Application Note 120830
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Breath analysis using Atmospheric Pressure Corona Discharge Ionization-Mass Spectrometry (APCDI-MS)

A) Introduction

Breath analysis for clinical diagnosis and therapeutic monitoring is considered to be a challenge and research activities by scientific groups are ongoing, continuously. Specific research efforts regarding the analysis of volatile organic compounds (VOCs) by mass spectrometry (MS) in this field have been made since more than one decade and special technologies as proton transfer reaction mass spectrometry (PTR-MS) and selected ion-flow-tube mass spectrometry (SIFT-MS) play a significant role.

We have recently reported about analyses performed with the miniaturized AMD Mini QuAS^{3}AR (www.amd-analysis.com) specifically suitable for the direct analysis of low atomic number elements in ESI-MS mode and for direct analysis of volatile organic compounds in ambient air by the application of Atmospheric Pressure Corona Discharge Ionization (APCDI). We were interested to evaluate the potential capabilities of this system for dedicated applications like breath analysis, too.

B) Summary

The described analytical results have been performed with the experimental version of a new bench-top double focusing mass spectrometer. The dedicated and miniaturized API-MS system is based on the original AMD QuAS^{3}AR Technology. The API Interface is suitable for ESI-MS, APCI-MS and GD-MS (www.amd-analysis.com). The interface and the mass analyzer are integrated with a multi-stage turbo pumping system. The API Interface was used without any modifications for the breath analyses in APCDI mode. Single breath exhalations were introduced via a Teflon pipe into the API chamber and real-time analyses by full scan techniques were performed. The laboratory air in the API chamber was replaced to a significant portion by introduction of the breath exhalation gas. The water content in the breath gas has been used as a reagent gas for the production of protonated molecular ions of VOCs under atmospheric pressure. The rudimentary sample introduction was accepted due to the orientating character of the analyses which should give an indication of the analytical potential of the system for possible future applications.

Due to the limited scope of the project emphasis has been directed to the analysis of the protonated ion at mass 59 of Acetone, known as one of the major components of the human breath and diabetes indicator. No quantifications in this context have been made but estimations of the detection limits for other possibly existing but not identified components (volatile parameters of diagnostic relevance). Determination of these components would require research efforts beyond the scope of this note. Several reproducible measurements have been performed using probands with a normal blood glucose level. We expect that the current detection limits of about 5 ppb can be extended to the upper ppt level using more sophisticated sample introduction methods.

Since full scan methodology is the method of choice, extension of the current evaluation system with an array detector for simultaneous ion detection should allow detection limits in the low ppt level using the applied APCDI method and the application of "fingerprint" algorithms" for disease pattern recognition may be possible.

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C) Methodology

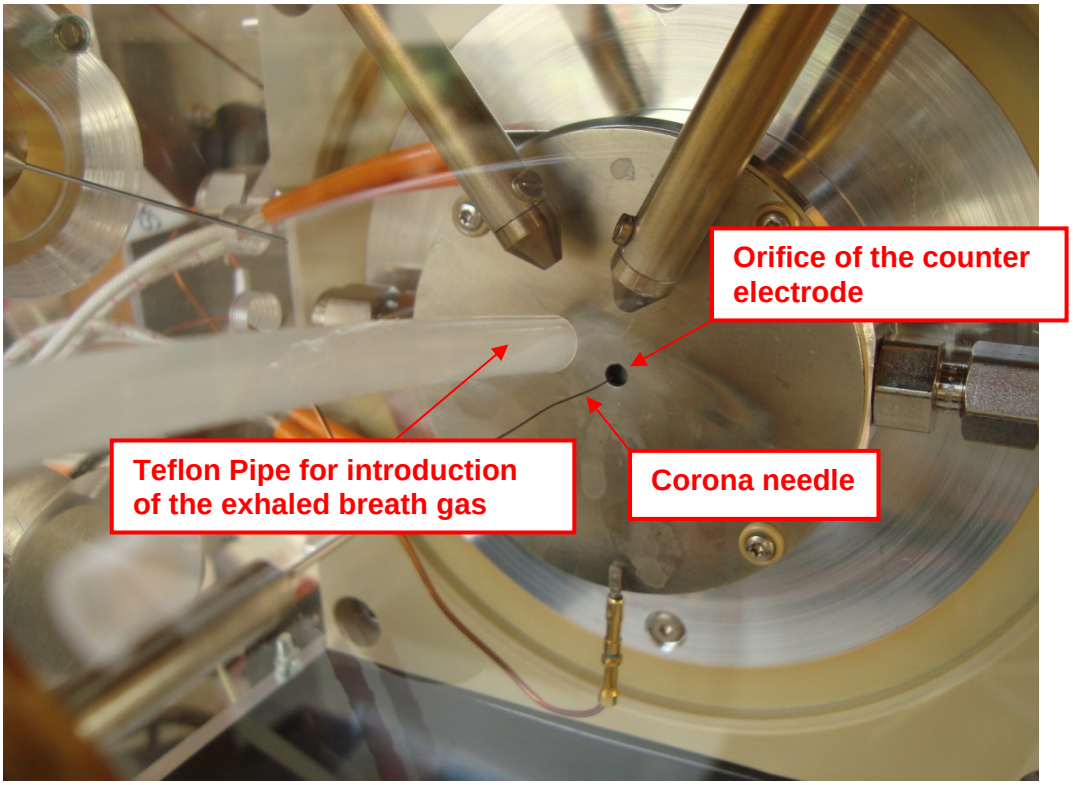

Fig. 1 In-side view of the API chamber

The AMD Mini QuAS^{3}AR mass analyzer, a miniaturized dedicated version of the AMD QuAS^{3}AR double focusing system, was equipped with an API Interface in APCDI mode (APCDI-MS system). System details of the evaluation model have been reported, previously (www.amd-analysis.com). The mass resolution was set to about R= 60 (10% valley).

The universal API chamber useable for ESI, APCI, GD and here for APCDI methods consists of a transparent 2 l box with the required infrastructure for the applications in question. In the context of the application described here the major components in use are indicated in the above figure.

The corona discharge needle is mounted in front of the counter electrode orifice and used at about + 4000 V, the counter electrode at + 450 V, the nozzle at 0 V and the analyzer is floating on - 2000 V. The temperature of the vacuum part of the API interface was kept at room temperature for the experiment described in figures 2-7 below.

The Teflon breath inlet pipe directly used by the exhaling person is not heated and can be exchanged easily for each experiment.

D) Results

D1) Discussion of a selected measurement out of various experiments

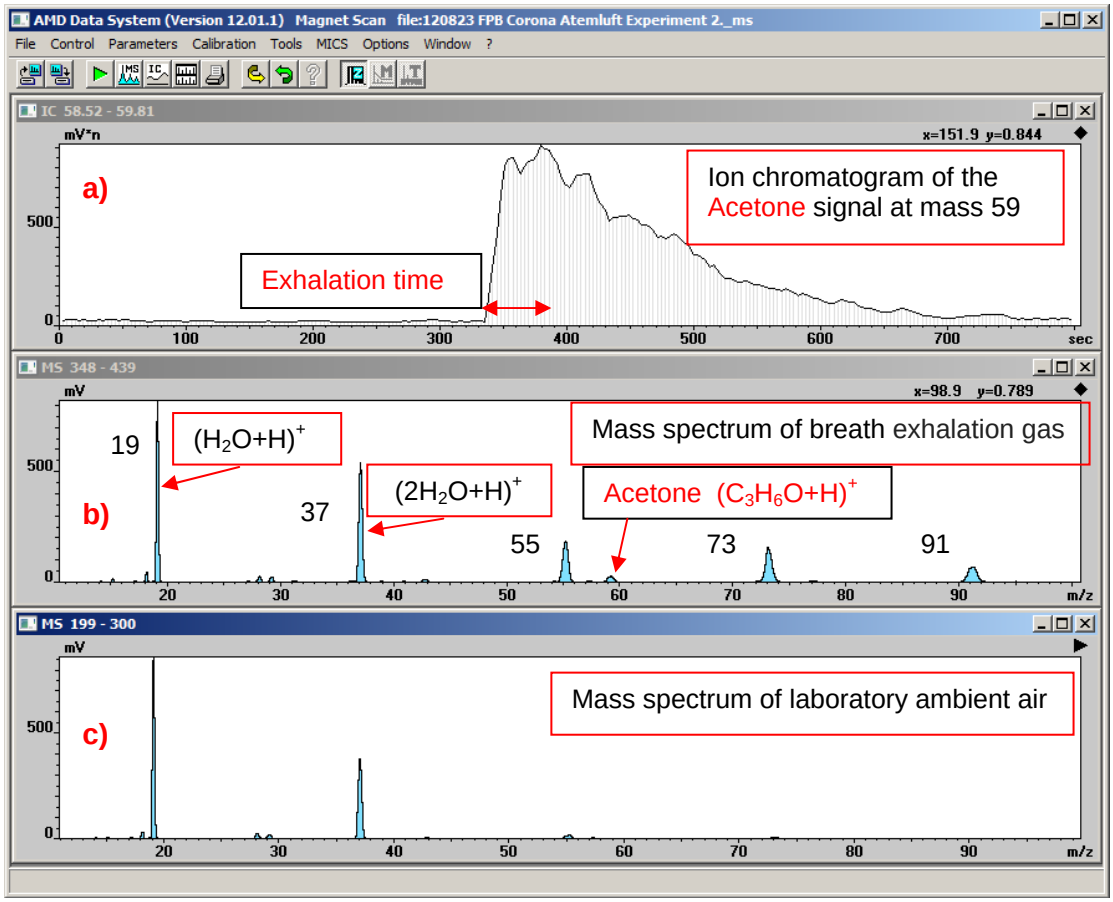

Fig. 2 **Full scale mass spectra** dominated by the protonated water signal and the formation of corresponding water clusters.

Above figure describes the initial situation of a mass spectrometric breath measurement using the **APCDI method** and the standard **API interface of the AMD Mini QuAS^{3}AR** system. Three single breath exhalations are sequentially introduced by one proband via a Teflon pipe into the API chamber exchanging the residual ambient air in the 2 l volume to a large extent within about 45 sec by the breath gas.

Section **a)** describes the trend of the breath concentration in the API chamber using the ion chromatogram of the protonated Acetone signal at mass 59. The ion chromatogram indicates that the breath gas is replaced by laboratory air with a quasi exponential function.

Section **b)** shows the mass spectrum (raw data) of the breath gas up to mass 100 yielding the prorонated water ion at mass 19 and corresponding water clusters at masses 37,55,73 and 91. The quasi molecular Acetone ion is just visible in this scale. The intensity of the water clusters in the breath spectrum is enhanced compared to those in the mass spectrum **c)** of the ambient air by increased aerosol content.

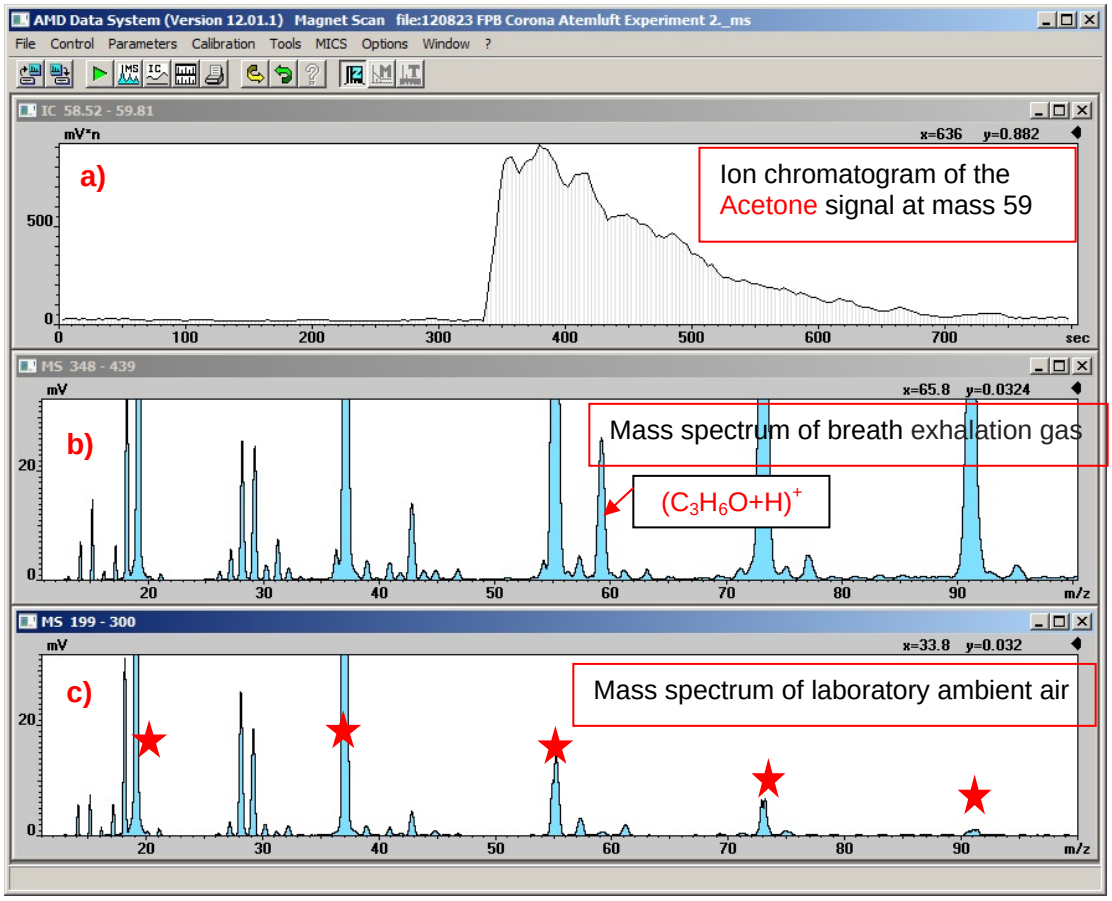

Fig. 3 **Extended scale mass spectra** showing intense ions of the breath gas in section **b)**

The figure shows that the most significant peak in the breath spectrum, the quasi molecular ion of Acetone, yields an intensity of about 26 mV. The smallest peaks detectable are shown in a figures 5,6 below. The protonated water ion at mass 19 and the water clusters are marked with ★.

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On the one hand side the production of water clusters may interfere with the relevant breath ions but on the other hand it may assist an unequivocal calibration and component identification. The cluster formation can be controlled by the Nitrogen counter flow and the interface temperature.

It is reported in the literature that in the breath of healthy persons the Acetone concentration is around 900 ppb. Therefore, it has been assumed in this case that the Acetone signal achieved here corresponds with this value since the blood glucose level of the proband for this experiment was slightly below 100 mg/dL.

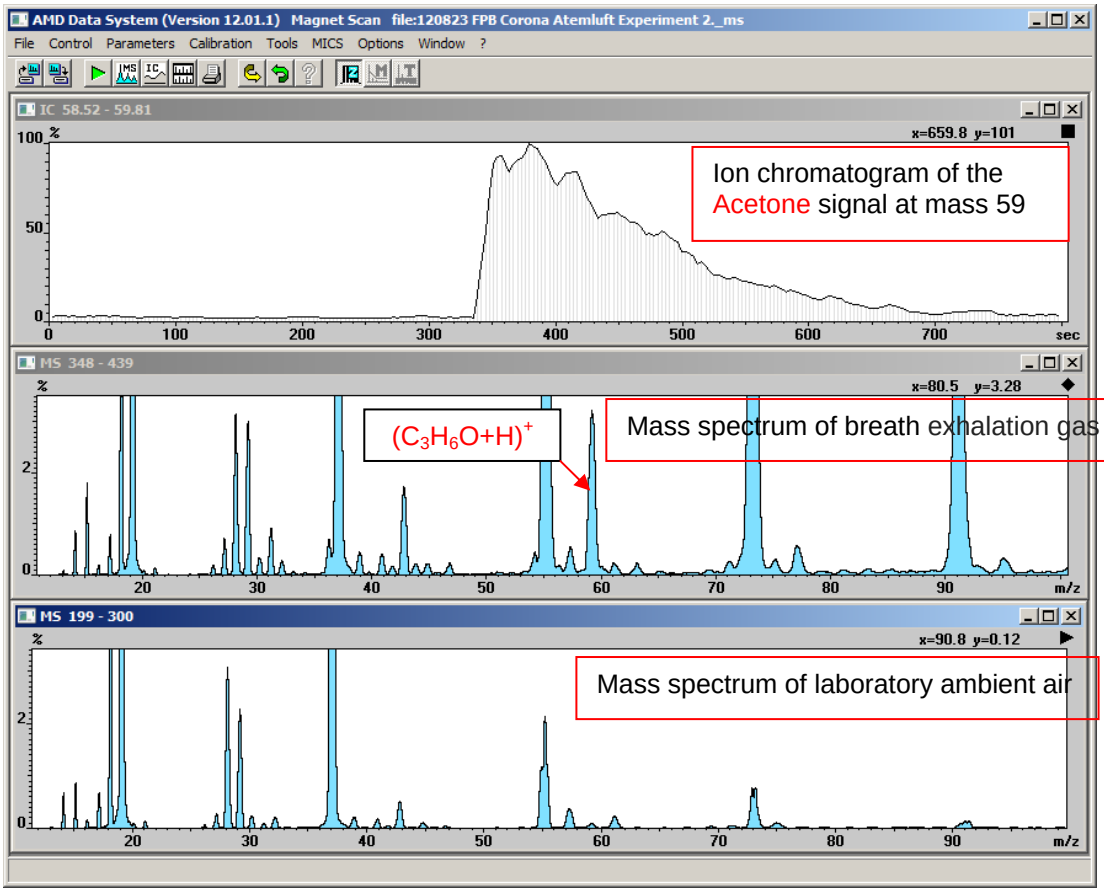

Fig. 4 **Normalized scale mass spectra** (100% = protonated water ion intensity at mass 19) of breath gas and ambient air

It may be noted from this figure that the highest peak related to the exhaled breath gas, namely the Acetone signal yields about 3 % of the protonated water ion signal as the highest peak in the spectrum.

The protonated water ion signal normalized to 100% has got a total signal height of 1V. The data system can handle a 10V signal as maximum, while the smallest signal above base line can be measured on a 10 µV level. The dynamic range for data acquisition therefore is $1{:}10^6$. For the mass spectra obtained this capacity wasn't used, fully.

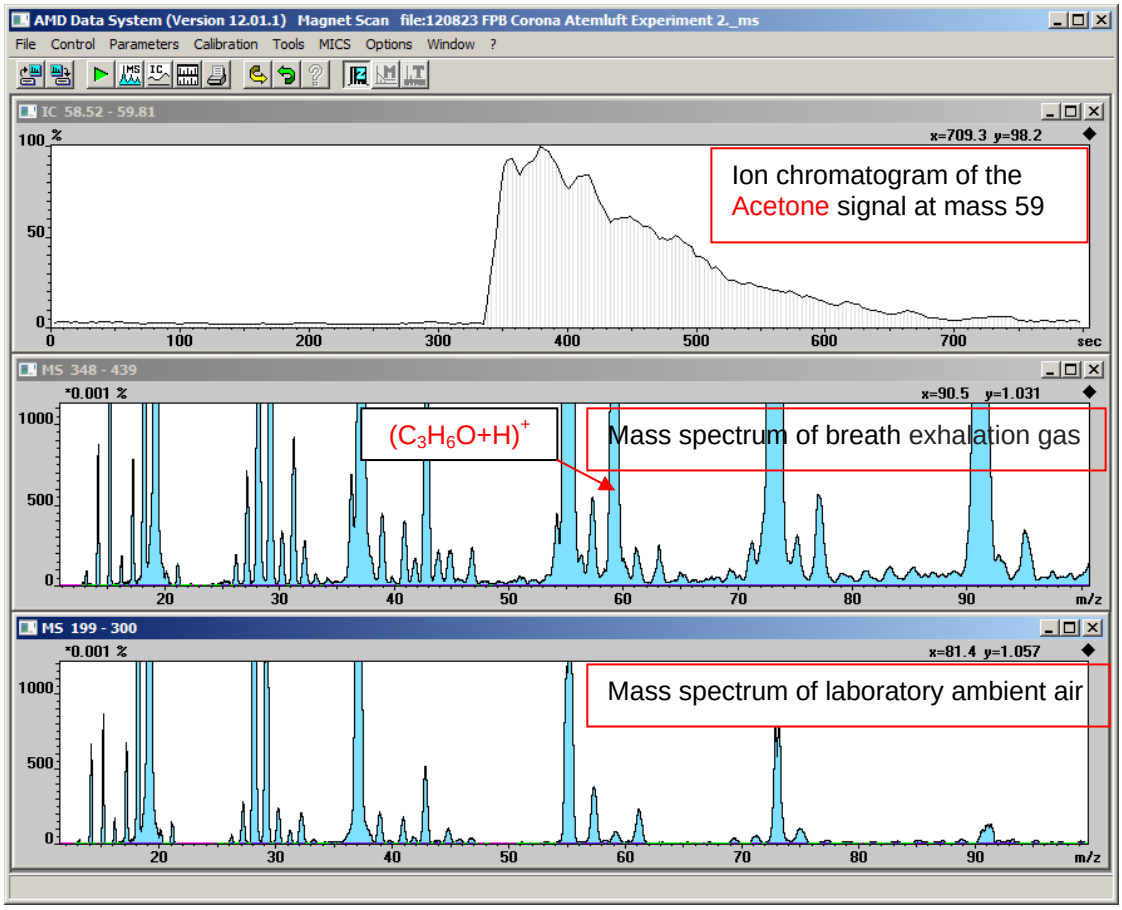

Fig. 5 **Normalized and further extended scale mass spectra** (100% = protonated water ion intensity at mass 19) of breath gas and ambient air

The intensity scale has been extended such that peaks in the order of 1/100 of the Acetone signal can be identified here at a 9 ppb level in the breath gas. The current detection limit for evaluable peaks is demonstrated in the following figure below.

Comparing the spectra of ambient air and breath gas at equal scales it is obvious at a first glance that the total sensitivity has increased for breath gas. But besides this, however, the pattern has changed. This is for instance obvious for a number of peaks at masses 15,31,43,44,47,59,63,77,95 which can be assigned to the breath gas.

While the existence of the Acetone peak can be considered to be save (confirmed by direct Acetone vapor sniffing) the unequivocal identification of other peaks would require significant research efforts beyond the scope of this note.

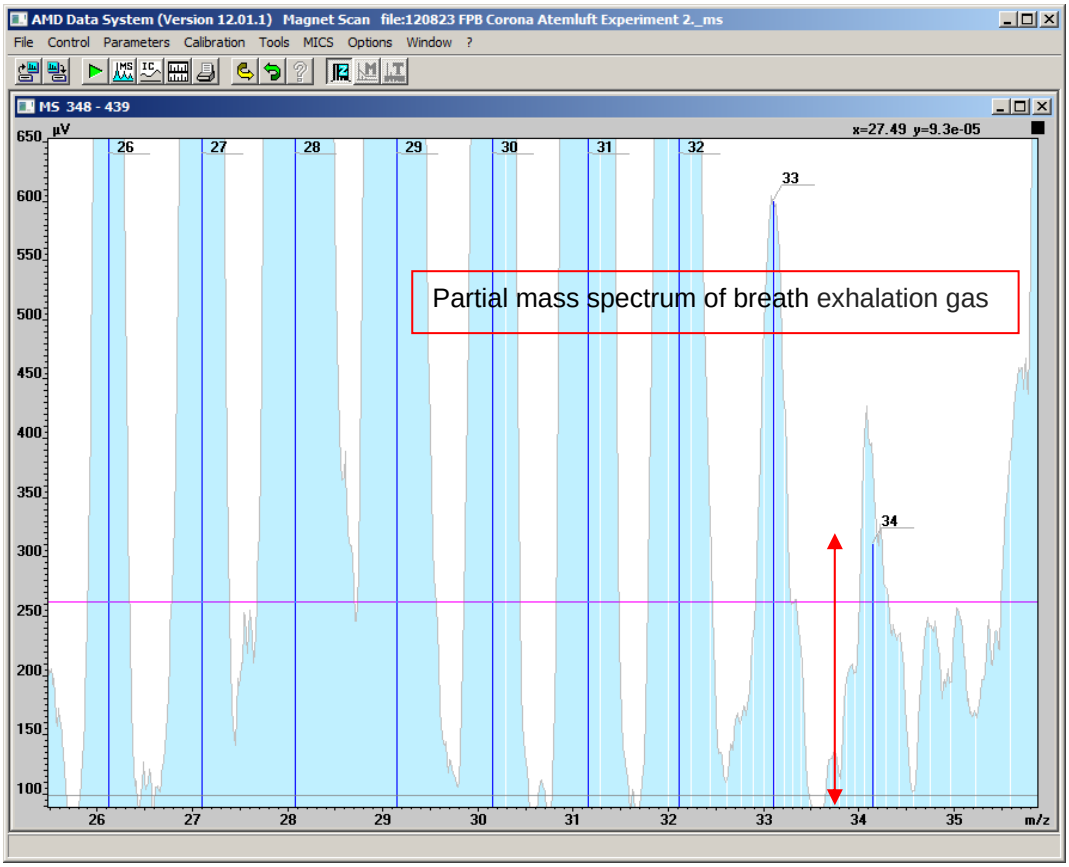

Fig. 6 Extraction of a portion of the mass spectrum of the breath gas at **LOD level.**

The intensity scale has been extended so far that the limit of detection (LOD) is reached for the current experimental set-up. A peak height of about 200 µV can be evaluated which results in relative terms to the Acetone signal (0.2 / 26 mV) in a ratio of 7.7 x 10^{-3} x 900 ppb.

Current LOD: 6.9 ppb

Potential for improvements is available by the application a of more efficient breath sample introduction. In addition it may be mentioned in this context that the measuring time per peak for the applied scan method and mass resolution conditions is in the order of 15 msec while the cycle time is about 3 sec. As a result the duty cycle is about 5 x 10^{-3}. The AMD Mini QuAS^{3}AR analyzer (straight focal plane) is prepared for the implementation of an array detector offering simultaneous ion recording for a wide mass range. The implementation of this technique will improve the duty cycle at least to a value of 50%. This will be an improvement of a factor of 100.

The execution of both potential measures should improve the LOD by more than two orders of magnitude. As a result we expect for an optimized breath analyzer on that basis to achieve an LOD in the low ppt range.

Perspective LOD: 10 - 20 ppt

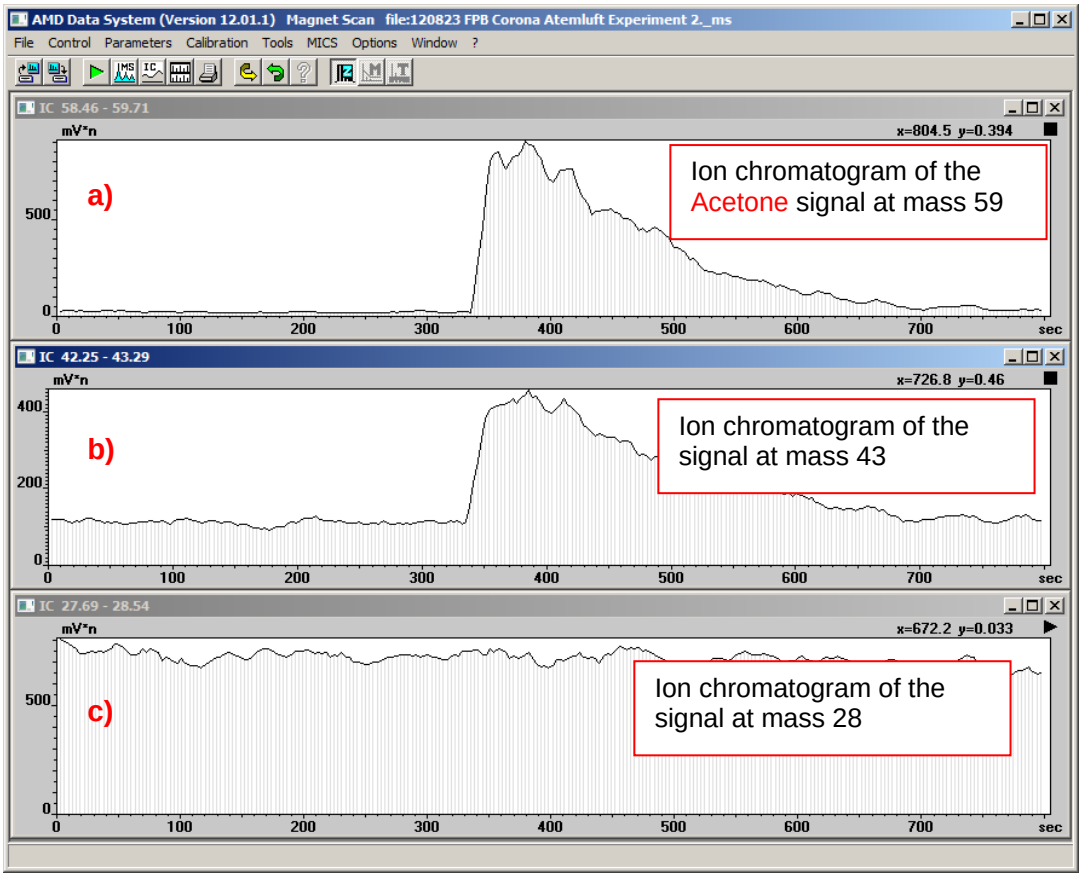

Fig. 7 Ion chromatograms of relevant signals for breath gas spectra and ambient air

This figure demonstrates that some useful information may be derived from ion chromatograms. The ion chromatogram of Acetone in trace **a)** indicates that the background level from the ambient is close to zero and the measured Acetone level clearly belongs to the breath gas.

The ion chromatogram of mass 43 in trace **b)** indicates that a constant level of this ion belongs to the ambient air and the signal increase belongs most probably to the breath gas. However, based on the current information level available it could also belong (at least partially) to an increased sensitivity gain by the higher water content of the breath gas due to optimized reagent gas reaction.

At least the constant ion chromatogram of mass 28 in trace **c)** indicates clearly, that this ion intensity is not influenced by the breath gas..

D2) Result of a specific experiment

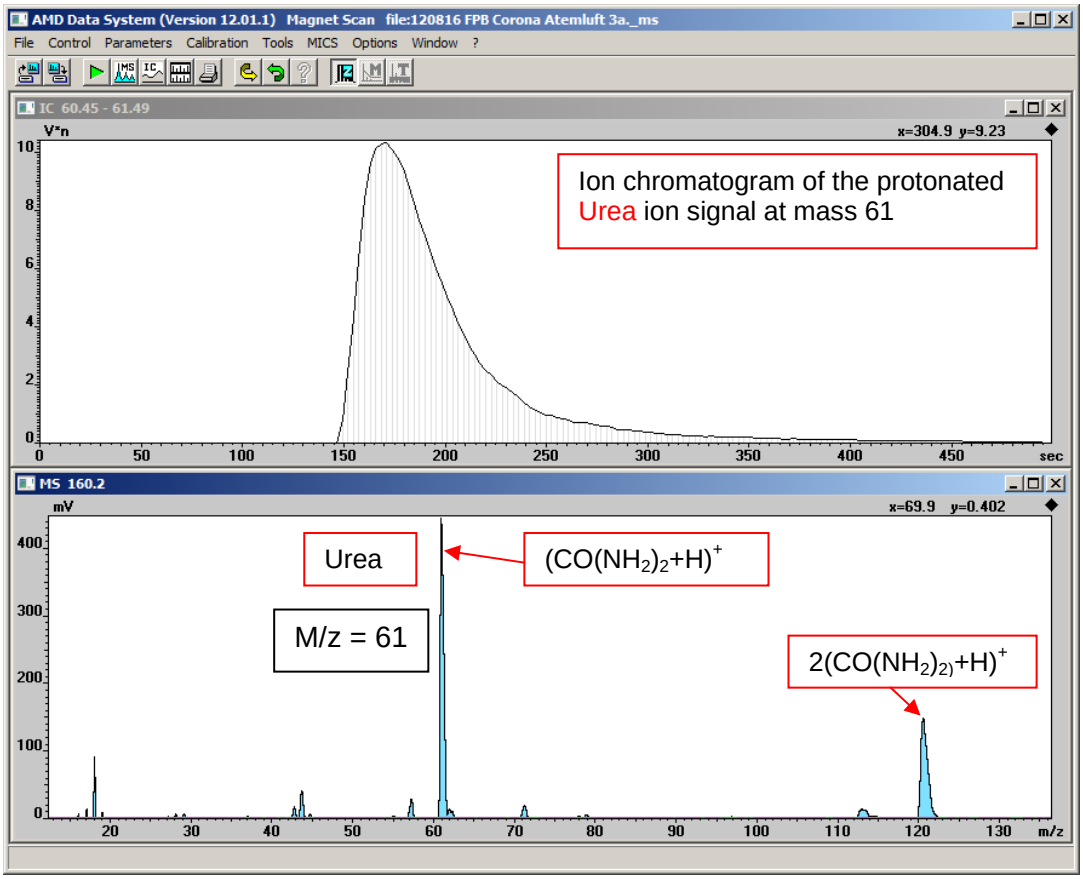

Fig. 8 Abnormal Ion chromatogram and mass spectrum

In one of our experiments a surprising result occurred represented by above figure. Another proband (blood glucose about 110mg/dl) produced during the on-line breath exhalation a significant amount of **saliva** which was automatically introduced into the API chamber via the Teflon pipe.

After some thoughts we came to the conclusion that the very intensive peak at mass 61 represents the protonated **Urea** ion. However, medical or other aspects for the existence (e.g. food related) have not been investigated and clarified in the context of this experiment, finally and unequivocal compound identification has not been performed. Therefore, there is some **remaining speculative risk for this conclusion.**

Nevertheless here are some aspects about the occurrence of this ion: Deviating from the experiments described before, the counter electrode and the front vacuum part of the API interface was hold at a temperature of about 140^{0}C in this experiment. Since Urea has got strong water-binding capacity we assume that the protonated **Urea** ion signal was produced by contact of the **saliva** with the heated counter electrode. The urea molecule was transferred into the gas phase and water acted as proton donator. The Urea concentration (ppm range) was such high that even the cluster ion at mass 121 was produced. Above considerations may be supported by the fact that the proton affinity (PA) of Urea is significant higher than the PA of water.

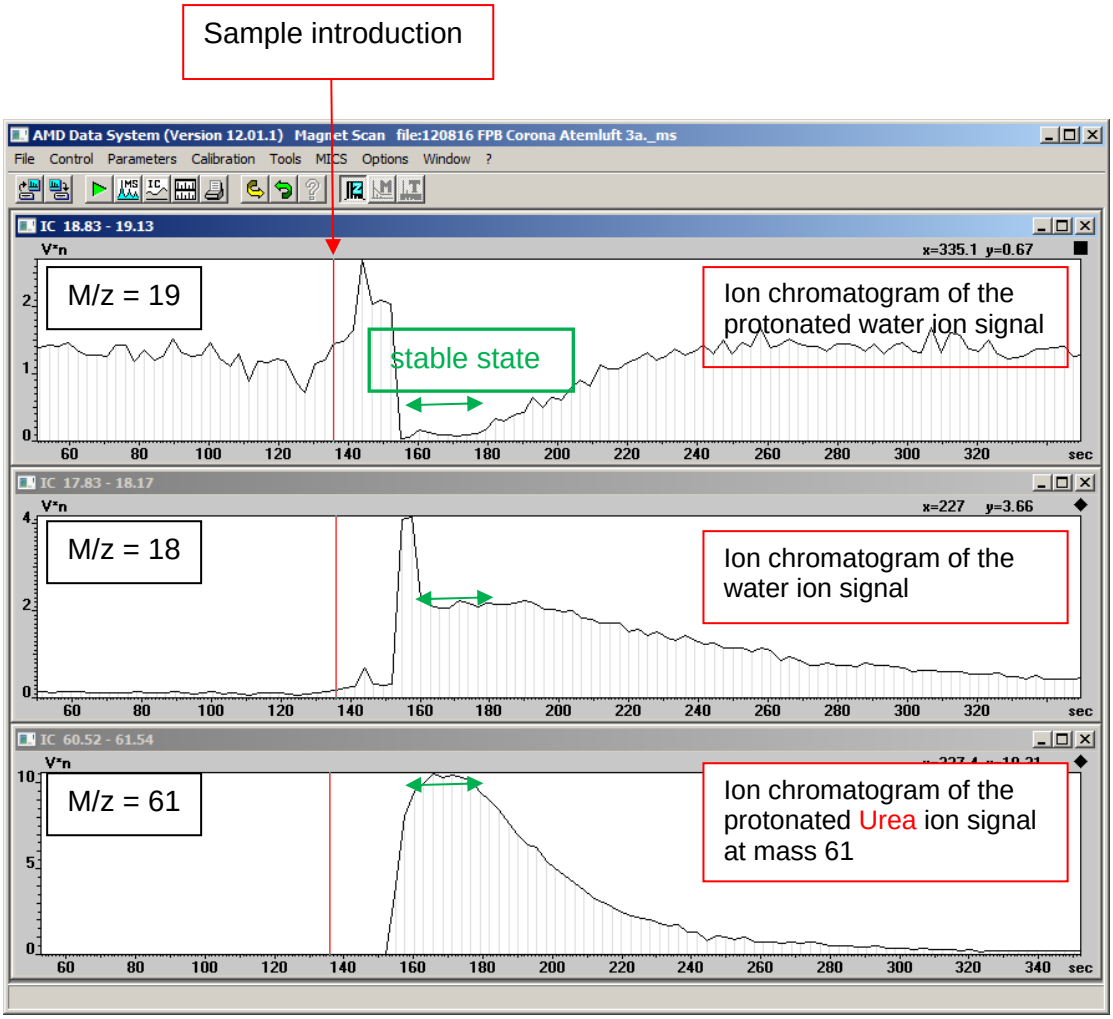

Fig. 9 Ion chromatograms of protonated water, water and Urea

The chromatograms in this figure show the trend of relevant ion formations on the time scale. The time period until 135 sec describes the ambient air section. After sample introduction during the period from 135 to 150 sec water content and water protonation increases. After 152 sec a rapid decrease of water protonation combined with a rapid increase of the non protonated water signal and a strong increase of the protonated Urea signal occurs. Beginning at 158 sec until 180 sec a stabilized vapor pressure and proton transfer to the Urea molecule situation takes place. After 180 sec begins a continuous return to the ambient air concentration in the API chamber.

E) Conclusions

The target of the measurements performed with the evaluation model of the miniaturized AMD Mini QuAS^{3}AR and described here was to achieve information on the system potential for applications in the health care segment. The project had orientating character and has been performed under limited resources. The simple way of introducing breath gas for online measurements can be improved with relative small technical investments which should contribute to achieve somewhat lower detection limits as reported, currently.

A break-through in this respect is expected by the introduction of an array detector for extraordinary enhancement of the scan duty cycle. The design of the analyzer is prepared for implementation of this attachment which should boost the sensitivity in full scan mode such that detection limits of VOCs in breath gas in the low ppt level should be possible. The question how far higher mass resolution, basically a parameter for improved specificity, will play a significant role besides intelligent pattern recognition (“fingerprint algorithms”) programs may be decided by the ongoing research efforts of the scientific community in this field.

We conclude that the results so far achieved with the AMD Mini QuAS^{3}AR equipped with a universal API interface give reason to assume the future applicability of the system for clinical diagnosis and therapeutic monitoring based on breath analysis.