Breath volatile organic compound (VOC) COPD study at two DZL centers - evaluation of site-specific VOC

A Gaida1, O. Holz1,2, S. Schuchardt3, C. Nell4, U. Boas3, A.R. Koczulla4, J.M. Hohlfeld1,2

1Fraunhofer ITEM, Clinical Airway Research, 2 Member of the German Center for Lung Research (BREATHE), 3Fraunhofer ITEM, Bio- and Environmental Analytics - Hannover/DE, 4Philipps-University Marburg, Pneumology, Member of the German Center for Lung Research (UGMLC), 5Philipps-University Marburg, Pneumology - Marburg/DE.

Introduction
One way to include the analysis of breath volatile organic compounds (VOC) into multicenter studies is the use of adsorption tube technology (i.e. Tenax TA) followed by a central thermal-desorption (TD) GC-MS analysis. In a two-center collaboration study (Marburg, Hannover) we collected breath samples of COPD patients and controls and evaluated the impact of site and shipment on VOC sample quality and composition. For this analysis we compared the VOC pattern of healthy subjects between sites. In addition, we assessed the level of Benzene, Toluene and Xylene (BTX) in controls and smokers to test a potential contamination during shipment and storage.

Results
The chromatograms of dual breath sample-sets showed major site specific VOC peaks which could not or only in traces be found in samples collected at the respective other site. Most of these VOCs could not be detected in the accompanied room air samples, excluding room air or shipment as potential sources. Among the VOCs that discriminated sites were 2-Butyl-1-octanol (observed in Marburg) and 1-Phenoxy-2-propanol (observed in Hannover), the latter being known to be part of general cleaning solutions used in Hannover.

No significant differences could be observed between control subjects of both sites with respect to the levels of BTX, which clearly discriminated active smokers from controls at both sites.

Conclusion
We found site-specific VOCs which are likely to be linked to different materials or cleaning procedures. These VOCs did not show a negative impact on the analysis of breath-related VOCs, but the results show, that it is necessary to evaluate site specific effects on sample-quality in multicenter-studies. The comparable level of BTX aromatics indicates that shipping of thermal-desorption tubes does not contaminate breath samples and that shipment between sites is feasible.

Methods
Tenax tubes were conditioned in Hannover and shipped to Marburg. Breath collection was performed at both sites using identical sampling devices (inhalation via A2 filter and exhalation (flow control) into a stainless steel reservoir), but different mouth pieces. 3L of breath were continuously drawn onto 2 separate Tenax adsorption tubes. Samples from Marburg were shipped back to Hannover over night, while samples from Hannover were immediately analyzed by TD-GC-MS. For this analysis we used data of 10 non-smoking subjects from each site. 75 commonly observed substances were chosen for single-ion analysis.

Fig. 3: Site related VOCs could neither in Marburg (top) nor Hannover samples (bottom) be detected in the respective room air.

Fig. 4: Mean (SEM) of base-ion peak counts (log) for benzene, toluene and xylene are shown for healthy controls and COPD subjects and compared between sites (left: Hannover, right: Marburg). Storage and shipping do not significantly affect sample composition.

Fig. 1 (left): Area of three overlayed chromatograms, showing an example of VOC-patterns associated with mouthpieces and/or cleaning procedures in Hannover samples. (right): Boxplots comparing Hannover and Marburg samples for 8 major compounds from this pattern (including median, IQR, mean and outlier values of n = 10 healthy subjects from each site).

Fig. 2 (left): Area of three overlayed chromatograms, showing examples of VOC-patterns associated with mouthpieces and/or cleaning procedures in Marburg samples. (right): Boxplots of the major peaks in this VOC pattern, comparing Marburg to Hannover samples.