

1 Article

2 Hybrid Analytical Platform Based on Field- 3 Asymmetric Ion Mobility Spectrometry, Infrared 4 Sensing, and Luminescence-Based Oxygen Sensing 5 for Exhaled Breath Analysis

6 L. Tamina Hagemann, Stefan Repp, Boris Mizaikoff*

7 Institute of Analytical and Bioanalytical Chemistry (IABC), Ulm University, Albert-Einstein-Allee 11, 89081
8 Ulm, Germany; boris.mizaikoff@uni-ulm.de.

9 * Correspondence: boris.mizaikoff@uni-ulm.de; Tel.: +49-731-50-22750

10 Received: date; Accepted: date; Published: date

11 **Abstract:** The reliable online analysis of volatile compounds in exhaled breath remains a challenge
12 as a plethora of molecules occur in different concentration ranges (i.e. ppt to %), and need to be
13 detected against an extremely complex background matrix. While this complexity is commonly
14 addressed by hyphenating a specific analytical technique with appropriate preconcentration and/or
15 pre-separation strategies prior to detection, we herein propose the combination of three analytical
16 tools based on truly orthogonal measurement principles as an alternative solution: field-asymmetric
17 ion mobility spectrometry (FAIMS), Fourier-transform infrared (FTIR) spectroscopy-based sensors
18 utilizing substrate-integrated hollow waveguides (iHWG), and luminescence sensing (LS). These
19 three tools have been integrated into a single compact analytical platform suitable for online exhaled
20 breath analysis. The analytical performance of this prototype system was tested via artificial breath
21 samples containing nitrogen (N₂), oxygen (O₂), carbon dioxide (CO₂) and acetone as a model volatile
22 organic compound (VOC) commonly present and detected in breath. Functionality of the combined
23 system was demonstrated by detecting these analytes in their respectively breath-relevant
24 concentration range and mutually independent of each other generating orthogonal yet correlated
25 analytical signals. Finally, adaptation of the system towards the analysis of real breath samples
26 during future studies is discussed.

27 **Keywords:** exhaled breath analysis; field-asymmetric ion mobility spectrometry; FAIMS; Fourier-
28 transform infrared spectroscopy; FTIR; luminescence sensing; infrared sensors; hyphenated
29 techniques; hybrid techniques; acetone; carbon dioxide; oxygen

31 1. Introduction

32 Breath contains a wide variety of molecules in largely different concentration ranges - from ppt
33 to percent - that are potentially useful for therapy monitoring and elucidation of metabolic pathways.
34 The analysis of such a complex sample by a single analytical techniques is almost impossible. Hence,
35 the combination of orthogonal analytical tools appears to be a viable strategy addressing this issue.
36 To date, predominantly preconcentration, e.g. via solid-phase microextraction (SPME) fibers and
37 needle trap devices (NTD), and/or pre-separation schemes, e.g. gas chromatography (GC) or
38 multicapillary (MCC) columns are implemented for addressing trace concentrations, and for
39 reducing the sample complexity. By combining pre-separation schemes with FID[1], mass
40 spectrometers, (e.g. TOF-MS[2]) or ion mobility based detectors, e.g. IMS[3] or DMS[4] potent
41 analytical tools have resulted. However, MS-based equipment - while being able to detect a very wide
42 variety of analytes - tends to be costly, bulky and frequently not suitable for online analysis. Also, if
43 only one type of detector is used, potentially useful analytes (i.e. biomarkers) that are not sensitive to
44 the selected detector type remain undetected.

45 Therefore, the integration of orthogonal detection schemes into a single hybrid analytical
46 platform is the next logical step. Only a few research groups have selected this path for exhaled breath
47 analysis. The probably most commonly selected approach is the use of electronic noses[5–10], i.e.
48 arrays of different colorimetric[8] or metal oxide sensors[9] individually responding to different types
49 of molecules. While these sensors arrays offer portable and rapidly responding breath detection
50 capabilities, specific biomarker identification and inter-device comparability remain challenging[11].
51 Vaks *et al.*[12] and Shorter *et al.*[13] both combined light sources emitting different wavelengths or
52 even wavelength regimes (i.e. subTHz, THz, IR) in order to broaden the scope of addressable analytes
53 in breath. However, even if these light sources complemented each other, hence providing
54 orthogonality to some extent the basic detection mechanism was essentially similar. Hence, molecules
55 not responding to the respective detection scheme (here, sufficient light absorption in the selected
56 wavelength regimes) will remain undetected. Consequently, truly orthogonal methods are based on
57 different physical principles generating the analytical signals, yet applied to the same sample. This
58 approach has already been proposed[14,15] and put into practice[10,16–20] by various research
59 groups. For example, Covington *et al.*[10] applied an eNose and GC-IMS to the same breath samples,
60 whereas Williams *et al.*[19] parallelly used non-dispersive infrared analysis and PTR-TOF-MS on the
61 same sample set. It is important to notice though that all above-mentioned groups except Monks *et al.*
62 [20] applied different analytical methods as standalone-techniques, i.e. the used analytical devices
63 were not integrated into a single setup. This entails extensive sample handling – and potentially
64 associated handling errors – and extended analysis times, e.g. required for separate sample injection
65 and limits application at the patient bedside. Furthermore, with the exception of Williams *et al.*[19]
66 *offline* breath analysis was performed frequently involving gas bags or sample storage, and thus
67 taking the risk of cross-contamination and sample degradation.

68 Only few groups have developed hybrid analytical devices that enable *online* breath analysis
69 based on truly orthogonal principles integrated in a single sensing platform. Tiele *et al.*[21] published
70 a portable device for CO₂ and O₂ detection that additionally measured temperature and pressure.
71 Miekisch *et al.*[22,23] presented a multidimensional sensing platform including hemodynamic
72 monitoring as well as comprehensive breath monitoring via capnometry, spirometry and PTR-TOF-
73 MS, all being integrated into the same online monitoring platform. While Miekisch *et al.* analyzed
74 human breath, our research team has focused on exhaled mouse breath analysis within a mouse
75 intensive care unit (MICU) at the Institute of Anesthesiologic Pathophysiology and Method
76 Development (IAPMD) at Ulm University Medical Center, which requires as an additional challenge
77 the analysis of exceedingly (i.e. few hundreds of microliters) small breath sample volumes[24–26]. In
78 order to gain metabolic insights, ¹²CO₂, ¹³CO₂ and O₂ concentrations as well as the respiratory quotient
79 (RQ) were evaluated using various analytical tools (iHWG-FTIR spectroscopy, interband cascade
80 laser based tunable diode laser absorption spectroscopy (TDLAS) and LS), which were all adapted to
81 the challengingly small breath volumes exhaled by a mouse or any comparable small animal model.
82 Besides these already quantifiable analytes in mouse breath, the detection of additional volatile
83 compounds such as acetone and H₂S is currently in development for therapy monitoring and to aid
84 in understanding the underlying metabolism of traumatized mice.

85 Hence, the present study aims at extending the scope of addressable analytes in mouse breath
86 beyond CO₂ and O₂ by combining FTIR and LS with FAIMS serving as truly orthogonal analytical
87 methods. The detection principles of iHWG based FTIR spectroscopy[27], LS[28] and FAIMS[29] have
88 been described in detail elsewhere. O₂ detection via LS was necessary, as O₂ is neither IR active nor
89 does it give rise to a FAIMS signal. Furthermore, CO₂ could not have been detected by the
90 luminescence sensor and is not ionizable by the ⁶³Ni FAIMS ionization source, and hence, not
91 detectable by FAIMS. In turn, it provides a signal via IR spectroscopy/sensing techniques. Last but
92 not least, the luminescence sensor does not respond to VOCs, and the sensitivity of the selected IR
93 approach would not have allowed for VOC detection at the breath-relevant ppt or ppb concentration
94 range, even though a wide variety of breath-relevant VOCs are IR-active. Hence, integrating FAIMS
95 into the diagnostic platform was essential for reliable and sensitive trace VOC detection.

96 Synthetic breath samples containing N₂, O₂, CO₂ and acetone as an exemplary breath VOC were
97 prepared and analyzed to demonstrate functionality of the developed hybrid prototype. The
98 presented data proves the feasibility of the integration of FAIMS, FTIR and LS into a single analytical
99 platform for simultaneous online analysis of O₂, CO₂ and acetone as a breath VOC representative. It
100 was shown that the detection of all analytes was possible in the respective breath-relevant
101 concentration range, and that FAIMS, FTIR and LS signals were independent of one another, yet
102 correlated as determined at the same time within the same sample.
103

104 2. Materials and Methods

105 2.1 Hybrid Analytical Platform

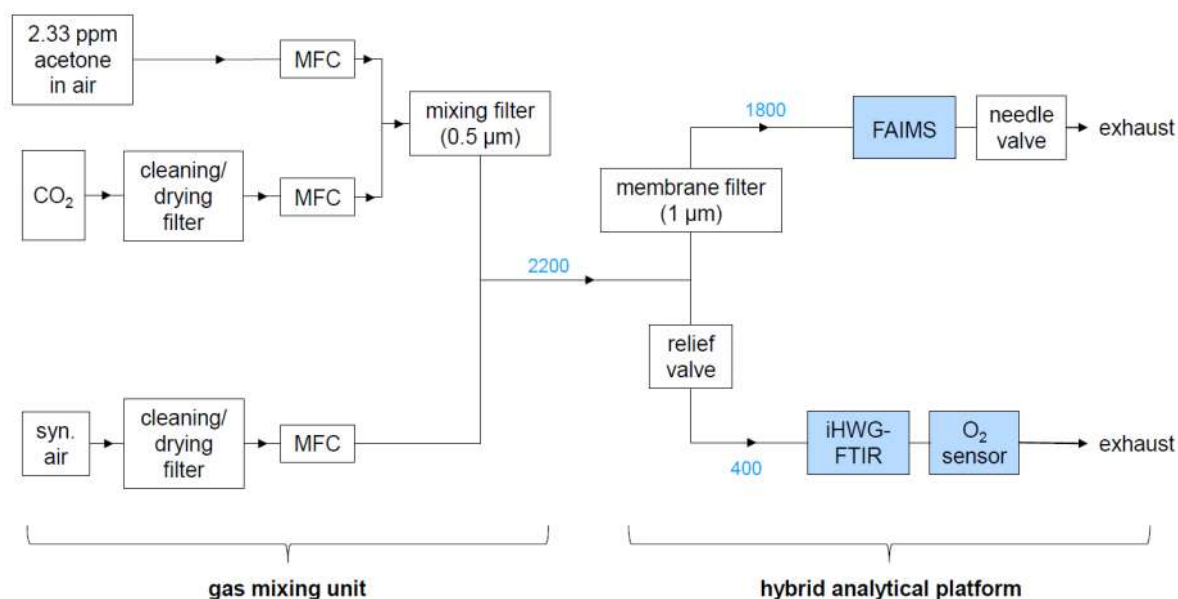
106 2.1.1 Gas Sample Preparation

107 A stock gas mixture of 2.33 ppm acetone in synthetic air (± 0.23 ppm, MTI Industriegase, Neu-
108 Ulm, Germany) was diluted down by synthetic air (produced with 20.5 vol.% O₂ grade 5.0, remains
109 N₂ grade 5.0, H₂O ≤ 5 ppmv, NO+NO₂ ≤ 0.1 ppmv, low molecular weight hydrocarbons C_nH_m $<$
110 0.1 ppmv, by MTI Industriegase, Neu-Ulm, Germany) and CO₂ (technical grade (DIN EN ISO 14175),
111 ≥ 99.8 vol.%, N₂ ≤ 1000 ppmv, H₂O ≤ 120 ppmv, MTI Industriegase, Neu-Ulm, Germany) to eight
112 samples, containing acetone concentrations between 0 and 20 ppb and a background concentration
113 of 3, 4 or 5 % CO₂ and 19.6 \pm 0.5 % O₂ (concentrations given here are volumetric concentrations). The
114 acetone, air and CO₂ flow were regulated by mass flow controllers (Bronkhorst El Flow Prestige, FG-
115 201CV-RBD-11-K-DA-000, 80 mL/min full scale capacity for acetone; FG-201CV-ABD-11-V-DA-000,
116 3000 mL/min full scale capacity for synthetic air; Vögtlin red-y smart series, type GSC-A9KS-BB22,
117 200 mL/min full scale capacity for CO₂). For cleaning and drying purposes, air and CO₂ were filtered
118 through active charcoal (# 20626, Restek, Bad Homburg, Germany), molecular sieve (5Å pore size,
119 # 8475.2, Carl Roth GmbH & Co KG, Karlsruhe, Germany) and sintered glass filter elements
120 (VitraPor®, 40-100 μ m, 4-5.5 μ m, 1.5 μ m). The dew point of air and CO₂ was measured to be -39.8°C
121 (humidity sensor SF52-2-X-T1-B, Michell Instruments, Ely, UK), corresponding to a water content of
122 192 ppm. The acetone sample gas was neither VOC filtered nor dried, since this would have caused
123 analyte loss. The water content in the acetone gas cylinder was assumed to be negligible due to the
124 dilution of acetone sample gas in comparatively big volumes of CO₂/air.

125 Acetone and CO₂ were mixed first, by leading their flow through a filter with 0.5 μ m pore size
126 (SS-2TF-05, Swagelok, Reutlingen, Germany) to induce turbulences for homogeneous mixing. The
127 combined acetone/CO₂ flow was then combined with the air flow. A schematic of the gas mixing unit
128 is displayed in Figure 1 (left half) in section 2.1.2 together with the hybrid FAIMS-FTIR-LS sensing
129 platform.

130 2.1.2 Hybrid FAIMS-FTIR-LS Platform and Concentration-Dependent Measurements

131 The hybrid analytical platform is displayed in Figure 1. Gas samples were provided by the gas
132 mixing unit displayed in the left half of Figure 1 and described in the previous section. The sample
133 flow produced by the gas mixing unit was constantly kept at 2200 mL/min. The relief valve (SS-
134 RL3S4, Swagelok, Reutlingen, Germany) between the gas mixing unit and the FTIR/O₂ sensor unit
135 was adjusted so that the flow reaching the FTIR/O₂ sensor unit was 400 \pm 10 mL/min and the flow
136 through the FAIMS PAD was 1800 \pm 30 mL/min. These flows were regularly checked on with a digital
137 flow meter (ADM1000, J&W Scientific, Folsom, CA, USA) at the outlet of the O₂ sensor and with the
138 flow sensor integrated in the FAIMS PAD, respectively. To minimize analyte adsorption along the
139 tubing walls, perfluoroalkoxy alkane (PFA) tubings (1/8" and 1/4" outer diameter, Swagelok,
140 Reutlingen, Germany) and heated (41 °C) Sulfinert tubings (#29242, Restek, Bad Homburg, Germany)
141 were used in order to minimize analyte adsorption.



142

143

144

Figure 1. Experimental setup comprising the gas mixing unit and the hybrid analytical platform.

Numbers in blue are gas flows in mL/min.

145

146

147

148

149

Before starting a measurement series, a hold time was adopted until flow and pressure had stabilized in the FAIMS device (1800 ± 30 mL/min, 0.800 ± 0.020 barg) to ensure reproducibility of the FAIMS data. In case the flow and pressure varied beyond the given limits, the needle valve at the exhaust of the FAIMS as well as the relief valve between FAIMS and FTIR were adjusted until flow and pressure had stabilized for at least ten minutes in the range defined above.

150

151

152

153

154

155

156

Each measurement series included eight acetone/ CO_2 /air gas samples. Prior to the analysis of an acetone/ CO_2 /air mixture, one sample containing pure air and one sample containing only air and CO_2 were recorded (see Table 1). During the pure air sample, the FTIR background was recorded, and the according FAIMS spectrum was used to ensure that the system had entirely cleaned down after the previous sample. The CO_2 /air measurement, on the other hand, served as a background spectrum for FAIMS. Before analysis, the respective sample gas was led through the setup for at least two minutes to ensure a constant analyte concentration in the whole setup and during the entire measurement.

Table 1: Overview on the measurement protocol within the hybrid setup.

order	injected sample	FAIMS	iHWG-FTIR	LS
1	pure air	verifying system cleanliness	background recording	-
2	CO_2 /air	background recording	-	-
3	acetone/ CO_2 /air	acetone signal recording	CO_2 signal recording	O_2 signal recording
4	repeated for all further samples of a measurement series in random order			

157

158

159

160

161

162

163

164

165

After recording a blank as the first sample of every measurement series, the remaining samples were analyzed in a random sample order that was different in each measurement series. The CO_2 concentration was constant (3, 4 or 5 %) within one measurement series. Three measurement series were recorded per CO_2 concentration. For all air, CO_2 /air and all acetone/ CO_2 /air samples, five FAIMS spectra (~ 19 min) and five FTIR spectra (~ 3.5 min) were successively recorded, while the sample was continuously flowing through the hybrid setup. Simultaneously, the O_2 concentration was continuously monitored for the duration of the FAIMS measurements.

166 2.2 Details on the Individual Analytical Methods

167 2.2.1 Field-Asymmetric Ion Mobility Spectrometry

168 FAIMS data were recorded with an OEM FAIMS PAD (Owlstone Inc., Cambridge, UK), using
169 the Lonestar software (version 4.912, Owlstone Inc., Cambridge, UK). After ionization by a ^{63}Ni
170 ionization source, analytes were detected by the FAIMS sensor (gap size 37 μm ; RF waveform:
171 267 ± 2 V maximum peak-to-peak voltage, 26 MHz \pm 26 Hz RF, 25 % Duty Cycle, 51 steps;
172 compensation voltage (CV) from -6 to +6 V (512 steps, \sim 4.5 s per full CV scan), flow 1800 ± 30 mL/min;
173 sensor temperature: 60 $^{\circ}\text{C}$). The sample gas was continuously flowing through the spectrometer at
174 1800 ± 30 mL/min as the data were recorded. The pressure could be regulated via the needle valve
175 (SS-2MG-MH, Swagelok, Reutlingen, Germany) at the FAIMS outlet and was set to 0.800 ± 0.020 barg.
176 A membrane filter at the inlet of the FAIMS device (polytetrafluoroethylene (PTFE) membrane, 1 μm
177 pore size), heated to 100 $^{\circ}\text{C}$ to avoid analyte accumulation in the filter, prevented particle
178 introduction into the FAIMS PAD. In order to avoid charge build up, the intersweep delay between
179 two subsequent recordings was set to 1500 ms. The obtained FAIMS spectra, also called dispersion
180 plots, displayed the ion current on the detector (z axis) in dependence on the CV (x axis) and the
181 percentage of the dispersion field (DF) which was scanned by varying the peak-to-peak-voltage
182 between 0 and 267 V stepwise.

183 2.2.2 Substrate-Integrated Hollow Waveguide Coupled Fourier-Transform Infrared Spectroscopy

184 CO_2 concentrations were monitored via iHWG coupled FTIR spectroscopy. The setup and gas
185 cell have been described in detail elsewhere[30]. Light from an ALPHA FTIR spectrometer (Bruker
186 Optik GmbH, Ettlingen, Germany) was coupled into an iHWG (aluminum, 7.5 cm optical path
187 length, 4x4 mm internal cross-section, produced by fine mechanical workshop West, Ulm University,
188 Ulm, Germany) and then onto the internal detector of the spectrometer via two gold-coated off-axis
189 parabolic mirrors (Thorlabs, MPD254254-90-M01, 2" RFL). Using the software OPUS (version 7.2,
190 Bruker Optik GmbH, Ettlingen, Germany), IR spectra were recorded in the spectral range from 4000
191 to 400 cm^{-1} at a spectral resolution of 2 cm^{-1} , with 20 averaged scans, and at a flow rate of
192 400 ± 10 mL/min. The Fourier transformation was done in OPUS, using the Blackman-Harris 3-term
193 apodization function. In order to exclude CO_2 from ambient air from the optical absorption paths, the
194 entire IR setup was housed in a plastic bag which was purged with synthetic air for at least 15 minutes
195 prior to as well as during each measurement series.

196 2.2.3 Oxygen Sensing

197 A flow-through O_2 sensor, detecting O_2 based on luminescence quenching, (FireSting O_2 , Pyro
198 Science GmbH, Aachen, Germany)[28] was used for monitoring the O_2 concentration, supported by
199 the Software FireSting Logger (version 2.365, PyroScience GmbH, Aachen, Germany). One data point
200 per second was recorded.
201

202 2.3 Data Processing

203 2.3.1 Field-Asymmetric Ion Mobility Spectrometry

204 Since a direct import of the FAIMS data (.dfm format) into Matlab was not possible, FAIMS data
205 were exported from the Lonestar software as text files and then imported into Matlab (R2018A, The
206 Mathworks Inc., Natick, MA, USA). For baseline correction, the average of the five repetitions of the
207 FAIMS dispersion plot of an CO_2 /air sample was subtracted from the average of the five repetitions
208 of the subsequent acetone CO_2 /air sample. Acetone monomer and dimer peak volumes were
209 approximated by respectively summing all intensity values in selected regions of the dispersion plot
210 (monomer: 68 to 72 % DF, -2.75 to -1.95 V CV; dimer: 46 to 50 % DF, -0.35 to +0.45 V CV). These
211 integration windows were chosen equally wide for monomer and dimer peak and based on a

212 compromise between achievable signal height and freedom from interferences with other spectral
213 components. The so obtained monomer and dimer peak volumes were then added together to obtain
214 the total acetone signal (from now on, only called "acetone signal"). Singly integrating the monomer
215 or the dimer peak would have distorted the FAIMS data evaluation: while the monomer peak was
216 very faint or even invisible at higher acetone concentrations, its contribution to the total acetone signal
217 at higher concentrations would not have been negligible.

218 After normalization with the mean acetone signal at the maximum measured acetone
219 concentration (20 ppb), the signal was averaged and the standard deviation was calculated. The
220 normalized and averaged acetone signal was plotted against the acetone concentration and an
221 asymptotic fit ($y=A \cdot B \cdot C^x$) was applied. Following IUPAC regulations[31], the concentration at the
222 limit of detection (LOD) and at the limit of quantification (LOQ) was estimated by inserting the signal
223 intensity at the LOD and the LOQ ($\mu_B + 3.29 \cdot \sigma_B$ and $\mu_B + 10 \cdot \sigma_B$, respectively, with average normalized
224 signal intensity of the blank μ_B and its according standard deviation σ_B) into the inverse of the
225 calibration function ($x=\ln((A-Y)/B)/\ln C$).

226 2.3.2 Fourier-Transform Infrared Spectroscopy

227 IR data were imported from OPUS into Origin Pro 2017G. An exemplary spectrum is shown in
228 Figure A1 in the Appendix. For baseline correction, each IR spectrum was shifted by the median of
229 the data set, since the latter suitably represented the baseline. The area under the baseline-corrected
230 IR peak at 2360 cm^{-1} between 2200 and 2450 cm^{-1} was averaged for the five repetitions recorded in a
231 row for each sample. The so obtained CO_2 signal was then normalized by division by the overall
232 maximum CO_2 signal and the normalized signal was averaged for the three repetitions of the
233 measurement series recorded for each CO_2 concentration (3, 4 and 5 % CO_2). The according standard
234 deviation was calculated.

235 2.3.3 Oxygen Sensing

236 For each measurement, the O_2 concentrations directly output by the FireSting Logger software
237 was averaged for the time span between 5 and 15 min after starting the O_2 measurement. O_2
238 concentrations recorded between 0 and 5 min were not included in the average, because the O_2
239 concentration reached an equilibrium after approximately 5 min (see Figure A2 in the Appendix).
240 The so obtained O_2 signal was then normalized by division by the overall mean O_2 signal; the
241 normalized signal was averaged for the three repetitions of the measurements series recorded for
242 each CO_2 concentration and the standard deviation was calculated.

243

244 3. Results and Discussion

245 3.1 FAIMS Results

246 As mentioned above, FAIMS dispersion plots of pure air and of CO_2 /air were recorded before
247 recording an acetone/ CO_2 /air containing sample (for further detail also see Table 1 in Section 2.1.2).
248 Figure 2 exemplarily shows a dispersion plot for each sample type collected in positive mode. The
249 dispersion plot of pure air (Figure 2a) mainly showed the reactant ion peak (RIP), which, in positive
250 detection mode, appears due to the formation of ionized clusters of water molecules present in the
251 carrier gas[32]. The faint vertical signal in Figure 2a at around 0 V CV was approximately constant
252 for all recorded dispersion plots. It could not be erased throughout the whole project and was likely
253 to be caused by substances emitted from the tubings and the FAIMS device itself. The CO_2 /air
254 dispersion plot (Figure 2b) also mainly showed the RIP. No clear analyte peak appeared, since CO_2
255 is not ionizable by the ^{63}Ni source. The faint additional trace at around -65 % DF and -3 V CV
256 assumably occurred because of contaminations from the CO_2 gas bottle that could not be entirely
257 removed by the used filters. The acetone/ CO_2 /air dispersion plot (Figure 2c) showed an intensity
258 decrease of the RIP as well as the appearance of two main additional peaks. Generally, once an
259 ionizable analyte like acetone is inserted into the FAIMS, one or two water molecules in the ionized

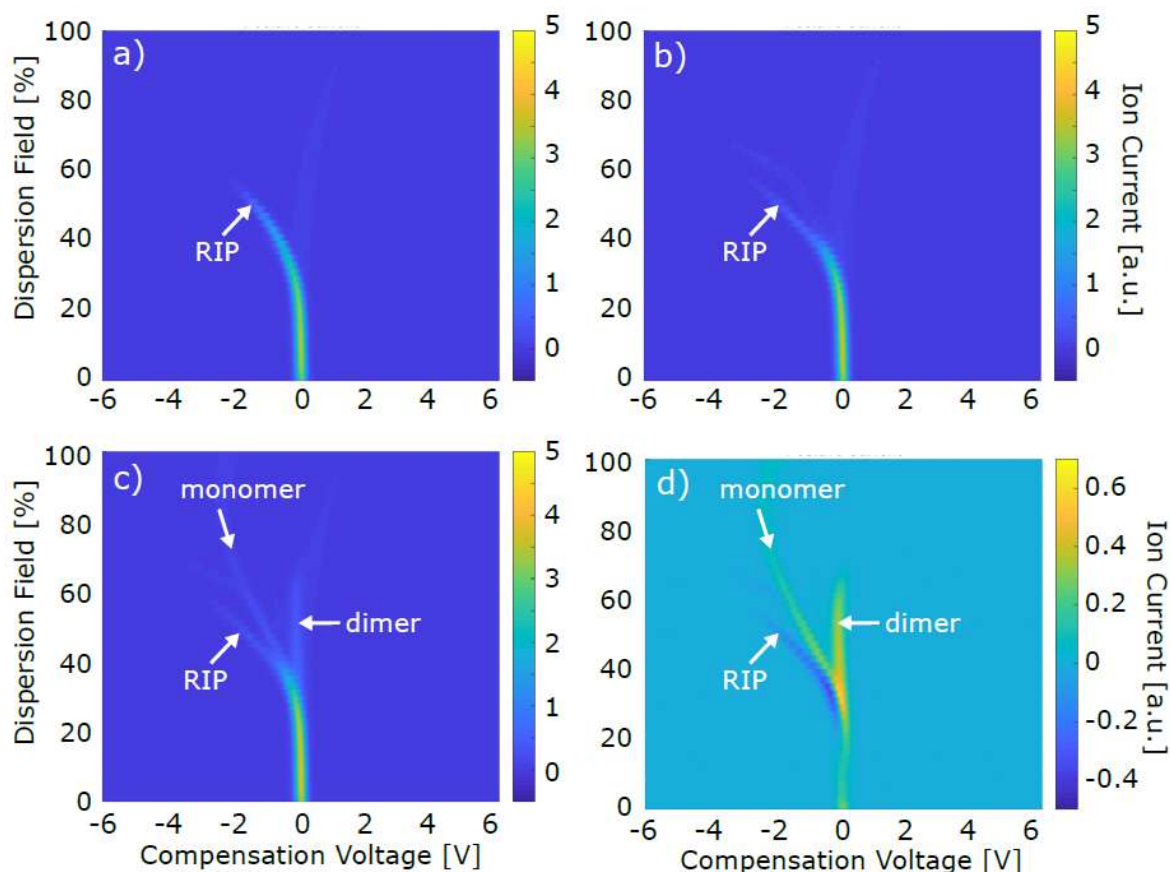


Figure 2. FAIMS dispersion plots. (a) pure air sample (b) CO₂/air sample (c) acetone/CO₂/air sample (d) background subtracted acetone/CO₂/air sample ((c) minus (b)). CO₂ and acetone concentration of these exemplary data were 4 % and 1 ppb, respectively. For the sake of clarity, not all four graphs wear all three axes labels.

260 carrier gas clusters are replaced by the analyte molecules. Hence, the RIP intensity decreases and a
 261 monomer and/or dimer peak appear, respectively. The tentative assignment of monomer and dimer
 262 peak, as it is indicated in Figure 2c, was based on the concentration-dependent behavior of both
 263 peaks: while the monomer peak intensity showed an intensity maximum at lower concentrations, the
 264 dimer peak constantly increased with increasing acetone concentration, as an additional water
 265 molecule in each monomer cluster was replaced by a second acetone molecule, thus forming a dimer
 266 cluster. The relative position of monomer and dimer peak also was in accordance with our
 267 expectations and thus substantiated our peak assignment: the lighter, less bulky and hence more
 268 mobile monomer cluster gave rise to a peak at a lower CV than the less mobile dimer cluster. The
 269 exact origin of the faint feature between monomer and dimer peak in Figures 2c and 2d
 270 (~50 %DF, -0.5 V CV) is unknown, but its potential effect is commented on in section 3.2. In order to
 271 obtain the net monomer and dimer signal, Figure 2b was subtracted from Figure 2c for background
 272 subtraction. The resulting data is shown in Figure 2d. The z axis of Figure 2d was varied compared
 273 to Figures 2a to 2c in order to make the monomer and dimer peak more clearly visible. At the position
 274 where the RIP appeared in Figure 2a to 2c, the signal intensity was negative in Figure 2d, since the
 275 RIP intensity decreased while acetone was present in the FAIMS sensing region.
 276

277 3.2 Co-Dependencies of Acetone, CO₂ and O₂ Signal

278 The normalized total acetone signal, composed of monomer and dimer peak volume, was
 279 plotted against the acetone concentration, shown for 3, 4 and 5 % CO₂ in Figure 3a. Due to saturation
 280 of the FAIMS detector, the acetone signal converged towards a maximum value for higher acetone
 281 concentrations. Thus, an asymptotic fit was applied. It is obvious from Figure 3a, that the acetone

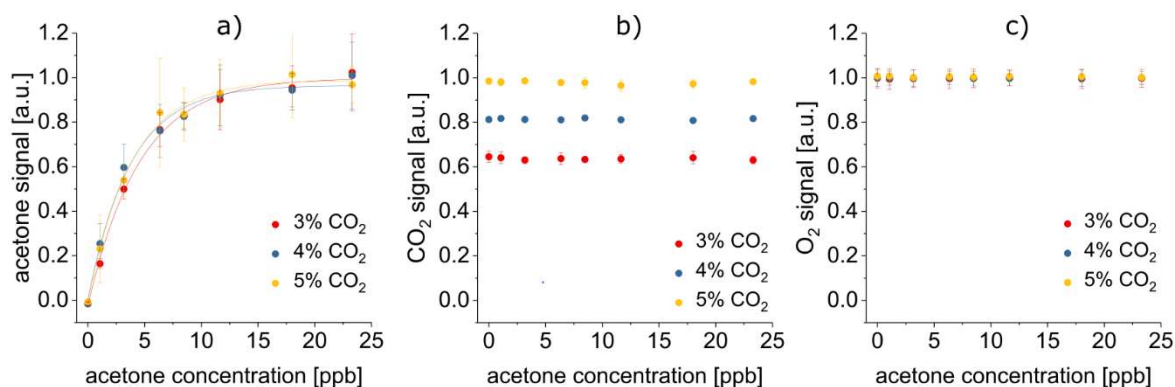


Figure 3: No mutual signal co-dependencies of acetone, CO₂ and O₂ were detected. All displayed error bars are 1 σ error bars. (a) Acetone signals recorded with FAIMS depend on the acetone concentration (asymptotic fit $y = A - B \cdot C^x$), yet is independent of the CO₂ content. (b) CO₂ signals recorded by iHWG-FTIR only vary depending on the CO₂ concentration. (c) O₂ signals recorded by LS are neither influenced by the acetone nor by the CO₂ content.

282 signal was statistically identical, regardless if the CO₂ concentration was 3, 4 or 5 %. Likewise, the
 283 according analytical figures of merit, i.e. LOD, LOQ, R² and parameters of the asymptotic fit, did not
 284 depend on the CO₂ concentration (see Table 2). Hence, the CO₂ concentration did not have any effect
 285 on the FAIMS results. Reversely, Figure 3b and 3c reveal, that the acetone concentration did neither
 286 affect the CO₂ nor the O₂ signal. Also, the O₂ signal did not change depending on the CO₂
 287 concentration, but stayed constant irrespective if 3, 4 or 5 % CO₂ were present. In conclusion, no
 288 mutual co-dependencies of the acetone, CO₂ and O₂ signal were detected.

Table 2: Analytical figures of merit of the concentration-dependent FAIMS measurements of acetone. No statistical difference between fit parameters A, B and C of the asymptotic fit (equation $y = A - B \cdot C^x$) at 3, 4 or 5 % CO₂. R², concentration at LOD and concentration at LOQ varied, yet with no clear trend visible depending on the CO₂ content. This indicates independence of the acetone signal from the CO₂ concentration.

	3 % CO ₂	4 % CO ₂	5 % CO ₂
fit parameter A	0.998 \pm 0.019	0.964 \pm 0.025	0.989 \pm 0.023
fit parameter B	1.024 \pm 0.025	0.962 \pm 0.038	0.997 \pm 0.034
fit parameter C	0.803 \pm 0.012	0.765 \pm 0.022	0.772 \pm 0.019
R ² >	0.995	0.989	0.992
LOD [ppt]	145	78	56
LOQ [ppt]	358	405	165

289 The FAIMS error bars shown in Figure 3a are relatively big compared to the FTIR and LS error
 290 bars in Figures 3b and 3c, respectively. Several different sources have presumably contributed to the
 291 acetone signal variance. First, three slight features apart from RIP, monomer and dimer peak were
 292 visible in the dispersion plots of the acetone/CO₂/air sample (see Figure 3c at ~65 % DF / -3 V CV, at
 293 ~50 % DF / -0.5 V CV and at ~75 % DF / +0.5 V CV). As mentioned before, these possibly appeared
 294 due to contaminations from the CO₂ gas bottle and due to evaporations from the tubings and the
 295 FAIMS itself. Even if they do not seem to have fundamentally impacted the obtained data, these
 296 contaminations might still have competed with acetone for the ionization energy in the FAIMS
 297

298 ionization region, therefore possibly altering the acetone signal intensity and increasing the
299 associated error bars. Furthermore, it cannot be excluded that slight humidity variations occurred,
300 additionally enhancing the variance of the acetone signal. Finally, the saturation of the FAIMS
301 detector at higher acetone concentrations can be assumed to also have made a contribution to the
302 signal variance.

303 3.3 Towards Real Breath Analysis

304 It is our goal to further develop the hybrid FAIMS-FTIR-LS platform towards online analysis of
305 mouse breath. Already with the current setup, the detection of the main breath components CO₂, O₂
306 and acetone, as a breath VOC representative, was possible in breath-relevant concentrations.
307 Especially the fact that breath VOC detection is possible down to LODs and LOQs in the low to
308 medium ppt range with this hybrid setup, makes it a promising tool for real breath analysis, since
309 breath VOCs most often occur in ppt to ppb concentrations[33]. Furthermore, O₂, CO₂ and acetone
310 signal were found to be mutually independent. This underlines the excellent orthogonality of FAIMS,
311 FTIR spectroscopy and LS, making their combination especially suitable for a complex matrix like
312 exhaled breath: simply by selecting a suitable combination of analytical methods, a first – at least
313 virtual – “preseparation” of the sample components has been undertaken, thus already simplifying
314 the analytical task.

315 Nevertheless, the hybrid setup and the experiments conducted with it need to be further evolved
316 before online analysis of real mouse breath is possible. First, unlike in our model samples, of course
317 more than one VOC is present in real breath. All these breath VOCs will compete for the FAIMS
318 ionization energy and therefore cause co-dependencies of their signals. To prevent this, preseparation
319 based on a GC or an MCC column will be integrated into the hybrid setup, enabling the VOCs to
320 reach the ionization region one by one. Since the contaminations discussed above (see Figure 2c) will
321 also be separated from the analytes via the GC or MCC column, the FAIMS signal variance may
322 additionally benefit from the preseparation scheme. Furthermore, alkanes, as an important class of
323 breath VOCs[34], cannot be detected with the current setup, because they are not ionized by the ⁶³Ni
324 ionization source. This problem could be overcome by taking advantage of the modular flexibility of
325 the FTIR detection unit: extending the optical path length of the iHWG and replacing the FTIR
326 spectrometer by a more intense light source like a tunable quantum cascade laser, the LOD/LOQ for
327 alkane detection via FTIR could be shifted to breath-relevant concentrations. Moreover, the samples
328 tested until now only contained minimal amounts of water, whereas real breath is oversaturated with
329 humidity. Since the FAIMS detection mechanism is based on ionized water clusters, changes in
330 humidity have a major effect on the FAIMS signal intensity. Here, chemometric data treatment in
331 dependence of the present water level or experimentally filtering out the humidity by a condenser as
332 proposed by Maiti *et al.*[35], which is explicitly suitable for dehumidifying breath without significant
333 VOC loss, could be possible solutions.

334 4. Conclusions

335 A compact hybrid sensing platform enabling orthogonal analysis of gas/vapor phase samples based
336 on FAIMS, FTIR and LS was presented, and its utility online analysis of synthetic breath samples
337 containing acetone, CO₂ and O₂ was demonstrated. It was shown that the signals of these compounds
338 were independent of one another, and that all three components could be detected at their respective
339 breath-relevant concentrations. The LOQ of acetone could even be lowered to the medium ppt
340 concentration range, which renders the method a promising approach for the potential analysis of
341 trace level breath VOCs. Yet, challenges according to nonetheless integrating additional analyte
342 preconcentration/-separation strategies and dealing with high humidity levels will need to be
343 resolved prior to the useful analysis of real-world exhaled breath samples, and will be addressed
344 during future studies.

345 **Abbreviations:** Å Angström, barg unit for gauge pressure in bar (pressure in bar exceeding atmospheric
346 pressure), CO₂ carbon dioxide, CV compensation voltage, DF dispersion field, FAIMS field-asymmetric ion

347 mobility spectrometry, FTIR Fourier-transform infrared spectroscopy, GC gas chromatography, IABC Institute
348 for Analytical and Bioanalytical Chemistry, IAPMD Institute for Anesthesiological Pathophysiology and
349 Method Development, iHWG substrate-integrated hollow waveguide, min minutes, LS luminescence sensing,
350 MCC multicapillary column, μL microliter, MICU mouse intensive care unit, N_2 nitrogen, O_2 oxygen, OEM
351 original equipment manufacturer, PFA perfluoroalkoxy alkane, ppb parts per billion, ppm parts per million, ppt
352 parts per trillion, PTFE polytetrafluoroethylene, RIP reactant ion peak, RQ respiratory quotient, TDLAS tunable
353 diode laser absorption spectroscopy, THz Terahertz, VOC volatile organic compound, $^\circ\text{C}$ degree Celsius.

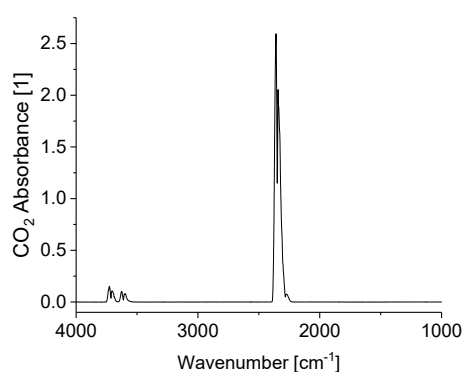
354 **Acknowledgments:** This work was supported by the Research training group PULMOSENS at Ulm University
355 (GRK 2203) and by the German National Academic Foundation (Studienstiftung des Deutschen Volkes).

356 **Author Contributions:** Conceptualization: B.M., L.T.H.; Design, establishment and optimization of setup:
357 L.T.H.; Design of experiment: L.T.H.; Final data acquisition: S.R.; Data evaluation: S.R., L.T.H.; Writing original
358 draft: L.T.H.; Writing-review and editing: B.M., S.R.; Funding acquisition: B.M., L.T.H.; All authors read and
359 approved the final manuscript.

360 **Conflicts of Interest:** The authors declare no conflict of interest. The founding sponsors had no role in the design
361 of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the
362 decision to publish the results.

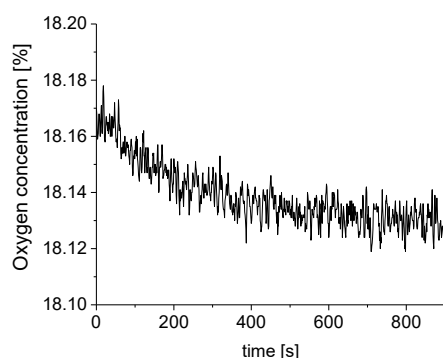
363 Appendix

364 The primary signals of CO_2 and O_2 recorded by FTIR spectroscopy and LS, respectively, are
365 shown in Figure A1 and A2.



366

367 Figure A1: IR spectrum of 4 % CO_2 . Acetone theoretically also is IR active, but is not detected here
368 due to its extremely low concentrations in the ppb range.



369

370 Figure A2: O_2 concentration as detected by the luminescence sensor.

371 References

372 1. Mueller, W.; Schubert, J.; Benzing, A.; Geiger, K. Method for analysis of exhaled air by microwave energy
373 desorption coupled with gas chromatography-flame ionization detection-mass spectrometry. *J.*
374 *Chromatogr. B Biomed. Appl.* **1998**, *716*, 27–38.

- 375 2. Smolinska, A.; Klaassen, E.M.M.; Dallinga, J.W.; Van De Kant, K.D.G.; Jobsis, Q.; Moonen, E.J.C.; Van
376 Schayck, O.C.P.; Dompeling, E.; Van Schooten, F.J. Profiling of volatile organic compounds in exhaled
377 breath as a strategy to find early predictive signatures of asthma in children. *PLoS One* **2014**, *9*, e95668.
- 378 3. Hüppe, T.; Lorenz, D.; Maurer, F.; Albrecht, F.W.; Schnauber, K.; Wolf, B.; Sessler, D.I.; Volk, T.; Fink, T.;
379 Kreuer, S. Exhalation of volatile organic compounds during hemorrhagic shock and reperfusion in rats:
380 An exploratory trial. *J. Breath Res.* **2016**, *10*, 16016.
- 381 4. Basanta, M.; Koimtzis, T. Sampling and analysis of exhaled breath on human subjects with thermal
382 desorption- gas chromatography- differential mobility spectrometry. *Int. J. Ion Mobil. Spectrom.* **2006**, *9*,
383 45–49.
- 384 5. Di Natale, C.; Macagnano, A.; Martinelli, E.; Paolesse, R.; D’Arcangelo, G.; Roscioni, C.; Finazzi-Agrò,
385 A.; D’Amico, A. Lung cancer identification by the analysis of breath by means of an array of non-
386 selective gas sensors. *Biosens. Bioelectron.* **2003**, *18*, 1209–1218.
- 387 6. Machado, R.F.; Laskowski, D.; Deffenderfer, O.; Burch, T.; Zheng, S.; Mazzone, P.J.; Mekhail, T.;
388 Jennings, C.; Stoller, J.K.; Pyle, J.; et al. Detection of lung cancer by sensor array analyses of exhaled
389 breath. *Am. J. Respir. Crit. Care Med.* **2005**, *171*, 1286–1291.
- 390 7. Peng, G.; Tisch, U.; Adams, O.; Hakim, M.; Shehada, N.; Broza, Y.Y.; Billan, S.; Abdah-Bortnyak, R.;
391 Kuten, A.; Haick, H. Diagnosing lung cancer in exhaled breath using gold nanoparticles. *Nat.*
392 *Nanotechnol.* **2009**, *4*, 669–673.
- 393 8. Mazzone, P.J.; Wang, X.F.; Xu, Y.; Mekhail, T.; Beukemann, M.C.; Na, J.; Kemling, J.W.; Suslick, K.S.;
394 Sasidhar, M. Exhaled Breath Analysis with a Colorimetric Sensor Array for the Identification and
395 Characterization of Lung Cancer. *J. Thorac. Oncol.* **2012**, *7*, 137–142.
- 396 9. De Vries, R.; Brinkman, P.; Van Der Schee, M.P.; Fens, N.; Dijkers, E.; Bootsma, S.K.; De Jongh, F.H.C.;
397 Sterk, P.J. Integration of electronic nose technology with spirometry: Validation of a new approach for
398 exhaled breath analysis. *J. Breath Res.* **2015**, *9*, 46001.
- 399 10. Tiele, A.; Wicaksono, A.; Kansara, J.; Arasaradnam, R.P. Breath Analysis Using eNose and Ion Mobility
400 Technology to Diagnose Inflammatory Bowel Disease — A Pilot Study. *Biosensors* **2019**, *9*, 55–70.
- 401 11. Fens, N.; van der Schee, M.P.; Brinkman, P.; Sterk, P.J. Exhaled breath analysis by electronic nose in
402 airways disease. Established issues and key questions. *Clin. Exp. Allergy* **2013**, *43*, 705–715.
- 403 12. Vaks, V.L.; Domracheva, E.G.; Pripolzin, S.I.; Chernyaeva, M.B. Multifrequency high precise subTHz-
404 THz-IR spectroscopy for exhaled breath research. *Terahertz Emit. Receiv. Appl. VII* **2016**, *9934*, 99340E–1
405 to 99340E–10.
- 406 13. Shorter, J.H.; Nelson, D.D.; McManus, J.B.; Zahniser, M.S.; Milton, D.K. Multicomponent Breath Analysis
407 With Infrared Absorption Using Room-Temperature Quantum Cascade Lasers. *NIH Public Access* **2010**,
408 *10*, 76–84.
- 409 14. Ibrahim, W.; Wilde, M.; Cordell, R.; Salman, D.; Ruskiewicz, D.; Bryant, L.; Richardson, M.; Free, R.C.;
410 Zhao, B.; Yousuf, A.; et al. Assessment of breath volatile organic compounds in acute cardiorespiratory
411 breathlessness: A protocol describing a prospective real-world observational study. *BMJ Open* **2019**, *9*,
412 1–13.
- 413 15. Inside the Breath Cluster <https://www.insidebreath.com/en/>, accessed on April 30th 2019.
- 414 16. Dharmawardana, N.; Ooi, E.; Goddard, T.; Woods, C.; Watson, D.; Yazbek, R. Characterization of breath
415 volatile organic compounds in patients with upper aerodigestive mucosal squamous cell carcinoma.
416 *IABR Breath Summit* 2018.
- 417 17. Gould, O.; Ratcliffe, N.; de Lacy, C.B.; Francis, N.; Young, N. Approaches to a simple breath test for the

- 418 early diagnosis of post-operative complications following colorectal cancer and segmental resection.
419 *IABR Breath Summit* 2018.
- 420 18. Shimouchi, A.; Jinno, N.; Taniguchi, K.; Okumura, N.; Miyamoto, Y. Volatile compounds in exhaled air
421 associated with scores of general physical and mental conditions. *IABR Breath Summit* 2018.
- 422 19. Williams, J.; Stönnner, C.; Wicker, J.; Krauter, N.; Derstroff, B.; Bourtsoukidis, E.; Klüpfel, T.; Kramer, S.
423 Cinema audiences reproducibly vary the chemical composition of air during films, by broadcasting
424 scene specific emissions on breath. *Sci. Rep.* **2016**, *6*, 1–10.
- 425 20. Wilde, M.J.; Cordell, R.L.; Salman, D.; Zhao, B.; Ibrahim, W.; Bryant, L.; Ruskiewicz, D.; Singapuri, A.;
426 Free, R.C.; Gaillard, E.A.; et al. Breath analysis by two-dimensional gas chromatography with dual flame
427 ionisation and mass spectrometric detection – method optimisation and integration within a large-scale
428 clinical study. *J. Chromatogr. A* **2019**, *1594*, 160–172.
- 429 21. Tiele, A.; Covington, J. Design and development of an end-tidal breath capture device. *IABR Breath*
430 *Summit* 2018.
- 431 22. Sukul, P.; Trefz, P.; Kamysek, S.; Schubert, J.K.; Miekisch, W. Instant effects of changing body positions
432 on compositions of exhaled breath. *J. Breath Res.* **2015**, *9*, 47105.
- 433 23. Sukul, P.; Schubert, J.K.; Kamysek, S.; Trefz, P.; Miekisch, W. Applied upper-airway resistance instantly
434 affects breath components: A unique insight into pulmonary medicine. *J. Breath Res.* **2017**, *11*, 47108.
- 435 24. Fortes, P.R.; Wilk, A.; Seichter, F.; Cajlakovic, M.; Koestler, S.; Ribitsch, V.; Wachter, U.; Vogt, J.;
436 Radermacher, P.; Carter, C.; et al. Combined sensing platform for advanced diagnostics in exhaled
437 mouse breath. *Front. Biol. Detect. From Nanosensors to Syst. V* **2013**, *8570*, 85700Q–1 to 85700Q–9.
- 438 25. Seichter, F.; Tütüncü, E.; Hagemann, L.T.; Vogt, J.; Wachter, U.; Gröger, M.; Kress, S.; Radermacher, P.;
439 Mizaikoff, B. Online monitoring of carbon dioxide and oxygen in exhaled mouse breath via substrate-
440 integrated hollow waveguide Fourier-transform infrared- luminescence spectroscopy. *J. Breath Res.* **2018**,
441 *12*, 036018.
- 442 26. Tütüncü, E.; Nägele, M.; Becker, S.; Fischer, M.; Koeth, J.; Wolf, C.; Köstler, S.; Ribitsch, V.; Teuber, A.;
443 Gröger, M.; et al. Advanced Photonic Sensors Based on Interband Cascade Lasers for Real-Time Mouse
444 Breath Analysis. *ACS Sensors* **2018**, *3*, 1743–1749.
- 445 27. Wilk, A.; Chance Carter, J.; Chrisp, M.; Manuel, A.M.; Mirkarimi, P.; Alameda, J.B.; Mizaikoff, B.
446 Substrate-integrated hollow waveguides: A new level of integration in mid-infrared gas sensing. *Anal.*
447 *Chem.* **2013**, *85*, 11205–11210.
- 448 28. Seichter, F.; Vogt, J.; Radermacher, P.; Mizaikoff, B. Nonlinear calibration transfer based on hierarchical
449 Bayesian models and Lagrange Multipliers: Error bounds of estimates via Monte Carlo – Markov Chain
450 sampling. *Anal. Chim. Acta* **2017**, *951*, 32–45.
- 451 29. Covington, J.A.; van der Schee, M.P.; Edge, A.S.L.; Boyle, B.; Savage, R.S.; Arasaradnam, R.P. The
452 application of FAIMS gas analysis in medical diagnostics. *Analyst* **2015**, *140*, 6775–6781.
- 453 30. Hagemann, L.T.; McCartney, M.M.; Fung, A.G.; Peirano, D.J.; Davis, C.E.; Mizaikoff, B. Portable
454 combination of Fourier transform infrared spectroscopy and differential mobility spectrometry for
455 advanced vapor phase analysis. *Analyst* **2018**, *143*, 5683–5691.
- 456 31. Inczédy, A. *IUPAC Compendium of Analytical Nomenclature: Definitive Rules. Chapter 18.4.3.7*; 3rd ed.; 1997;
- 457 32. Eiceman, G.A.; Karpas, Z. *Ion Mobility Spectrometry*; 2nd ed.; Taylor & Francis Group LLC: Boca Raton,
458 FL, USA, 2005;
- 459 33. Phillips, M. Method for the collection and assay of volatile organic compounds in breath. *Anal. Biochem.*
460 **1997**, *247*, 272–278.

- 461 34. Cheepsattayakorn, A.; Cheepsattayakorn, R. Breath tests in respiratory and critical care medicine: From
462 research to practice in current perspectives. *Biomed Res. Int.* **2013**, *2013*, 702896.
- 463 35. Maiti, K.S.; Lewton, M.; Fill, E.; Apolonski, A. Sensitive spectroscopic breath analysis by water
464 condensation. *J. Breath Res.* **2018**, *12*, 046003.
- 465