without losing the time resolutions, that were necessary for substance determination under high respiratory rates, dwell times were adapted to the expected biomarker concentrations. For that reason, dwell time for acetone—typically occurring in levels above 100 ppb—was limited to 10 ms.

Table 1 Experimental conditions for PTR-MS measurements

Ion channel	Multi Ion Detection	Dwell time	Annotation
1	m/z 21	20 ms	^{18}O isotope of primary ions H_3O^+ (m/z 19)
2	m/z 37	10 ms	Protonated water cluster (dimer), precursor ion
3	m/z 32	10 ms	Ionised oxygen
4	m/z 45	20 ms	Protonated acetaldehyde
5	m/z 59	10 ms	Protonated acetone
6	m/z 69	20 ms	Protonated isoprene
7	m/z 83	20 ms	Protonated fragment of hexanal
8	m/z 37	10 ms	Protonated water cluster (dimer)
	Dead time	~70 ms	Data transfer and processing

Data processing algorithm

The following data processing algorithm was applied to distinguish inspiratory from alveolar phases. According to its lower proton affinity than water, the PCO_2, which enables easy recognition of the different phases of the breathing cycle, cannot be determined by PTR-MS. Therefore, the water dimer signal (*m/z* 37) was applied to distinguish between inspiratory and alveolar phases. The water cluster ions ($H_3O^+(H_2O)_n$ n=1,2,…) are detected, based on proton transfer between H_3O^+ and water molecules. The abundance depends on the electric field along the drift tube and on the amount of water vapour in the sample [18]. As the expiratory phase contains more water vapour than the inspiratory phase, an increased water cluster signal is shown during expiratory phases compared to inspiratory phases. The associated VOC signals, measured between both water cluster signals (*m/z* 37) of each cycle, were selected as the inspiratory phase or the alveolar phase or none of them via the average and the gradient of both water cluster signals. All *m/z* 37 signals of individual volunteer during the entire measurement were used to calculate the mean signal for *m/z* 37.

The selection was performed using the following automatic data processing algorithm. Signals of associated VOC were only valid as expiratory phases if the water cluster signals from the same cycle complied with following conditions:

1. $m/z\ 37_{\text{first}} >$ average of all m/z 37 value
2. $|\text{Gradient}| < 2.5\%$

$$|\text{Gradient}| = \left[\frac{m/z\ 37_{\text{first}} - m/z\ 37_{\text{last}}}{m/z\ 37_{\text{first}} + m/z\ 37_{\text{last}}}\right] \times 2$$

The corresponding conditions for the inspiratory phase are:

1. $m/z\ 37_{\text{first}} <$ average of all m/z 37 value
2. $|\text{Gradient}| < 2.5\%$

$$|\text{Gradient}| = \left[\frac{m/z\ 37_{\text{first}} - m/z\ 37_{\text{last}}}{m/z\ 37_{\text{first}} + m/z\ 37_{\text{last}}}\right] \times 2$$

Both requirements (1 and 2) mean:

1. Only if the individual *m/z* 37 signal was greater than the mean of total *m/z* 37 values, the associated VOC signals were attributed to an alveolar phase. Otherwise, the signals were considered to belong to inspiratory or mixed expiratory phases.
2. Data points were assigned to inspiration or alveolar phase, only if the gradient of both $H_3O^+H_2O$ (*m/z* 37) signals from the same cycle was less than a fixed value of 2.5%. Only if both conditions were fulfilled, data were labelled as alveolar or inspiratory. Otherwise, the data were regarded as mixed expiratory and were not taken into account. The principle of the algorithm using two *m/z*=37 measurements per cycle for discrimination is demonstrated graphically in Fig. 2.

Off-line reference analysis

Breath gas samples were pre-concentrated by means of solid-phase micro extraction (SPME), separated by gas chromatography, identified and quantified by ion-trap mass spectrometry (VARIAN Star 3900 CX, Saturn version 5.51) as described before [19]. SPME fibers (Carboxen/PDMS) were purchased from SUPELCO (Bellefonte, USA). For SPME-GC-MS analyses, solution of 2,3-dimethyl,buta-1,3-diene (SUPELCO Bellefonte, USA) was added as an internal standard into the breath samples. A CP-Pora Bond GC-column was used (25 m×0.32 mm, internal diameter× 5 μm film thickness, Varian, USA), and electron impact ionisation (70 eV) was applied for mass spectrometry. CO_2-controlled sampling [20] and SPME-GC-MS analysis have been described before in detail in [21, 22]. In brief, alveolar breath gas was selected automatically by a custom-made CO_2 triggered sampling device (Innsbruck University, [20]) and collected into a Tedlar® bag during the whole time of each adjusted workload (approx. 2.5 min). After the end of each workload step the Tedlar® bag was exchanged and 10 mL of the samples were transferred immediately from the Tedlar® bag into 20 mL evacuated silanised headspace vials. For high respiratory rates (>40/min), the automated sampling procedure did not work continuously anymore. Ambient air samples were collected for each time point in parallel, for both sampling procedures and all samples were made in duplicate.

For statistical analysis linear regression for VOC concentrations measured by means of PTR-MS vs. those determined by means of SPME-GC-MS was performed. Regression analysis was done using SigmaStat 3.5/ SigmaPlot 10.0.

Calibration and analytical parameters

The calibration gas mixtures were purchased from Ionimed (Innsbruck, Austria). Standard gas mixtures were diluted with clean synthetic air (Linde Group, Munich, Germany) in Tedlar® bags. The detection limit was determined using a seven-point calibration (six repetitions) using calibration gas mixtures. For SPME-GC-MS measurements concentrations from 6 to 1060 ppb for acetone, from 1.3 to 505 ppb for isoprene, from 8 to 212 ppb from acetaldehyde and from 1.6 to 99 ppb from hexanal were measured. Each substance was identified by mass spectrum and retention time and compared with reference substances. Quantification was done using selected ions (*m/z* 44 for acetaldehyde, *m/z* 58 for acetone, *m/z* 68 for isoprene and *m/z* 56 for hexanal). The linearity of PTR-MS measurement was investigated for a concentration range from 10 to 1,000 ppb for acetone, 10 to 1,000 ppb for isoprene, 4 to 150 ppb for acetaldehyde and 2 to 50 ppb for

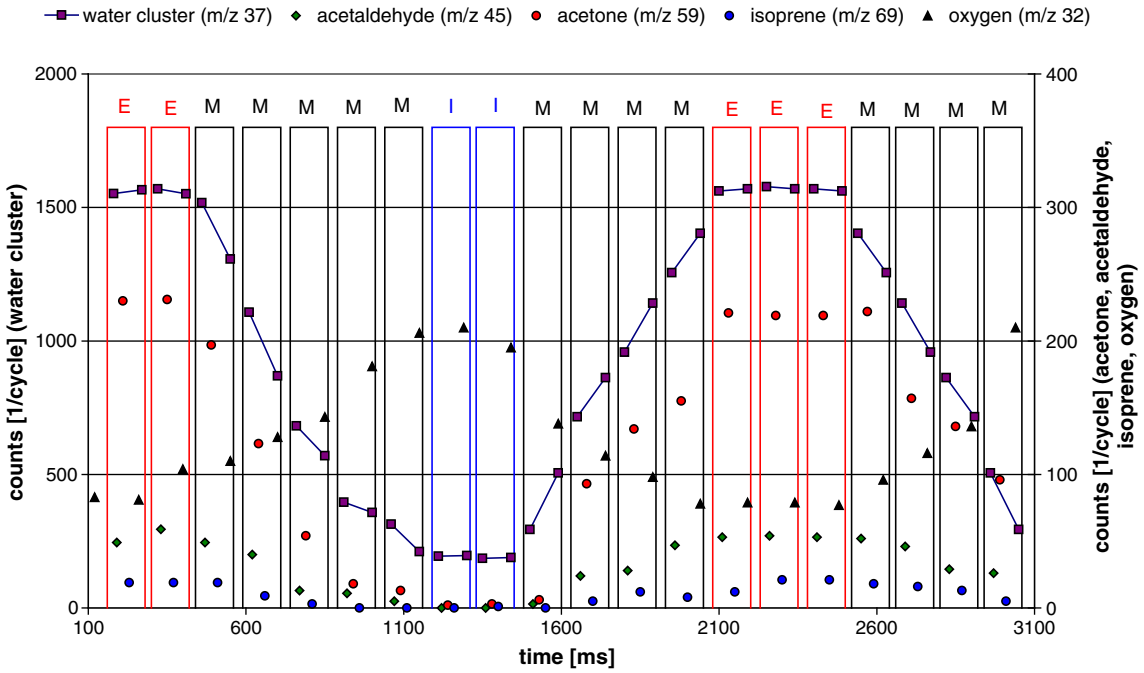

Fig. 2 PTR-MS signal assignment to different phases of the respiratory cycle. *Each box* represents a single PTR-MS measurement cycle with a *m/z* sequence as listed in Table 1. Signals within the *black boxes* (*M*) were assigned to mixed expiratory phases when the first water cluster signal of a cycle differed from the second by more than 2.5%. Signals were attributed to alveolar phases (*red boxes*, *E*) when the gradient between the first and the second water cluster signal was less than 2.5%, and when both signals were greater than the mean of the averaged water clusters. *Blue boxes* indicate cycles recognised as inspiratory phases (*I*). In these cases, values of both water cluster signals were less than the averaged signal and the gradient between the first and the second water cluster signal was less than 2.5%

hexanal. Limit of detection (LOD) were defined as the signal of three times noise ($S/N=3$), and limit of quantification (LOQ) is per definition signal of nine times noise ($S/N=9$).

Results and discussion

Application of the PTR-MS data processing algorithm yielded a clear distinction between alveolar and inspiratory phases, even under high respiratory rates up to 60/min during physical exercise. Without any additional equipment reliable quantification of blood-borne biomarkers in the alveolar phase and (ambient) VOCs in the inspiratory phase was possible at any time during the experiment. This was confirmed by an established automatic CO_2 controlled alveolar sampling method and laboratory based SPME-GC-EI-MS measurements. During physical exercise both methods, continuous PTR-MS monitoring and discontinuous, laboratory based SPME-GC-MS analyses, showed good correlation during phases without rapid physiological changes. However, when concentrations of volatile substances changed abruptly (e.g. during warming-up phase), results of time-averaged alveolar sampling and GC-MS and real-time PTR-MS diverged.

The reliable recognition of respiratory phases is a crucial issue in breath analysis. Identification of respiratory phases by means of the data algorithm is shown in Fig. 2. Hexanal is not displayed in Figs. 2–4, because the measured concentrations in exhaled breath were below the LOD of PTR-MS. Reliability of the algorithm was confirmed by the fact that (a) O_2^+ signals were minimal during alveolar phases and maximal during inspiratory phases, (b) signals of endogenous VOCs were maximal during alveolar phases and minimal during inspiratory phases (Fig. 2). Oxygen can be regarded as a marker of gas exchange, and for obvious physiological reasons, has lower concentrations in the expiratory phases than during inspiration.

Only alveolar concentrations reflect substance concentrations in blood, other substances originating from bronchial epithelia have maximum concentrations in dead space air. Contaminants from ambient air on the other hand show maximal concentrations in the inspired air and must be taken into account. Hence, only unequivocal description of the respective phase as alveolar, mixed expiratory or inspiratory will provide valid and reproducible results in breath analysis. Alternative approaches to identify the alveolar plateau during exhalation have been recently described. Automatic real-time sampling on

the basis of respiratory flow was realised by means of a flow-controlled shutter mechanism which guaranteed that only end-tidal exhalation segments were drawn into the mass spectrometer for analysis [23]. Valid inhalations/exhalations were selected by software algorithm using a C++ interface (PROCESS FLOW) for consistent on-line shutter control [23]. Automatic CO_2 controlled sampling [24], continuous automatic sampling on the basis of measured respiratory flow [23] as well as buffered end-tidal (BET) sampling approaches [25] were implemented

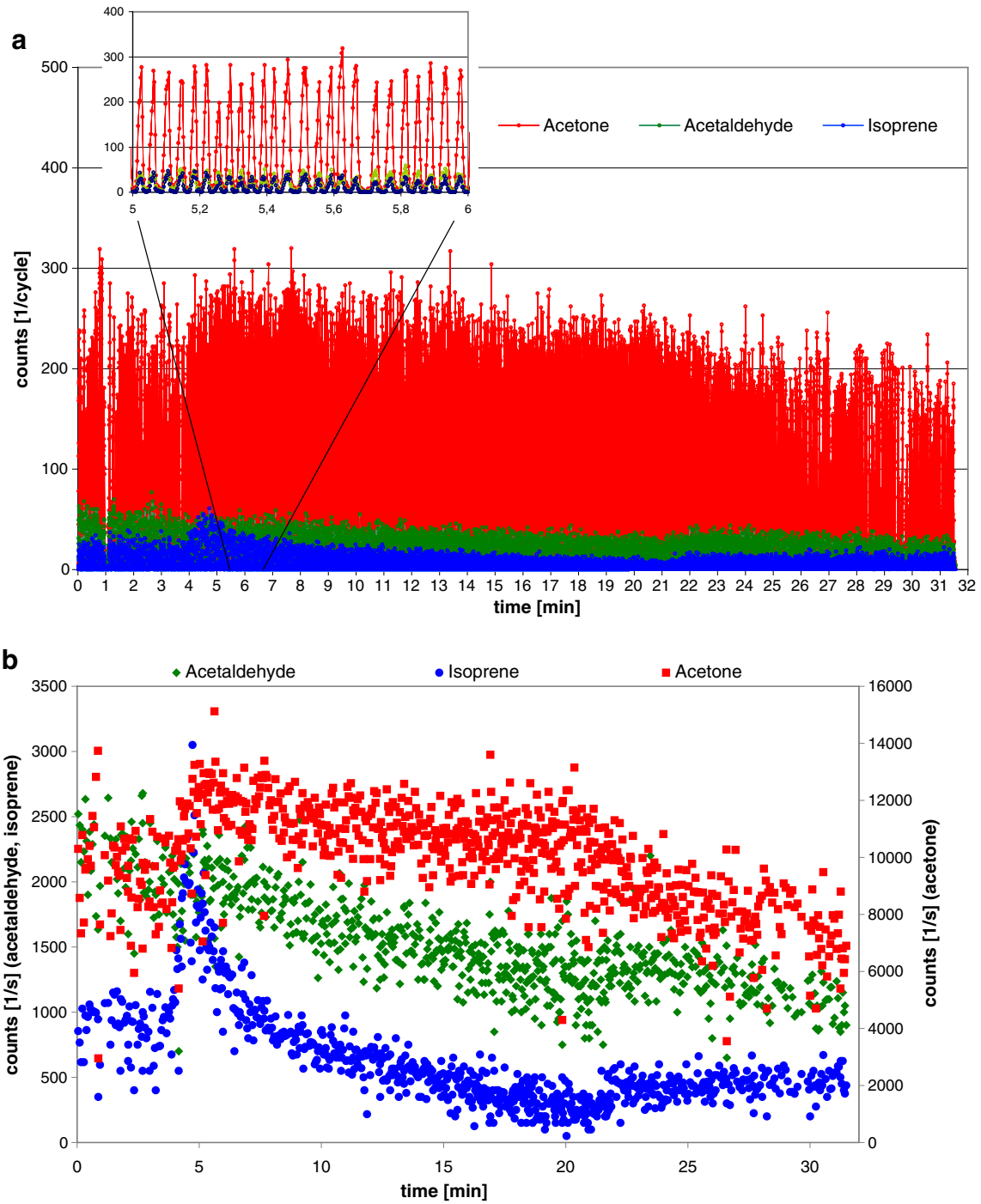

Fig. 3 Raw data (**a**) for acetone (*red line*), acetaldehyde (*green line*) und isoprene (*blue line*) without application of the data processing algorithm during exercise of one volunteer. *Insert* shows data on an enlarged timescale. **b** shows the effects of the data processing algorithm when alveolar data were extracted

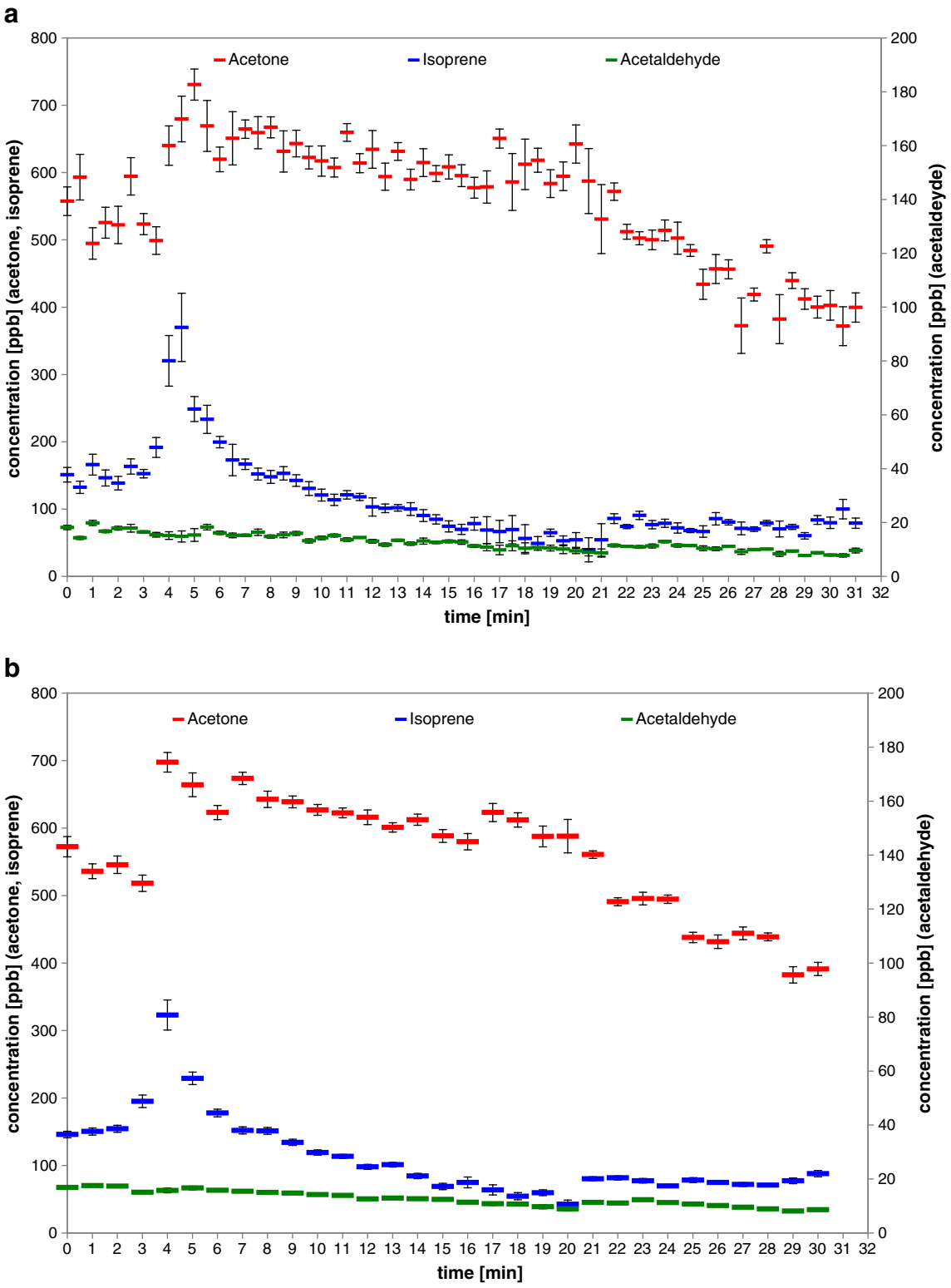

Fig. 4 Time courses and effects of time-weighted averaging for acetone (*red line*), isoprene (*blue line*) and acetaldehyde (*green line*) during exercise from the same volunteer as shown in Fig. 3. Data were attributed to alveolar (**a**–**c**) or inspiratory (30 s, **d**) breath phases by means of the algorithm. Alveolar concentration courses of substances after time-weighted averaging of 30 s (**a**), 60 s (**b**) and 120 s (**c**) are shown

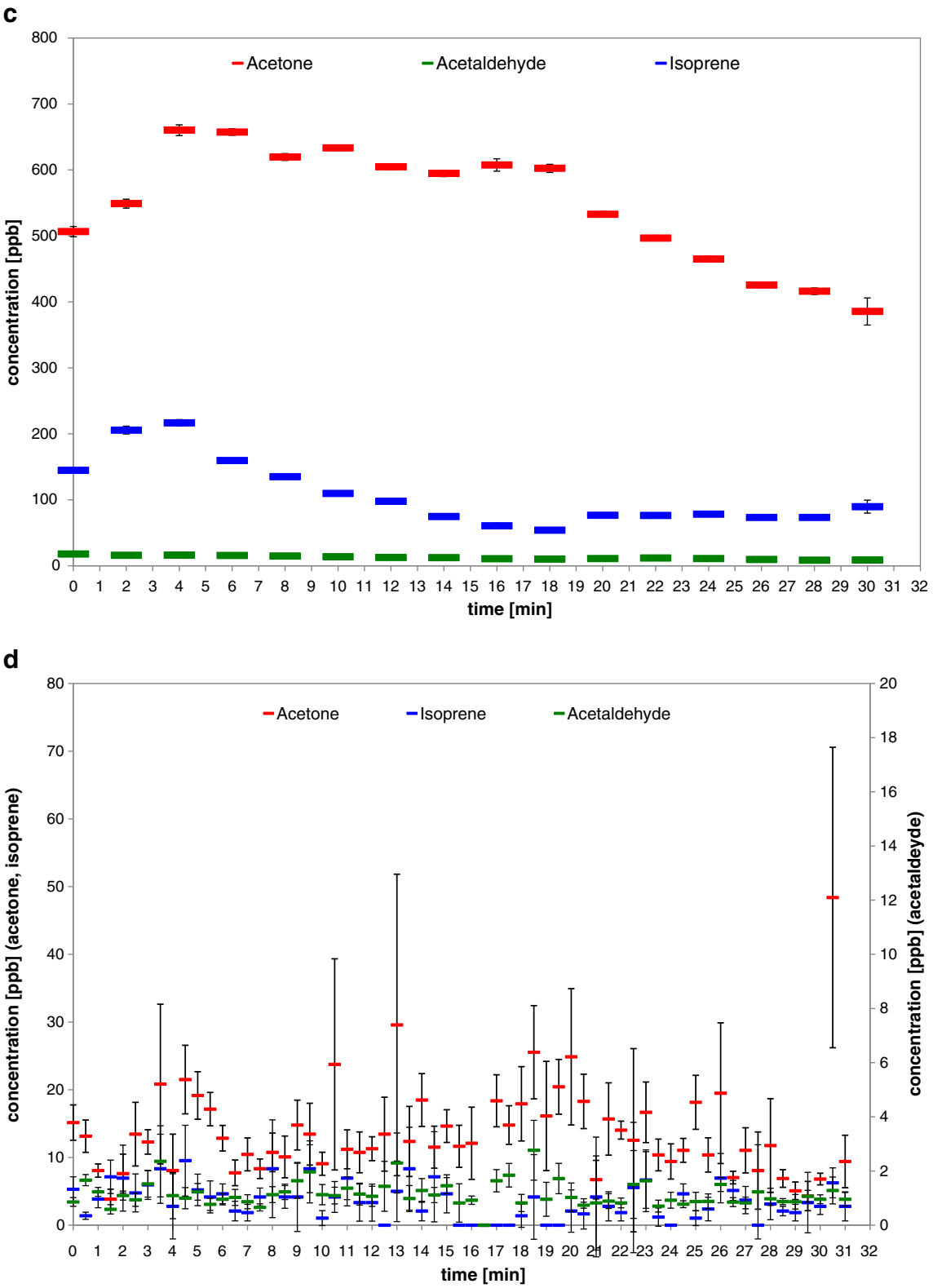

Fig. 4 (continued)

in combination with a PTR-MS on-line measurement system. In BET, the last fraction of a single exhalation gas sample is stored in a specially tailored tube. This buffering technique allows for extended measurement period, i.e. more *m/z* per breath can be measured, or an improved LOD is achieved [25]. In contrast to the previously published methods [23–25], the new method did not require any additional equipment. Compared to the above-mentioned methods, the algorithm described in this study does not only provide breath-to-breath recognition of the alveolar phase but it also enables simultaneous monitoring of inspired substance concentrations. This is especially important for clinical studies with partially high background concentrations. In previously described methods [22–24], inspired substance concentrations had to be determined by separate analyses of ambient air. Another drawback of external sampling systems is the need to store the samples intermittently, with the possible risk of artefact formation and adsorption of target compounds at the vessel walls.

The introduced algorithm is particularly useful if mass-spectrometric detectors with sequential analysis such as quadrupoles, magnetic sectors and ion-traps (the latter allowing an easy access to tandem mass spectrometry), which inherently suffer from limited time resolutions, are applied. Although mass-spectrometric techniques using time-of-flight mass-analysers as detectors can overcome the difficulty with limited time resolution [26, 27], the application of this algorithm can be used for unambiguous identification of inspiratory and alveolar expiratory phases for any on-line mass-spectrometric instrument applied for on-line real-time breath gas analysis.

Application of the data processing algorithm during exhaustive exercise is shown in Fig. 3. Signal intensities in Fig. 3a are shown as counts/cycle instead of counts/s in order to display all substances on a single scale (counts/cycle=counts/s×cycle time). As can be seen in Fig. 3b the clear recognition of respiratory phases represents only the first step of data analysis. The selected dwell time determined the achieved LOD in the PTR-MS measurement. The relatively high scattering (Fig. 3b) of signals for acetone, acetaldehyde and isoprene was caused by very short dwell times and the resulting counting statistics. To overcome this problem, time-weighted averaging was applied. Figure 4a and d show the results for moving average with a time constant of 30 s (Fig. 4a, alveolar phases only and Fig. 4d, inspiratory phases only), 60 s (Fig. 4b, alveolar phases only) and 120 s (Fig. 4c, alveolar phases only). A time-weighted averaging of 60 s (Fig. 4b) showed the same concentration gradient as 30 s (Fig. 4a), but with a significantly lower standard deviation (SD). However, compared with 60 s, the time-weighted averaging of 30 s still showed noisy data, where most of its SD areas were overlapping (e.g., isoprene signals between 5 min and 15 min), and hampered data interpretation. In contrast, a time-weighted averaging of 120 s (Fig. 4c) yielded the lowest SD but fast concentration changes (e.g. rapid change of isoprene at 5 min) were not reflected any more. As can be seen in Fig. 4d inspired concentrations of

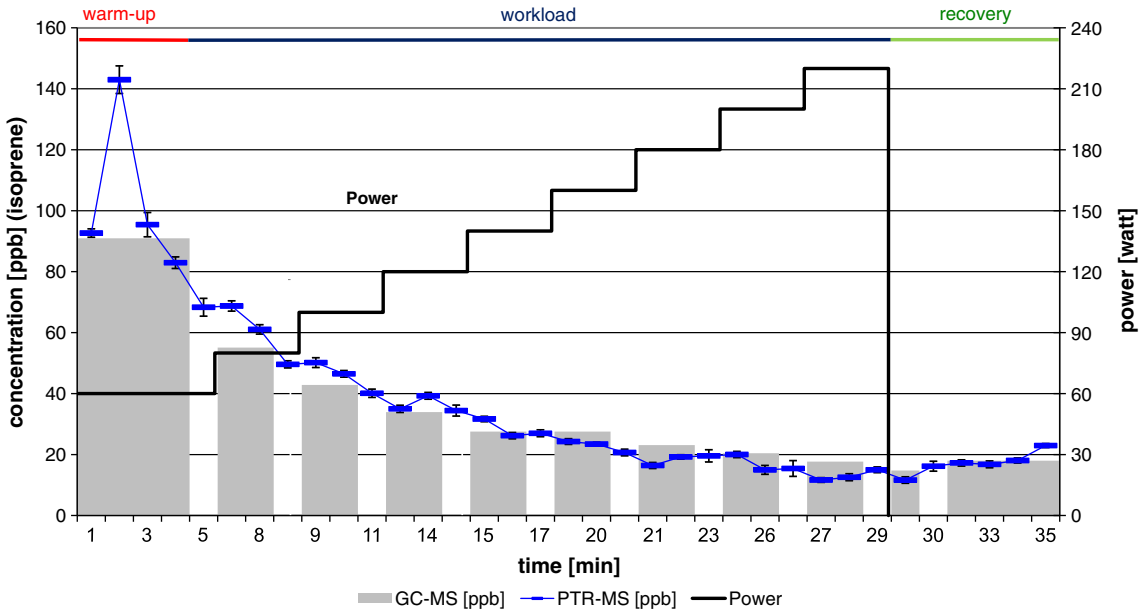

Fig. 5 Concentration profiles of isoprene from one healthy volunteer during exercise on a stationary bicycle. Alveolar concentrations obtained by means of the PTR-MS on-line method are represented by *blue data points*. Results of time-averaged alveolar sampling and SPME-GC-MS analysis are indicated as *grey bars*

all described substances were more than an order of magnitude lower than expired concentrations. Therefore, only alveolar concentrations were presented in Figs. 4a–c, 5 and 6.

The simultaneous real-time measurement of a large number of substances at trace level by means of PTR-MS is difficult, due to the limited time resolution of the quadrupole mass analyser and unequivocal identification of substances in the sample is not always possible. In addition, interpretation of PTR-MS signals may become difficult, due to association and clustering processes, unwanted ion-molecule reactions such as primary ion

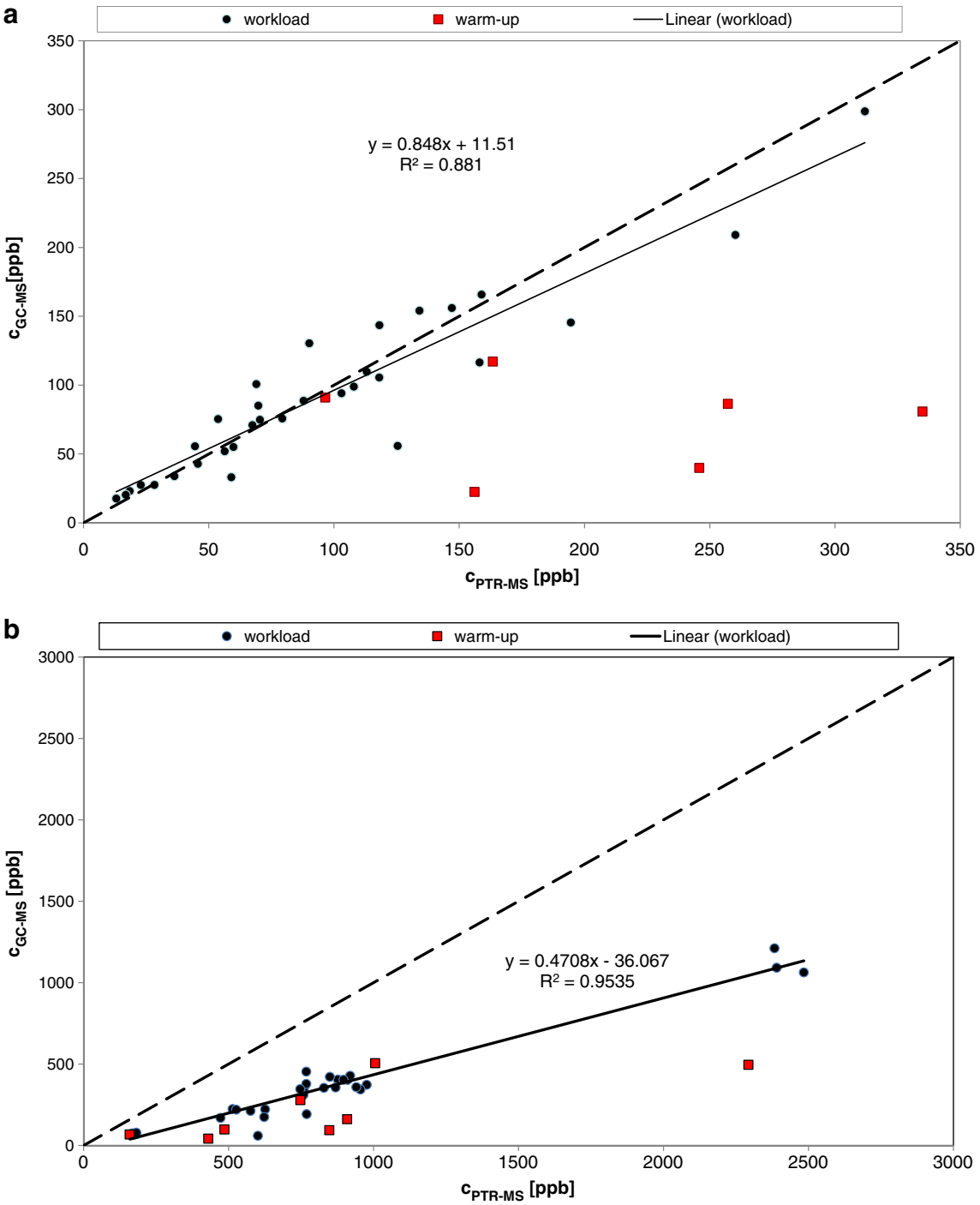

Fig. 6 Correlation between PTR-MS and SPME-GC-MS data for isoprene (panel **a**) and acetone (panel **b**). *Red squares* represent data from the warming-up phase, and *blue dots* represent data acquired during workload

switching reactions and fragmentations. Because quadrupole PTR-MS is an instrument with relative low selectivity, the focus of application is the fast monitoring, rather than the identification of unknown compounds. Therefore, cross-validation with selective instruments, like GC-EI-MS, brings additional information and often is still necessary to confirm results. Table 2 shows LOD and LOQ for the target analytes in alveolar breath achieved by PTR-MS and by the SPME-GC-MS reference method. Furthermore, the ranges of expiration and inspiration of the considered VOCs (acetone, isoprene, acetaldehyde and hexanal) and room air concentrations are given as measured by PTR-MS.

In Fig. 5, exhaled isoprene concentrations from one healthy volunteer during exercise on a stationary bicycle as measured by both methods, the PTR-MS on-line and the SPME-GC-MS off-line approach are depicted. Concentrations measured by PTR-MS and SPME-GC-MS are in good accordance. In agreement with previous published studies we found a marked increase of isoprene concentrations at the onset of exercise followed by steadily decreasing concentrations during workload in all investigated volunteers [28, 29]. Due to its low affinity for blood (Ostwald blood: gas partition coefficient at body temperature=0.75 [30]), this effect has mainly been attributed to an impairment of hemodynamic and respiratory parameters (e.g. heart rate and minute ventilation) or ventilation–perfusion effects [23]. Recent investigations of King et al. [23], however, suggest that this effect is due to effects of isoprene load (purge out) from working muscle compartment, receiving high fractions of cardiac output during onset of ergometer challenge [31]. Especially during the phases of rapid physiologically induced concentration changes (warm-up phase) results of PTR-MS and GC-MS diverged (Fig. 6a—red squares). Since alveolar sampling in Tedlar® bags took 2–3 min off-line GC-MS data have to be considered as being time-averaged. Therefore, concentration changes occurring within less than the sampling time could not be resolved by the applied GC-MS method. This effect can also be seen when linear regression of PTR-MS vs. GC-MS data was calculated for all experiments (Fig. 6a). There was good correlation between both methods when physiological parameters changed continuously without inducing abrupt changes of exhaled substance concentrations (Fig. 6a; blue dots). It can be seen clearly from Fig. 6a (red squares) that results from PTR-MS and GC-MS diverged during warm-up phase when abrupt changes of physiological parameters (e.g. cardiac output) induced rapid changes of exhaled substance concentrations.

Acetone concentrations determined by means of PTR-MS showed good correlations with results from GC-MS analysis (Fig. 6b, blue dots). However, GC-MS values were consistently lower than PTR-MS results. This is most probably due to condensation or adsorption effects at the Tedlar® bags material. Concentrations of water soluble substances such as acetone rapidly decrease in Tedlar® bags [32, 33] as water and polar substances condensate on the wall of the bags [34, 35] and polar compounds such as acetone may diffuse through these materials [35, 36]. The effect of inspiration is negligible because inspired substance concentrations were more than 20 times lower than the expired ones (Fig. 4d).

Without any additional equipment, the algorithm enabled reliable identification of alveolar and inspiratory phases. Since the applied system uses side stream measurement and, therefore, does not affect the flow in the main breathing system, the setup can be used for quantification of blood-borne biomarkers in healthy and diseased individuals. As results of this study confirmed that the algorithm worked well even under high respiratory rates, the setup can be applied even in small children and in patients with low tidal volumes and high respiratory rates. The results of this study emphasise the advantages of continuous and direct measurement. This is especially true for rapid and sudden changes of VOC concentrations and for substances with limited storage stability. The presented data processing algorithm can further promote the use of on-line mass spectrometry for real-time breath gas analysis.

Table 2 Analytical parameters (LOD, LOQ) for the determination of selected biomarkers by means of real-time PTR-MS and GC-MS

	PTR-MS		SPME-GC-MS		PTR-MS		
Compound	LOD (ppb)	LOQ (ppb)	LOD (ppb)	LOQ (ppb)	Range ex (ppb)	Range ins (ppb)	Ambient air (ppb)
Acetone	5.5	16.5	6	17	130–2600	5–210	0–15
Isoprene	1.2	3.6	1.3	4	13–540	0–24	0–6
Acetaldehyde	1	3	8	29	2–31	0–4	0–5
Hexanal	1	3	1.6	4.9	<LOD	<LOD	<LOD

In addition, concentration ranges (PTR-MS data) in expired, inspired and ambient air are shown

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