SUPPORTING MATERIAL for

Detection of Metabolites of Trapped Humans using Ion Mobility Spectrometry Coupled to Gas-Chromatography

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EXPERIMENTAL

The Trapped Human Experiment

The CO_2 concentration profile is used to visualise the development of the particular experiments. It is the ideal parameter for this purpose: due to the controlled conditions, the only source of CO_2

is the particular volunteer in the void. After a steep increase of about 60 min CO_2 concentration, it reaches a plateau of approx. 4000 ppm which rapidly decreases again when the volunteers has left the void after 6 hours (see Fig. 1). During experiment 8, the gas sensor system including CO_2 was disconnected for changing the sampling points, thus causing a temporary decrease of concentration while both GC/IMS were still connected to the void. During experiment 9, the volunteer temporarily left the void to urinate which can also be seen by a breakdown of concentration in the CO_2 profile.
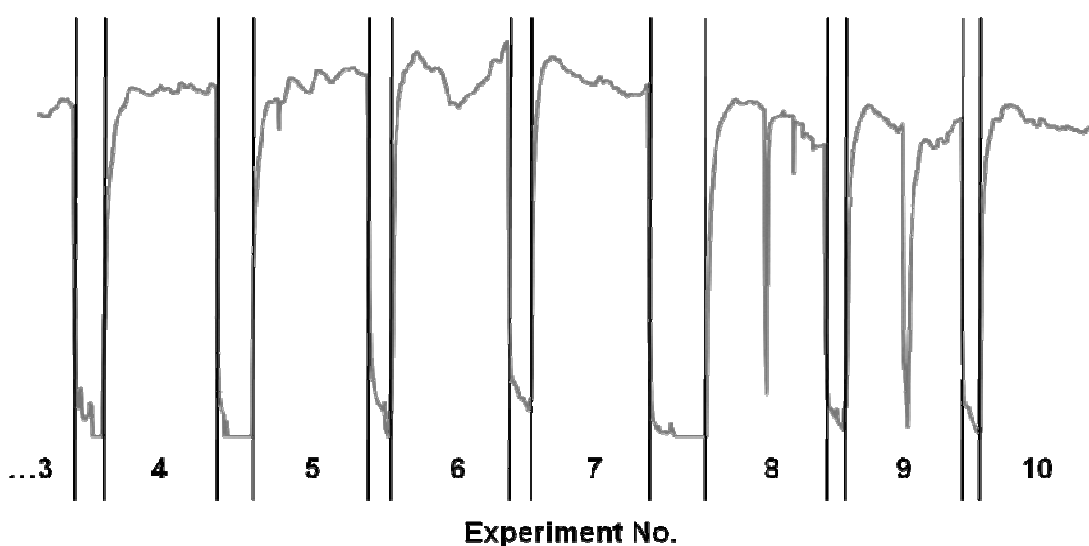
Figure 1. CO_2 concentration profile during the simulation of trapped humans in the frame of THE. Each experiment lasted 6 hours; the experiment no. is indicated. The concentration drop during experiment 8 exists due to a change of the sampling point of the sensor. During experiment 9, the volunteer left the void temporarily causing another concentration drop.

Ion Mobility Spectrometry coupled to Gas Chromatography

The ions are accelerated by a weak electric field (200 - 400 V cm^{-1}) towards the detector – in general a Faraday-plate. An ion gate is opened, e.g. every 100 ms, for typically 100-300 µs and

an ion cloud is allowed to enter the drift region, thus starting to travel towards the detector. A drift gas flow in the opposite direction (nitrogen or clean and dry air) causes collisions of the ions with the present gas molecules. Hence, the ions reach a constant resulting drift velocity depending on their charge, mass and shape. The drift velocity is characteristic for every analyte molecule and can be calculated from the measured drift time at a known drift length.

Normalising the drift velocity to the electric field obtains the ion mobility K (se eq. 1). However, K is still dependent on the density of the drift gas which is influenced by temperature and pressure. Therefore, a further normalisation to pressure and temperature is applied, thus leading to the so-called reduced ion mobility K_0 (se eq. 2), which is a unique characteristic of the analyte and independent on the experimental conditions:

$$K = \frac{L_D}{t_D} \cdot \frac{1}{E} \qquad\qquad (eq.1)$$

$$K_0 = K \cdot \frac{p}{p_0} \cdot \frac{T_0}{T} \qquad\qquad (eq.2)$$

with   K   - ion mobility in cm² V^{-1} s^{-1}

         K_0 - reduced ion mobility in cm² V^{-1} s^{-1}

         L_D - drift length in cm

         t_D - drift time in s

         E   - electric field in V cm^{-1}

         p, T   - ambient temperature and pressure

         p_0, T_0 - reference temperature (273,2 K) and pressure (1013,2 hPa)

ISAS and G.A.S. GC/IMS were operated under slightly different experimental conditions to to increase the information obtained from the particular experiments.

Table 1. Experimental setup of both ISAS and G.A.S. GC/IMS.

ISAS GC/IMS		*G.A.S. BreathSpec®*
Pre-separation		
150 mL/min	**Flow$_{MCC}$**	100 mL/min
20 cm	**Length$_{MCC}$**	20 cm
40 °C	**Temperature$_{MCC}$**	40 °C
OV-5, min. polar	**Type$_{MCC}$**	OV-1701, polar
IMS		
^{63}Ni, 550 MBq	**Ion Source**	^{3}H, 100 MBq
12 cm	**Length$_{Drift}$**	5 cm
100 mL/min	**Flow$_{Drift}$**	500 mL/min
ambient	**Temperature$_{Drift}$**	40 °C
320 V/cm	**Electric Field**	400 V/cm
Sampling		
8 mL	**Volume$_{Loop}$**	5 mL
40 °C	**Temperature$_{Loop}$**	40 °C
C1A	**Sample Point**	C1B

**Thermal desorption gas chromatography/mass spectrometry (TD-GC/MS)**

For the identification of unknowns in the GC/IMS spectra, additional random samples (2L) of the atmosphere in the void were drawn on adsorption tubes (TenaxR, SKC Inc., USA) for later analysis using thermal desorption gas chromatography/mass spectrometry (TD-GC/MS) immediately after a GC/IMS analysis. The adsorbed analytes were desorbed thermally and the sample was injected splitless at 250 °C into an Agilent Technologies 6890N GC system connected with an Agilent Technologies 5973 mass-selective detector (MSD, Gerstel, Mühlheim, Germany). The initial oven temperature of 35 °C was maintained for 2 min and then increased to 250°C by 7 °C/min and finally held for 27 min. A HP-5MS capillary column (60 m, 0.25 mm, 0.25 m film thickness; Wicom, Heppenheim, Germany) was used for compound separation with helium as carrier gas at a constant flow rate of 1.0 ml/min. The electron ionisation mode was performed with 70 eV, and a mass range of m/z 33-450 was detected.

RESULTS & DISCUSSION

For validation of the conclusions drawn from the detection of particular analytes identified as sign of life, all data was compared to the slope of CO_2 concentration as presented in Fig. 1. During the controlled experiments, the trapped volunteers were the only source of CO_2 and therefore the slope of carbon dioxide can be considered as the ultimate sign of life for the simulation setup. In real disaster events, this is not necessarily true as CO_2 could also derive from combustion or fires. All volunteers gave additional information on their actual health state, medication, personal hygiene and recent digestion. In the GC/IMS spectra obtained during the

experiment, all together 46 signals were investigated – all of them following the development of the experiments and the CO_2 slope in particular. Some exemplary signals are discussed here.

Octanal as a first example was detected during all experiments but with a higher concentration during experiment 6 compared to the others (see Fig. 2). Octanal was already found in samples of human breath[27], which explains the elevated concentration during the particular experiments. On the other hand, it is also used as a solvent which could be the reason for the elevated concentration in experiment 6 where the volunteer declared that a specific perfume was applied.
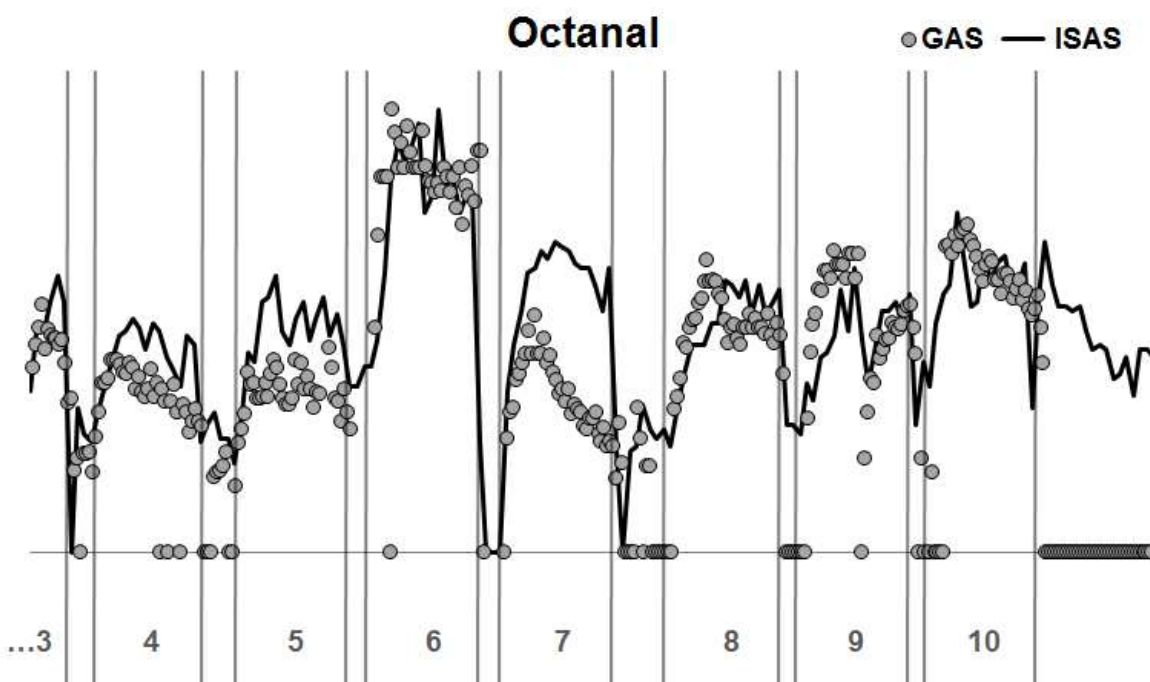

Figure 2. Slope of Octanal concentration during THE as determined by the GAS (grey circles) and ISAS (black line) GC/IMS in samples from the void.

In Fig. 2, a good agreement of both instruments provided by GAS and ISAS can be observed. The minor differences may be explained by the slightly different experimental setup. Over all investigated experiments, the particular volunteers can be differentiated similarly as from the

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slope of the CO_2 concentration (see Fig. 1) but with significantly individual maximum concentrations for Octanal. This might be the case due to individual metabolism and metabolic status of the different volunteers.

Not all signals appeared during all experiments. Exemplarily, peak 5 (see Fig. 3) – which is not yet identified – was observed during 2 particular experiments only. From the additional data obtained from the volunteers, one could assume that the signal is related to the fish or to a medicament which has been consumed by both volunteers exclusively but this can be validated only after identifying the relevant analyte. However, obviously it cannot be expected that this signal will be detected for any trapped person and therefore the peak was not considered for further evaluation.
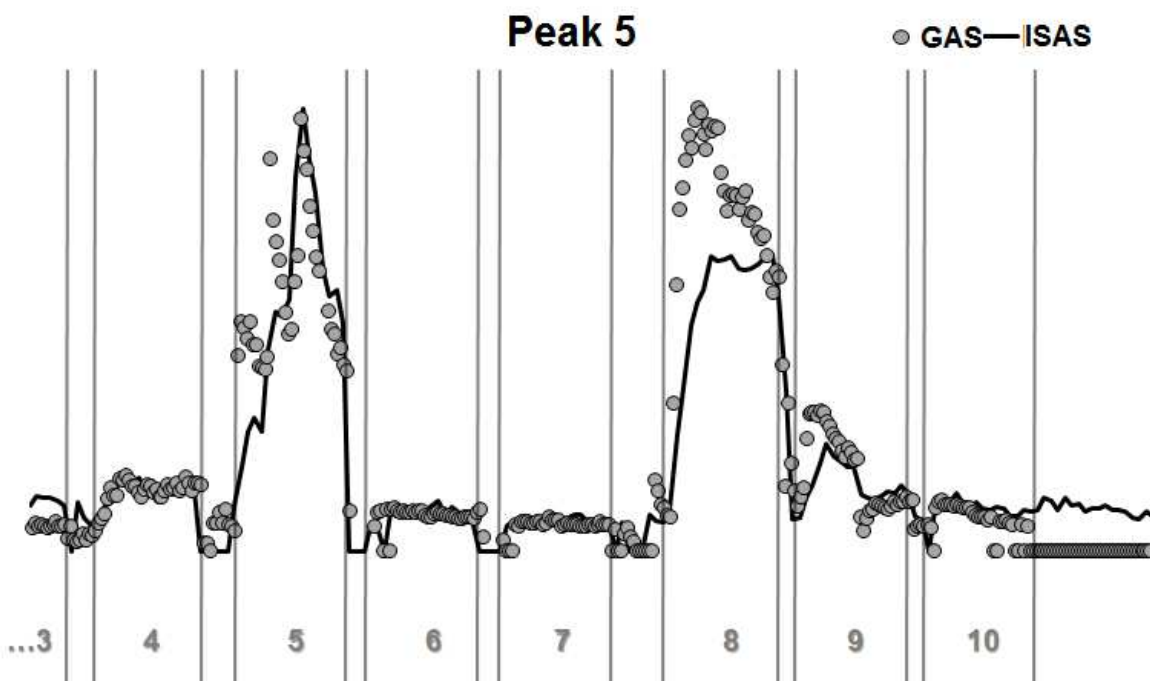
Figure 3.   Peak 5, a presently not identified substance which was detected during 2 experiments only and therefore is not considered as a human metabolite or a sign of life respectively.

The signal of 4-Methyl-2-pentanone (see Fig. 4) was used for validation of the correlation of the GC/MS analysis of the samples to the GC/IMS data. This analyte is supposed to be present in paint and glue and might be related to the construction of the void. Therefore, significant increase can be observed during the experiments most probably due to accumulation in the closed void. E.g. for experiment 6, a good correlation of the signal intensities of both methods can be observed. This procedure gives additional support for a valid identification of the unknown peaks.
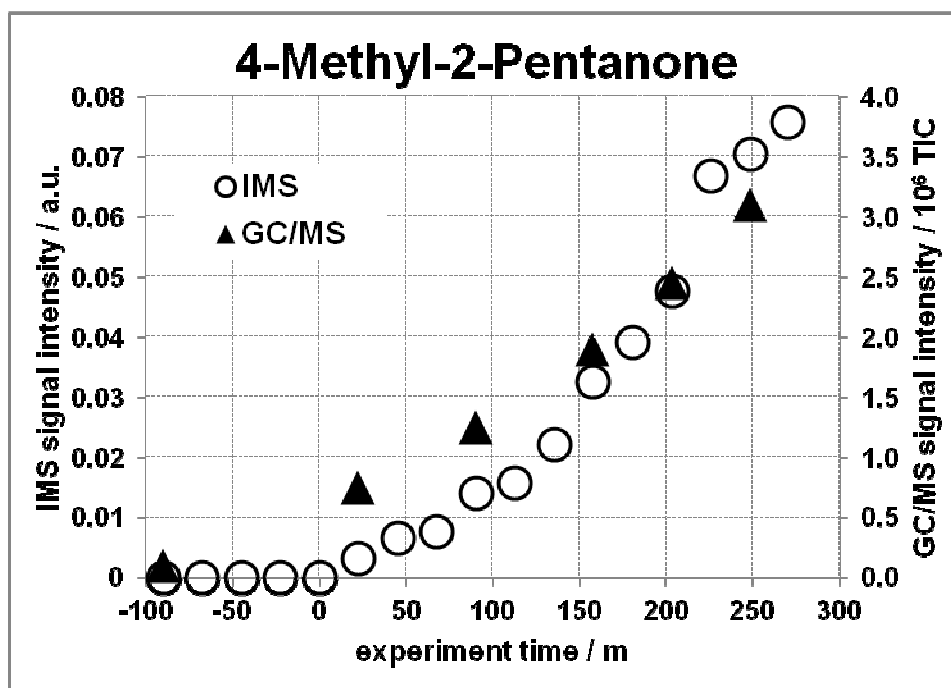
Figure 4.   Slope of the signal intensity of 4-methyl-2-pentanone during experiment 6 as detected by GC/MS and ISAS GC/IMS.

To obtain information on the concentration range of the detected analytes, the GC/IMS was calibrated with the reference analytes using the HovaCAL calibration gas generator[26] as described in the experimental section. The result of such a calibration is shown in Fig. 5 for

acetophenone at 80 and 100 % relative humidity as an example. The slope shows the typical non-linear behaviour of the dependency of signal intensity to concentration of an ion mobility spectrometer. The limit of detection is in the range of 100 ppt$_V$ and only minor influence of the humidity can be observed.

Acetophenon - calibration curve
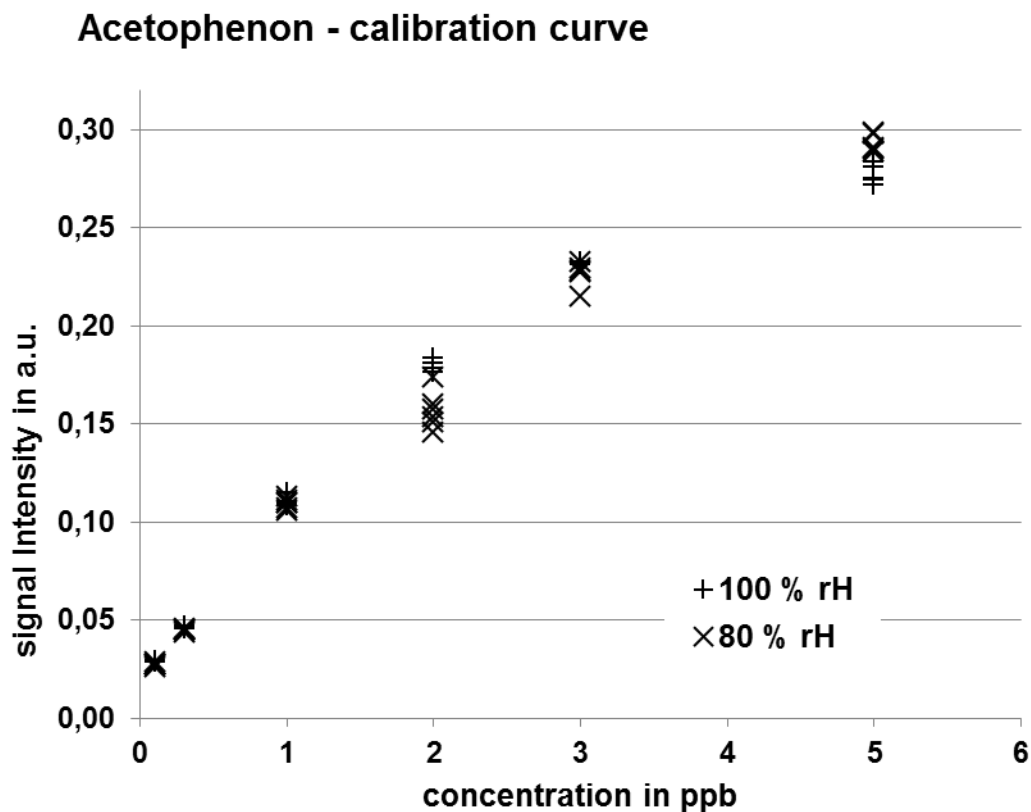
Figure 5. Acetophenone calibration curve for 80 % and 100 % relative humidity obtained from the ISAS GC/IMS.

ABBREVIATIONS

K   ion mobility

K_0 reduced ion mobility

L_D drift length

t_D drift time

E   electric field

T   temperature

T_0 standard temperature

p   pressure

p_0 standard pressure