Int. J. Ion Mobil. Spec. (2011) 14:177–185
DOI 10.1007/s12127-011-0070-0

TECHNICAL REPORT

MCC/IMS as potential noninvasive technique in the diagnosis of patients with COPD with and without alpha 1-antitrypsin deficiency

Rembert Koczulla · Akira Hattesohl · Severin Schmid · Bertram Bödeker · Sasidhar Maddula · Jörg Ingo Baumbach

Received: 20 June 2011 / Accepted: 27 June 2011 / Published online: 20 July 2011

Background

Chronic obstructive lung disease (COPD) is characterized by a not fully reversible and usually progressive airflow limitation. The disease is associated with an inflammatory response of the lungs to noxious particles, mainly cigarette smoke but also alpha 1-antitrypsin (AAT) deficiency predisposes to COPD. The usual clinical practice for diagnosing COPD is following symptoms, performing lung function and the assessment of responses to inhaled pharmacological agents. These tests have been standardized and are generally considered as informative [1]. The tests are time consuming. Still the quality of the tests is influenced by experience and may differ depending where the tests are performed. Further, it is suggested that every COPD patient is once screened for AAT deficiency (AATD). Alpha 1-antitrypsin deficiency is a co-dominant inherited disorder that is diagnosed by low serum levels, genotyping and phenotyping of AAT.

Usually the serum AAT levels are analyzed first. If the serum levels are decreased further procedures like genotyping (with polymerase chain reaction) and phenotyping (isoelectric focusing) are suggested. The costs of these diagnostic procedures are high [2] and therefore often not carried out. Thus, there is a need for novel diagnostic methods that are simple, fast and cost-effective and maybe performed bedside. Within the past decade, cellular and molecular techniques have been utilized as options for the diagnosis and monitoring of COPD and AAT deficiency [3].

The analysis of exhaled breath (EB) has been used to noninvasively obtain information about inflammatory processes within the lung. EB contains a complex mixture of volatile organic compounds (VOCs), which can be detected using gas chromatography–mass spectrometry (GC-MS) [4]. Electronic noses (eNoses) use a concept essentially different from GC-MS. In addition, they allow the online recognition of complex VOC mixtures via composite nanosensor arrays in combination with learning algorithms [5, 6]. Another approach is the ion mobility spectroscopy (IMS), were about 10 ml human breath will be analyzed directly and without any pre-enrichment. Two different types of IMS, such coupled to multi-capillary columns (MCC/IMS) [7–15] and differential mobility spectrometers [16, 17] were used.

It is currently assumed that COPD with or without AAT deficiency shows different molecular and cellular characteristics due to the pathophysiological inflammation present in AAT deficiency [18]. Thus, it may be that the VOC profile or smellprint is different in the EB of patients with COPD

R. Koczulla (✉) · A. Hattesohl · S. Schmid
Zentrum für Innere Medizin - Pneumologie,
Universitätsklinik Gießen und Marburg,
Standort Marburg, Baldingerstraße,
35043 Marburg, Germany
e-mail: koczulla@med.uni-marburg.de

B. Bödeker
B&S Analytik, BioMedicalCenter,
Otto-Hahn-Str. 15,
44227 Dortmund, Germany

S. Maddula · J. I. Baumbach
Department Microfluidics and Clinical Diagnostics, KIST Europe,
Campus E 71,
66123 Saarbrücken, Germany

Table 1 Characteristics of ion mobility spectrometer (BioScout 2010)

Parameter	Value
Ionization source	^{63}Ni (555 MBq)
Electric field strength	320 V/cm
Length of drift region	12 cm
Diameter of drift region	15 mm
Length of ionization chamber	15 mm
Shutter opening time	300 μs
Shutter impulse time	100 ms
Drift gas	synthetic air (20.5 % O_2 (4.5), 79.5 % N_2 (5.0))
Drift gas flow	100 ml/min to 300 ml/min
Pressure	101 kPa (ambient pressure)
Multi-capillary column	OV-5, polar
Column temperature	40 °C

with and without AAT deficiency. Based on this possibility, it was the aim of our study to compare smellprints between COPD patients with confirmed AAT deficiency and COPD patients without AAT deficiency. In another approach also the influence of AAT augmentation therapy should be studied. Patients with severe AATD can be treated with weekly AAT infusions (60 mg/kg body weight). This therapy regimen is based on studies showing an increase of AAT lung levels as well as an increase of the antineutrophil elastase capacity in the epithelial lining fluid of the lung [1]. Considering this, an influence of the augmentation on the smellprint of AATD patients seems

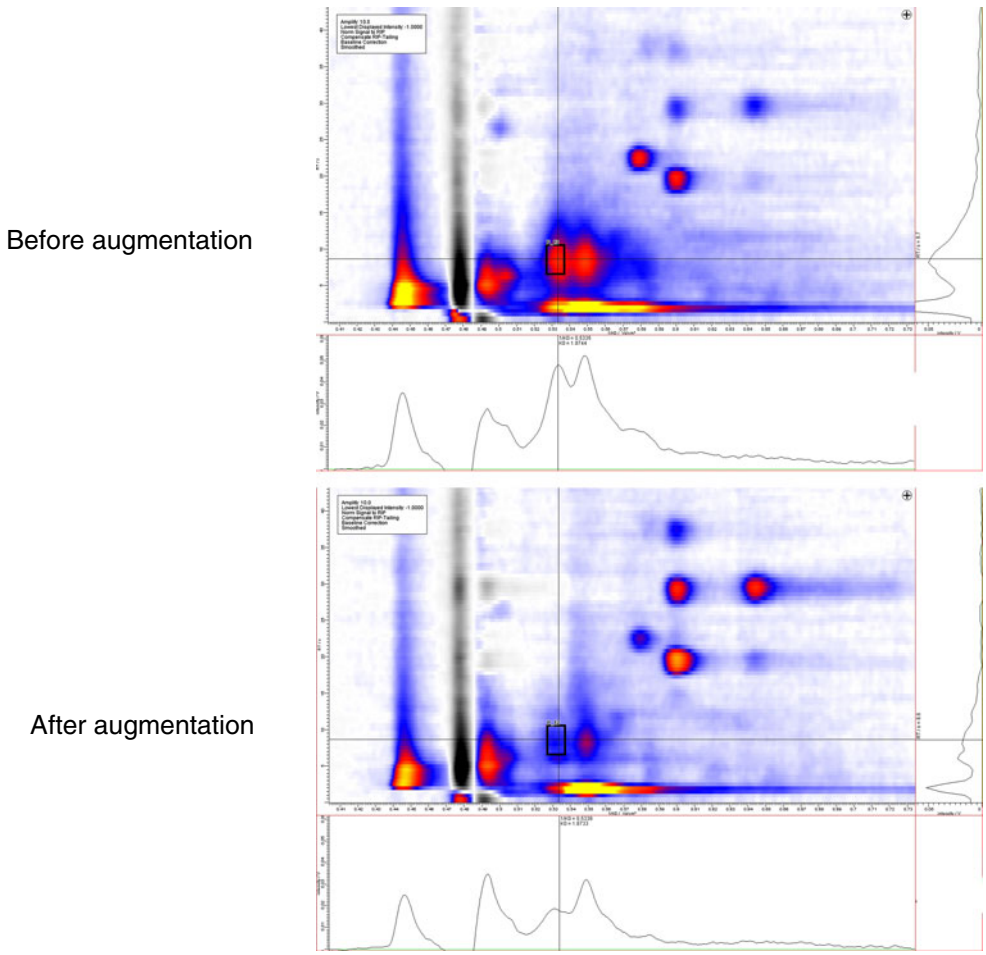


Fig. 1 IMS chromatograms before and after AATD augmentation

reasonable. Hence, the second aim of the study was to detect the influence of intravenously given AAT on the composition of VOCs in EB of AATD patients.

Patients

We included patients with genetically proved severe AATD (PiZZ genotype). All patients were fasting for at least 2 h. Patients had to wash out their mouth with water. For sampling the patients breathed into the device. For every breath the first expiratory 40 ml were discarded to exclude air of the oral cavity. The patients breathed until 10 ml of expiratory air (excluding the oral cavity) were obtained by the device for sampling. From AATD patients breath samples were taken twice, directly before and 2 h past their infusion or augmentation. The numbers of breath samples included within the two different cases investigated are:

- Before and after substitution: 2
- COPD without AATD: 8
- COPD based on AATD: 17.

Method

The IMS coupled to a multi-capillary column (MCC/IMS) used was a BioScout (B&S Analytik, Dortmund, Germany) consisting of the MCC/IMS and a SpiroScout (Ganhorn Medizin Electronic, Niederlauer, Germany) as sample inlet unit. The major parameters are summarized elsewhere [8–12, 19–23]. In this spectrometer a 550 MBq ^{63}Ni β-radiation source was applied for the ionization of the carrier gas (air). It was connected to a polar multi-capillary column (MCC, type OV-5, Multichrom Ltd, Novosibirsk, Russia) used as the pre-separation unit. In this MCC the analytes of exhaled breath were sent through 1000 parallel capillaries, each with an inner diameter of 40 μm and a film thickness of 200 nm. The total diameter of the separation column was 3 mm. The relevant MCC parameters are listed in Table 1.

All subjects were requested to exhale through a mouth piece connected to a Teflon tube. In each case end-tidal breath, controlled by a flow sensor, was collected in a sample loop of 10 ml volume. The sample air was collected and transferred to the multi-

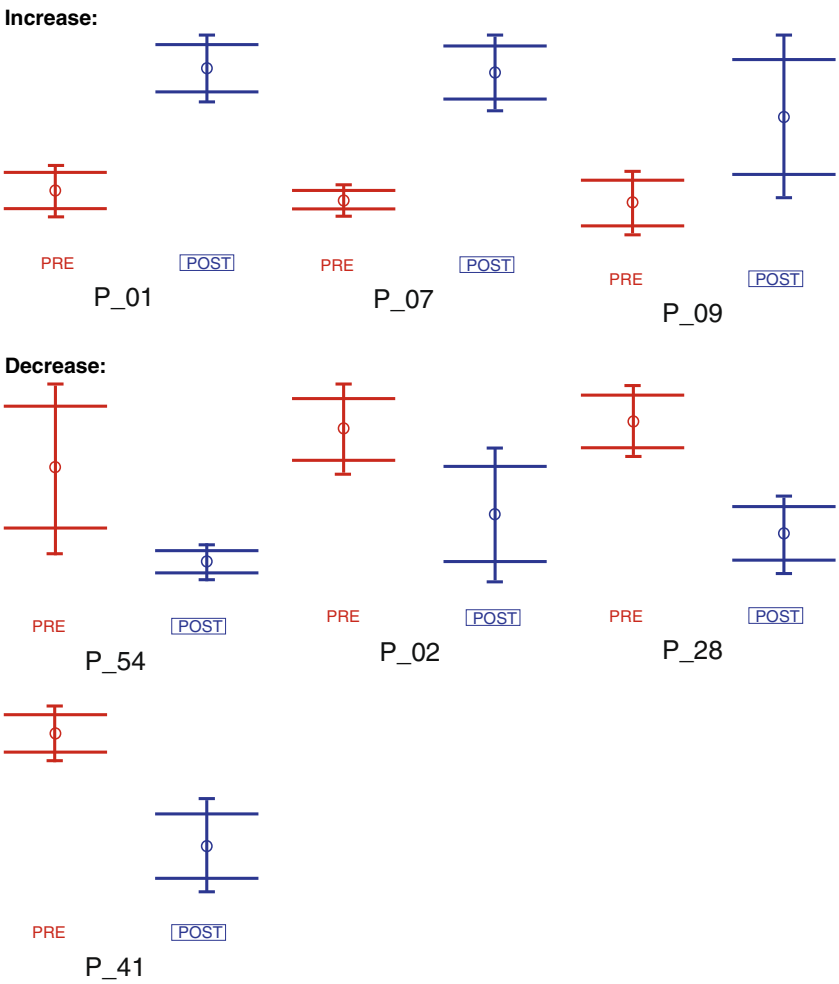


Fig. 2 Box-and-whisker plots related to increasing and decreasing signals, before and after AATD augmentation

Table 2 Position of the signals discriminating AATD before and after augmentation

#	Area	Norm U	$1/K_0$ VS/cm^2	RT/s	Nearest analyte
1	P_01	0.000	0.579	1.7	Butanole
2	P_02	0.000	0.550	2.3	Acetone
3	P_07	0.000	0.584	6.0	2-Hexanone
4	P_09	0.000	0.607	6.3	1-Pentanole
5	P_10	0.000	0.569	6.3	1-Butanole
6	P_11	0.000	0.531	6.2	2-Butanone
7	P_12	0.000	0.532	8.6	2-Propanole
8	P_13	0.000	0.578	8.1	2-Hexanone
9	P_14	0.000	0.550	8.6	3-Pentanone
10	P_18	0.000	0.562	17.0	2,5-Dimethylpyrazine
11	P_22	0.000	0.581	23.0	1,2,4-Trimethylbenzene
12	P_28	0.000	1.073	29.4	unknown
13	P_41	0.000	0.608	45.8	unknown
14	P_42	0.000	0.641	51.7	unknown
15	P_44	0.000	0.576	55.6	1,2-Butandiole
16	P_50	0.000	0.598	73.6	unknown
17	P_54	0.000	0.664	77.4	Menthon
18	P_56	0.000	0.735	77.7	unknown
19	P_60	0.000	0.668	84.4	unknown
20	P_62	0.000	0.607	88.5	unknown
21	P_64	0.000	0.616	96.9	unknown
22	P_65	0.000	0.760	95.4	unknown

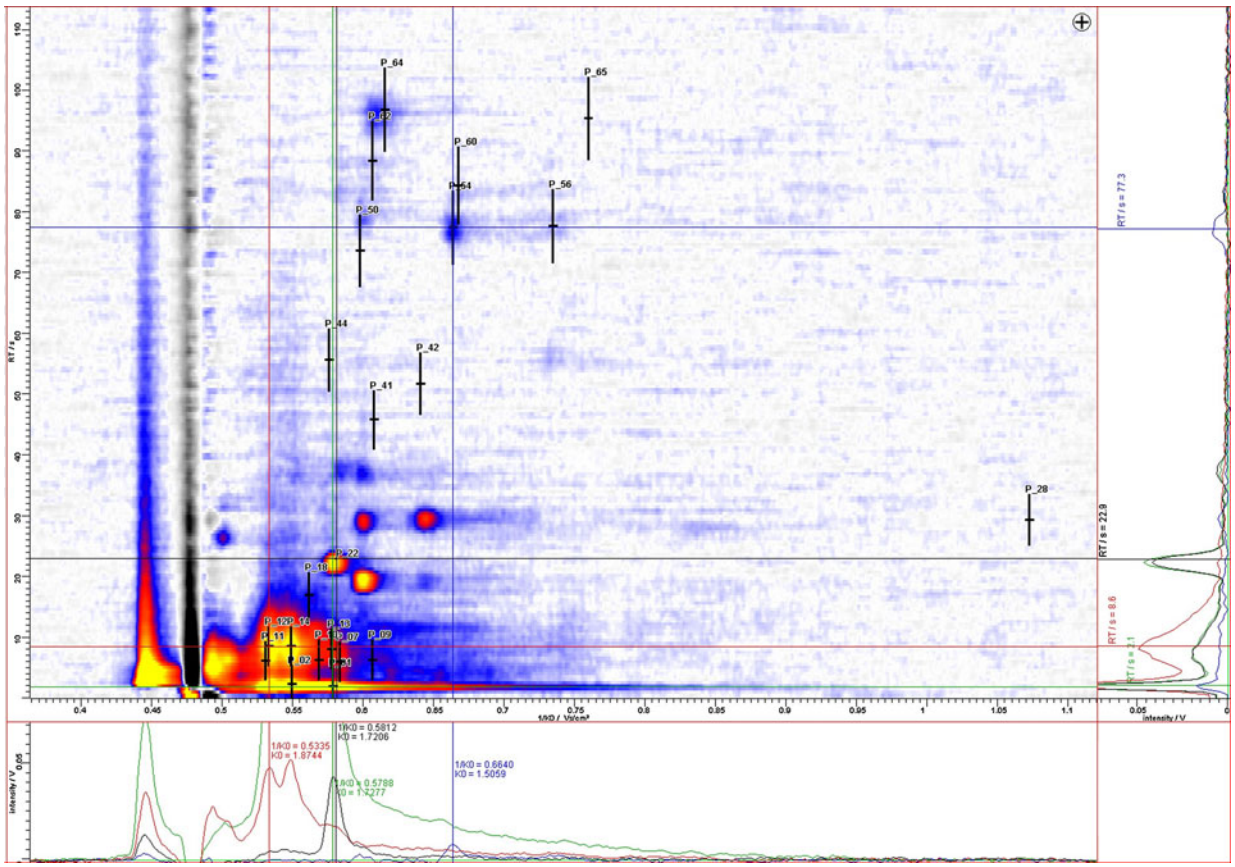


Fig. 3 Signals of potential relevance to AATD within the IMS chromatogram

capillary column for a first chromatographic separation after reaching 3 times 10 ml above the dead volume. Using the software VOCan 1.4 (B&S Analytik, Dortmund, Germany) the dead volume was adjustable and fixed in the present case to 500 ml. The expiration was controlled by a CO_2 sensor element integrated in the SpiroScout and recorded for each subject.

The peaks were characterized using the software Visual Now 2.2 (B&S Analytik, Dortmund, Germany), which is described elsewhere [8, 24–27]. All peaks found are characterized by their position with drift time (corresponding $1/K_0$-value), retention time and their concentration represented by the peak height. For both groups and each of the peaks a box-and-whisker plot was realized.

A preliminary relation between the peak position and the identity of the analyte was obtained using the database BSIMSDB 1.4 (B&S Analytik, Dortmund, Germany), but parallel measurements using e.g. GC/MSD (gas chromatography/mass selective detector) should be realized with respect of further confirmation.

Results

We compare two different cases

- IMS chromatograms before and after substitution
- AATD and COPD (without AATD), here we describe differences between COPD without AATD and COPD based on AATD.

The IMS chromatograms before and after AAT augmentation are compared in Fig. 1. The signal with the highest rank sum is marked by black rectangles. The box-and-whisker plots related to increasing and decreasing signals, before and after AAT augmentation are shown in Fig. 2. Totally, 22 different signals were found with rank sum 0.00, the best value. The positions were reported in Table 2 and shown in Fig. 3. It should be noted, that because of the preliminary status of the study and the rather low number of subjects included so far, the findings need confirmation within a larger group, but should encourage investigations of exhaled breath to identify potential biomarkers.

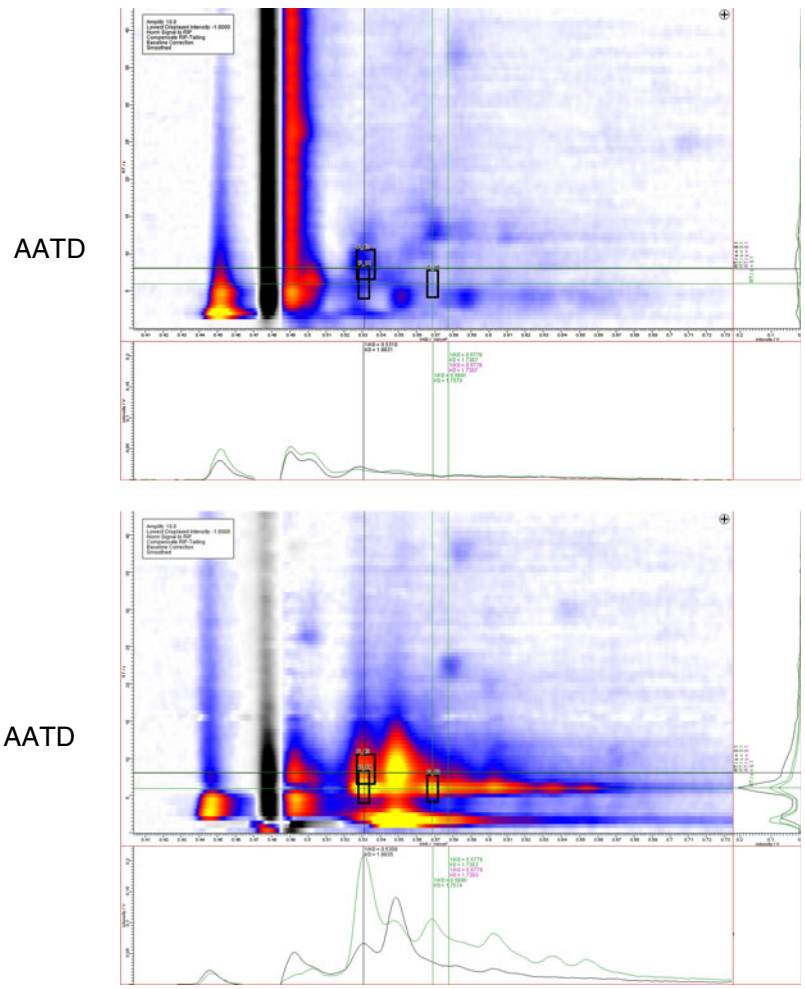
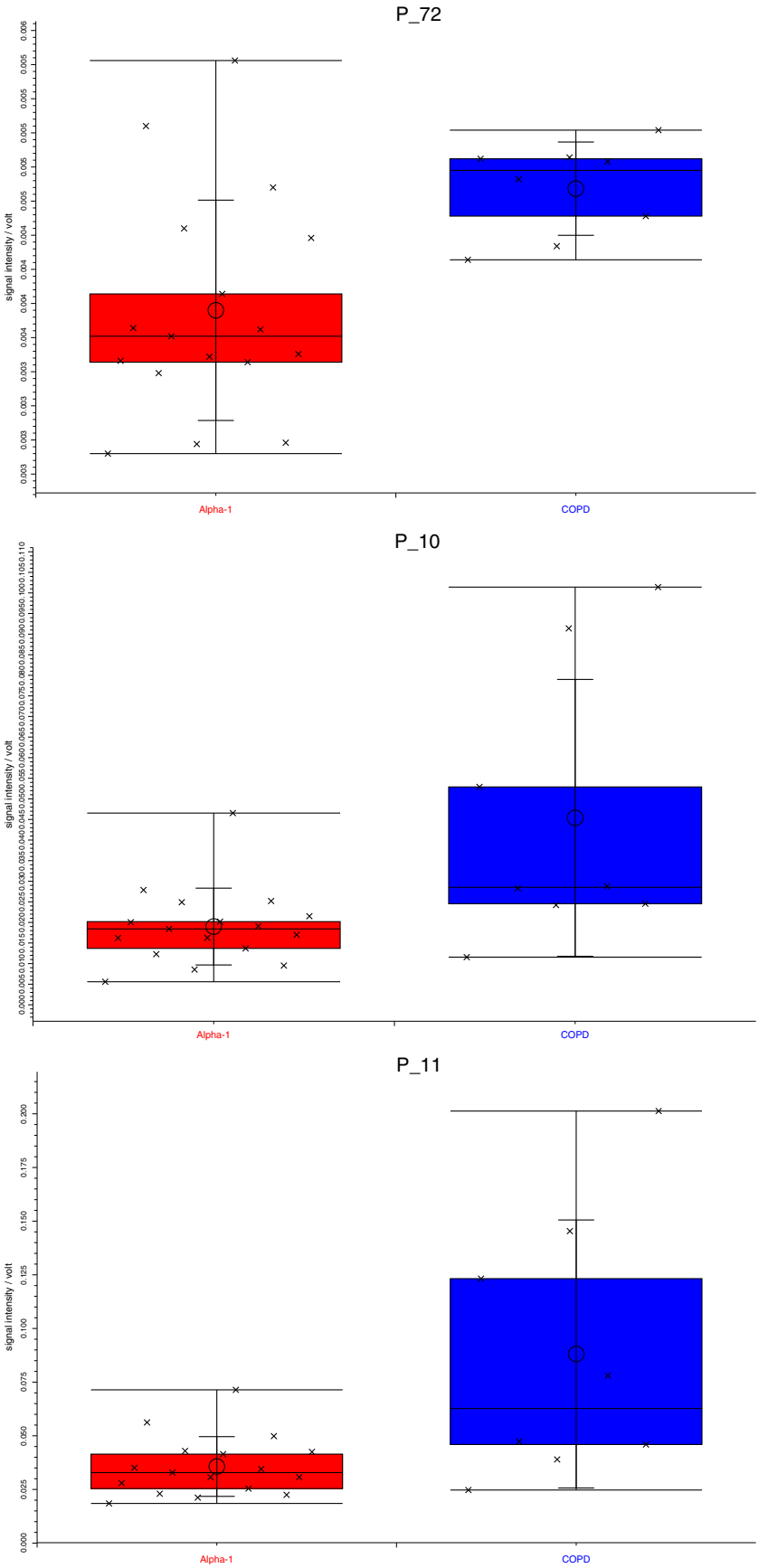


Fig. 4 Signals of AATD and COPD without AATD within IMS chromatograms

Fig. 4 (continued)



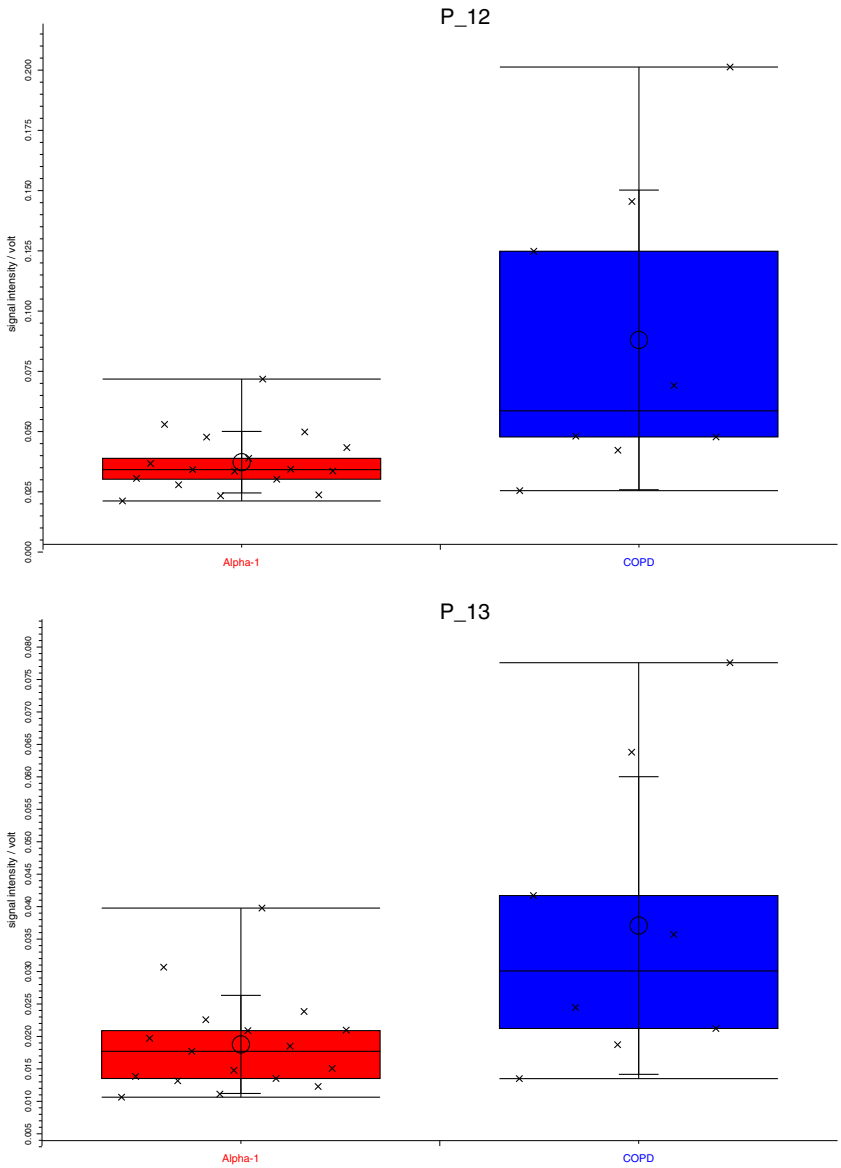


Fig. 5 Box-and-whisker plots of signals potentially separating AATD and COPD without AATD

Furthermore, the signals P_10, P_11, P_12 and P_13 should be considered also in relation to case two, dealing with the difference between COPD without AATD and COPD based on AATD.

Typical IMS chromatograms for case two are shown in Fig. 4. Some peaks useful for discrimination between COPD with and without AATD are marked. The box-and-whisker plots for the five signals with the lowest rank sum with respect to a potential separation between the two groups investigated are shown in Fig. 5 and described in Table 3. In nearly all cases the signal within the group of COPD without AATD is higher than in AATD.

In the group of COPD with and without AATD the numbers of patients were higher than in case before and after substitution. Therefore, in Table 4 the values of accuracy, sensitivity, specificity and the positive and negative predictive value are shown for the five peaks with the rank sum less than 0.2. In addition, the best thresholds calculated on the peak height scale are shown. The accuracy is in all cases higher than 75 %. For peak P_72 the sensitivity was 100 %, for all other peaks 75 % or higher. The values obtained seem

Table 3 Position of the signals discriminating AATD and COPD without AATD

#	Area	Norm U	$1/K_0$ VS/cm^2	RT/s	Nearest analyte
1	P_72	0.169	0.627	208.1	unknown
2	P_10	0.176	0.569	6.3	1-Butanole
3	P_11	0.184	0.531	6.2	2-Butanone
4	P_12	0.191	0.532	8.6	2-Propanole
5	P_13	0.199	0.578	8.1	2-Hexanone

Table 4 Accuracy, sensitivity, specificity, positive and negative predictive values (PPV, NPV) for the peaks found with the lowest rank sum

	P_10	P_11	P_12	P_13	P_72
Best threshold	0.024	0.046	0.042	0.021	0.004
True positive	13	14	12	13	12
False positive	1	2	1	2	0
True negative	7	6	7	6	8
False negative	4	3	5	4	5
Sensitivity (sens)	0.765	0.824	0.706	0.765	0.706
Specificity (spec)	0.875	0.750	0.875	0.750	1.000
NPV	0.650	0.700	0.632	0.684	0.600
PPV	0.636	0.667	0.583	0.600	0.615
a = sens - (1 - spec)	0.640	0.574	0.581	0.515	0.706
Accuracy	0.800	0.800	0.760	0.760	0.800

to be promising for a preliminary study with just 25 cases. Generally, the finding needs further confirmation and a higher number of subjects should be included within the study.

Conclusions

Two different case studies were investigated using MCC/IMS: before and after AAT substitution and COPD with and without AATD. For the first case, from two patients, 22 different signals were found with rank sum 0.00, the best value to differentiate. In case two, the 17 samples with AATD and eight with COPD without AATD could be separated by five peaks.

Our preliminary results demonstrate, that distinct patterns of a small number of IMS peaks are found to be useful to separate the classes under investigation. Therefore, MCC/IMS seems to be a promising method for the noninvasive identification as shown before for lung cancer and sarcoidosis patients [19]. But, because of the comparatively low number of subjects included in the preliminary study, a higher number should be investigated. In addition, the relations of the peaks to the analyte need further confirmation, e.g. using parallel measurements using GC/MSD.

Acknowledgements The financial support of the Ministry of Education, Science and Technology (MEST) of the Republic Korea is acknowledged thankfully. Part of the work on this paper has been supported by Deutsche Forschungsgemeinschaft (DFG) within the Collaborative Research Center (Sonderforschungsbereich) SFB 876 "Providing Information by Resource-Constrained Analysis", project TB1 "Resource-Constrained Analysis of Spectrometry Data". In addition, the work was supported partly by the German Federal Ministry of Economics and Technology based on a decision of the German Bundestag within the project KF2368102AKO.

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