

# Elucidating the composition of humic-like substances in atmospheric aerosol particles via 2D liquid chromatographic fractionation and ultra-high resolution mass spectrometry

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## Introduction & Motivation

Organic carbon (OC) contributes up to 70% of the total particle mass of atmospheric aerosol particles (Kanakidou et al. 2005) and plays an important role for cloud formation and the earth radiative budget, as it alters microphysical properties and the ability of particles to serve as cloud condensation nuclei (Kristensen et al. 2012). Moreover, it is expected to be involved in a wide range of human health issues (Mauderly and Chow 2008) and can have an impact on ecosystems (Fuzzi et al. 2006). Despite its importance, only a small fraction has been identified on a molecular level, due to the high complexity with thousands of substances. OC is dominated by a class of substances often referred to as humic-like substances (HULIS), which contributes up to 80% of the water-soluble organic carbon (Zheng et al. 2013). Elucidating its composition is therefore crucial to understand aerosol properties and its role e.g. in the cloud formation process. At the same time, the high complexity of the chemically unresolved mixture is challenging.

To overcome this challenge, we developed an offline 2D-chromatographic method combining size-exclusion chromatography (SEC) and reversed phase high performance liquid chromatography (RP-HPLC) for the fractionation of HULIS extracts (Spranger et al. 2017). The here presented results of the 2D-method as well as ultra-high resolution electrospray ionisation Fourier transform ion cyclotron mass spectrometry (UHR-ESI-FTICR-MS) are gained from ambient filter samples from the TROPOS research station Melpitz (Germany; 51° 31' 38" N, 12° 56' 8" O) from different seasons (summer S, winter W) with different air mass influences (west W, east E).

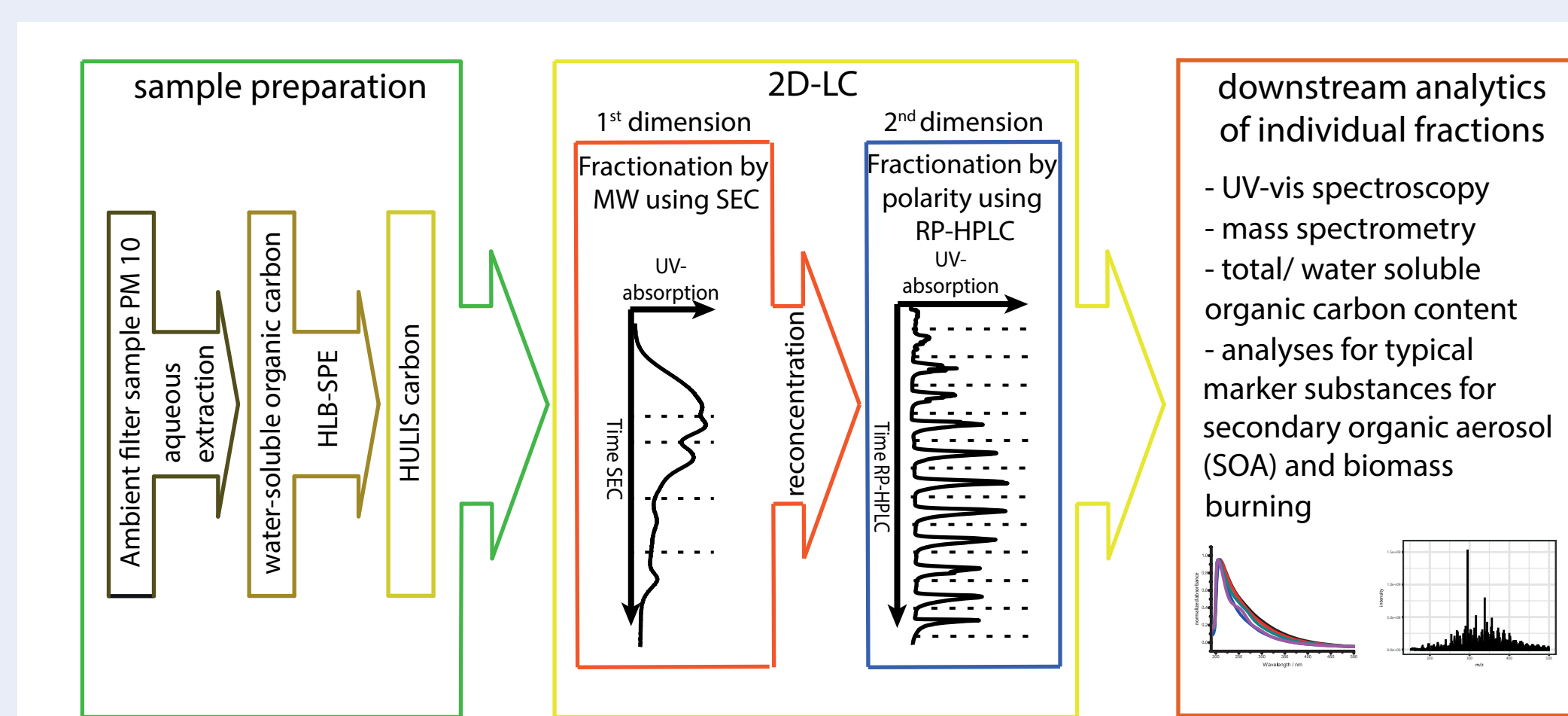


Fig. 1: Schema of the used analytical protocol for filter samples

## Method development and results of 2D-LC

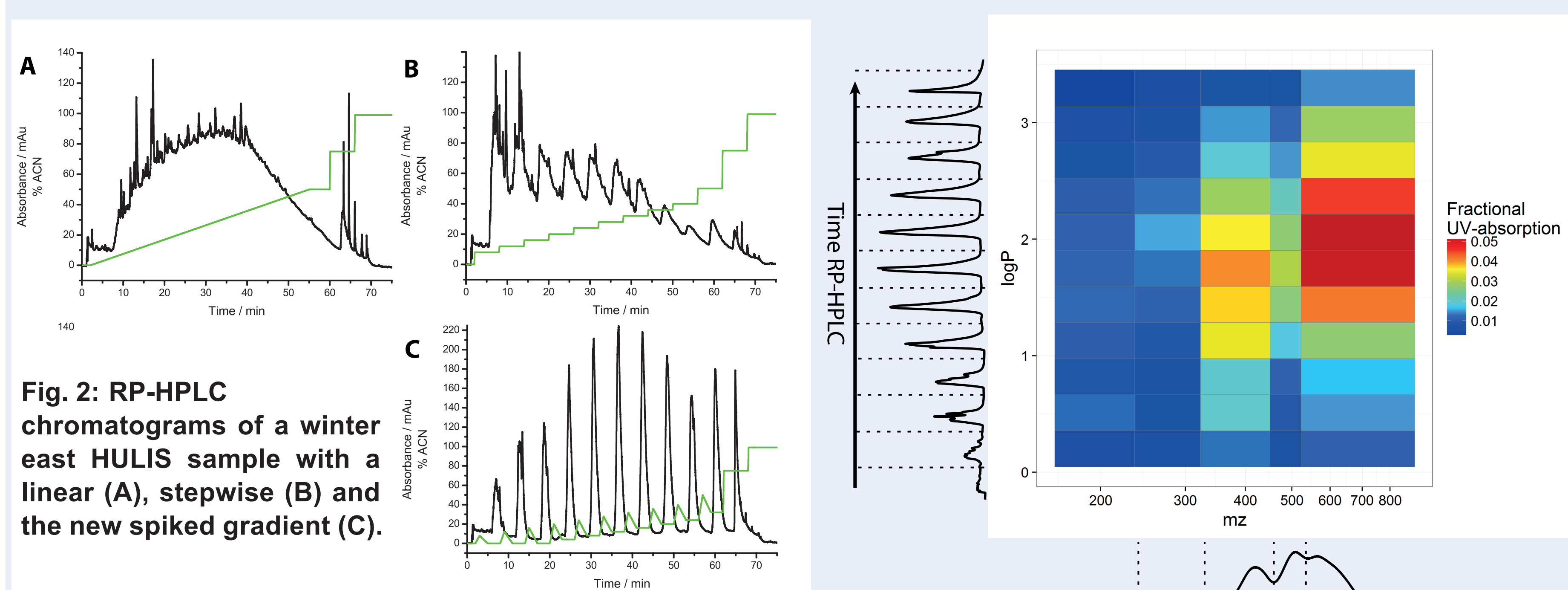


Fig. 2: RP-HPLC chromatograms of a winter east HULIS sample with a linear (A), stepwise (B) and the new spiked gradient (C).

### RP-HPLC

- linear gradient (Fig. 2A) leads to a broad peak with no separation of fractions
- a stepwise gradient (2B) leads to a weak fractionation with a purity of fractions around 70% for a 5-step gradient and less for a 10-step gradient
- newly developed „spiked gradient“ leads to a highly reproducible separation into 11 fractions with a fraction purity between 95-99% (2C)
- the expected polarity ranges of the fractions were calculated using a variety of standard substances with calculated log P values (octanol-water-confident)

### SEC

- mobile phase consists of water/ MeOH 80/20 with  $\text{NH}_4\text{HCO}_3$  as additive (other tested, but worse results)
- 75 mM  $\text{NH}_4\text{HCO}_3$  shows best results (Fig. 3)
- mass ranges calibrated with a variety of standard substances

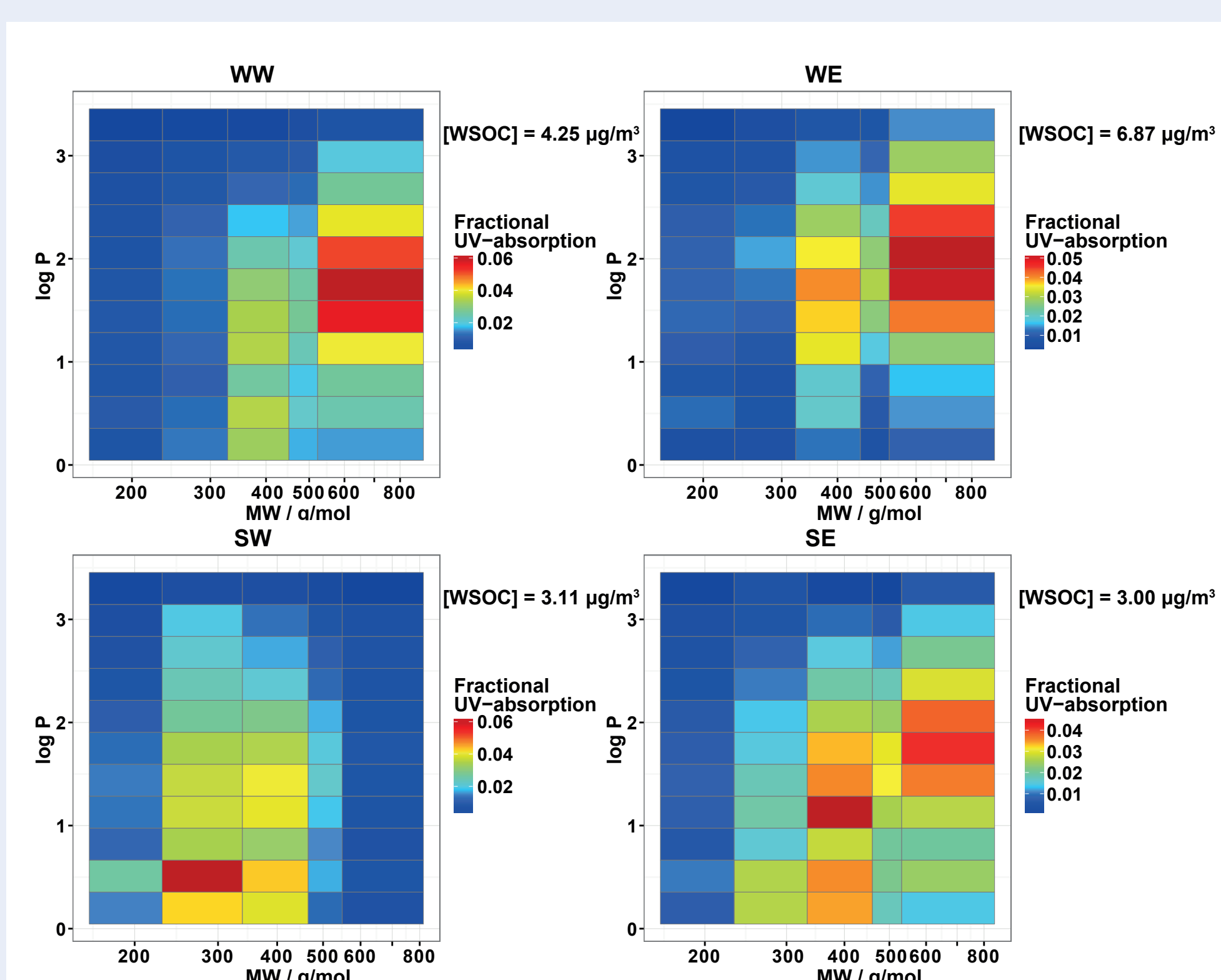


Fig. 4: 2D heat maps of WW, WE, SW and SE. The color scale is set to the corresponding maximum value of each 2D chromatogram.

## Conclusion

A new two-dimensional chromatographic approach is presented to separate the very complex mixture of atmospheric HULIS into 55 fractions with different polarity and molecular size ranges using optimized SEC and a newly developed unique spiked gradient profile for RP-HPLC. The method provides an easy and relatively fast characterization of HULIS and similar complex mixtures of water-soluble organic material. The measurements of four ambient HULIS samples illustrate the capability of the method to reveal large differences in HULIS composition even in samples from one and the same sampling site, which need to be considered in studies related to the properties and atmospheric impacts of HULIS and organic carbon. The reduced complexity of each individual fractions improves downstream analytics. For ESI-MS measurements, ~3 times the amount of individual MF could be assigned compared to a simple bulk analysis. This highlights the necessity of a strong chromatographic separation prior to MS measurements for these very complex mixtures.

## UHR-ESI-FTICR-MS

### Setup and sample preparation

- 55 fractions of one SE sample were collected after 2D-LC; solvent was evaporated and fractions were redissolved in water/ MeOH 50/50 (38 fractions measured)
- measurements were carried out on a Bruker solarix XR 12T MS with direct infusion ESI
- solvent blanks as well as blank samples for each RP-HPLC fraction (from a field blank with sample preparation and SEC fractionation) were measured; resulting mass formulas were removed from all samples

### Advantages of fractionated samples

- 6500 molecular formulas (MF) could be assigned for the bulk sample
- 17800 MF could be assigned for all measured fractionated samples; an increase by a factor of 2.7 due to reduction of ion suppression in ESI and hence sensitivity enhancement of low concentrated HULIS compounds
- Van-Krevelen plot shows majority of additional molecular formulas with low O/C ratio (Fig. 5), which are usually suppressed in non-fractionated bulk samples

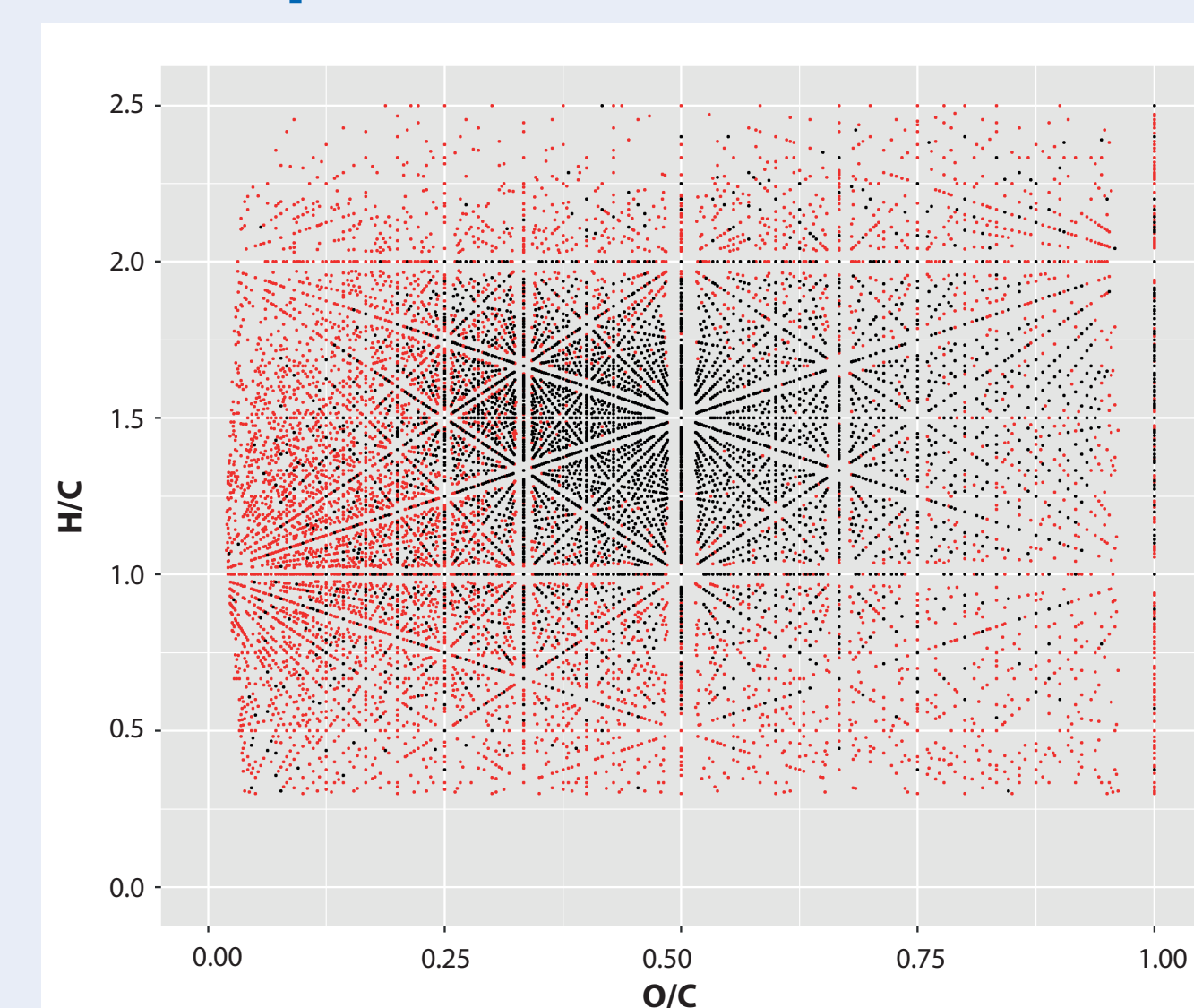


Fig. 5: Van-Krevelen plot for bulk sample (black) and sum of fractions (red)

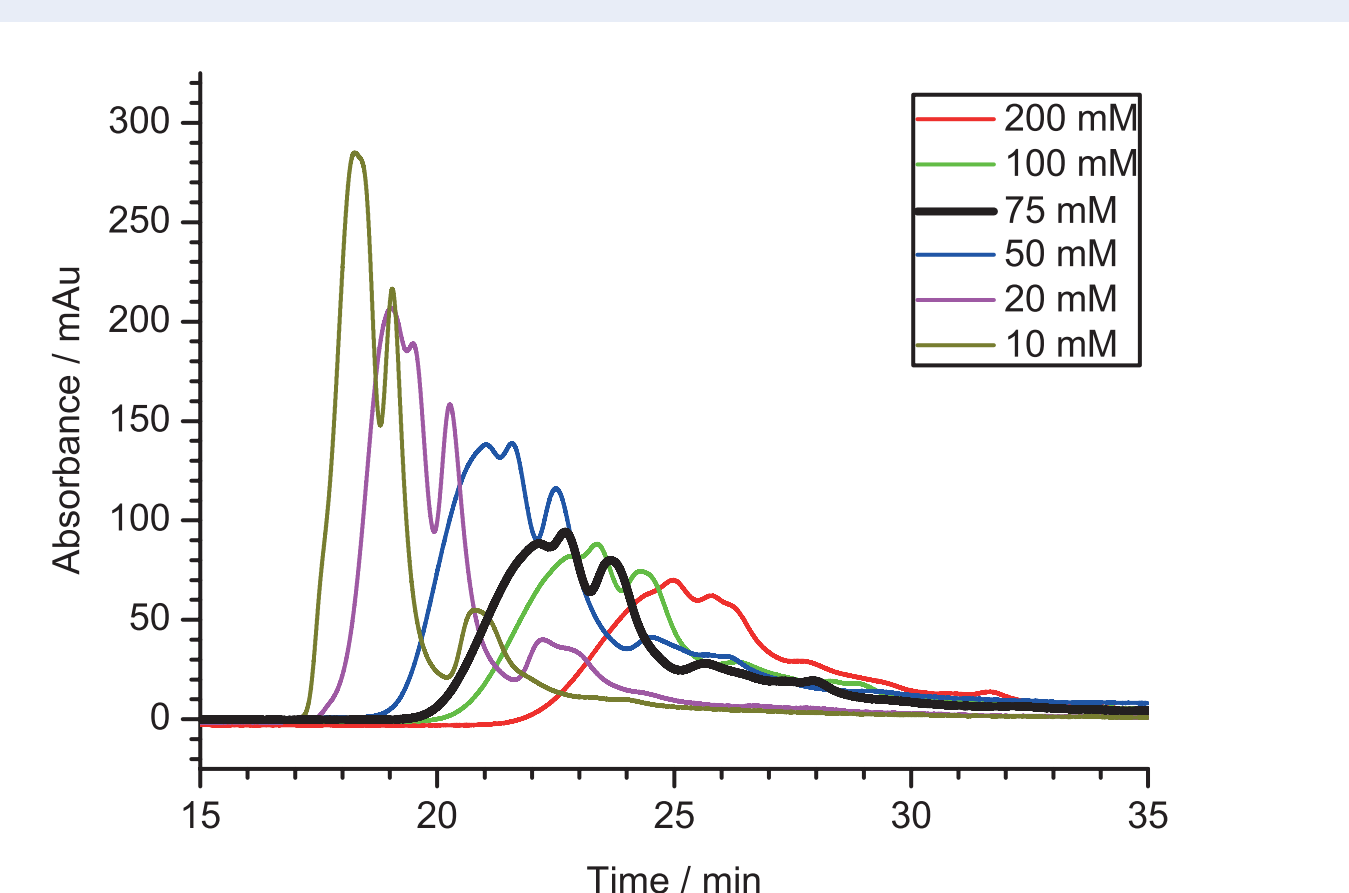


Fig. 3: SEC chromatograms of a winter east HULIS sample with different concentrations of ammonium hydrogen carbonate.

## Results

- heat maps of four different ambient samples highlight minor and major differences between the samples
- in winter, most intense signals for large molecules with low polarity
- for WW intensity is slightly shifted towards higher polarity, especially for medium sized molecules
- in summer, smaller and more polar molecules dominate
- for SW only 6% of absorption arises from large molecules (24% for SE, ~33% for WE & WW)
- sharp and intense maximum for small (229-337 g/mol) and very polar ( $\log P \sim 0.5$ ) molecules for SW
- in general, 4 characteristic areas could be observed in the 2D heat maps
- results also approved for larger dataset from the same sampling site

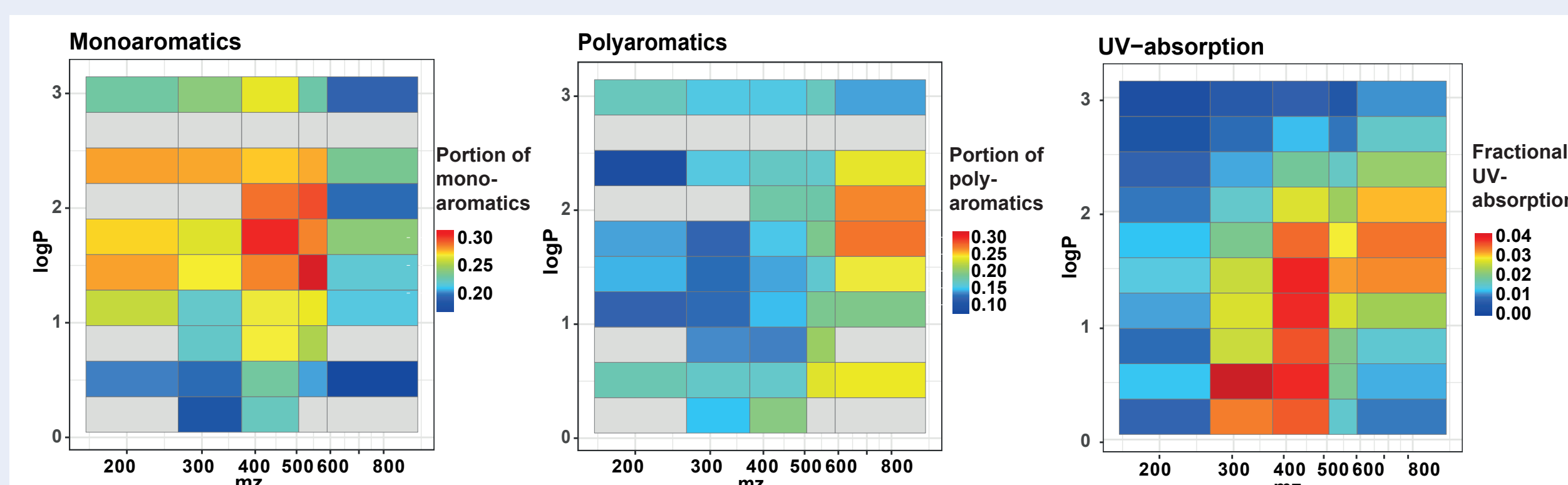


Fig. 6: 2D heat maps showing the amount of mono- and polyaromatic MF in each fraction and the corresponding UV-absorption

- MF assignment enables to calculate whether a molecule is aromatic (mono- or poly-) or not (approach by Yassine et al. 2014 was used)
- only for the largest molecules, a high percentage of polyaromatics could be observed (Fig. 6), also correlating with the UV-absorption of these fractions
- high amount of monoaromatics for medium sized molecules with medium polarity
- intense maximum in UV-absorption for highly polar, small sized molecules with low aromaticity, implies strong biogenic influence
- lowest calculated carbon oxidation state for small sized molecules indicates freshly emitted aerosol or local, mostly biogenic sources (Fig. 7)

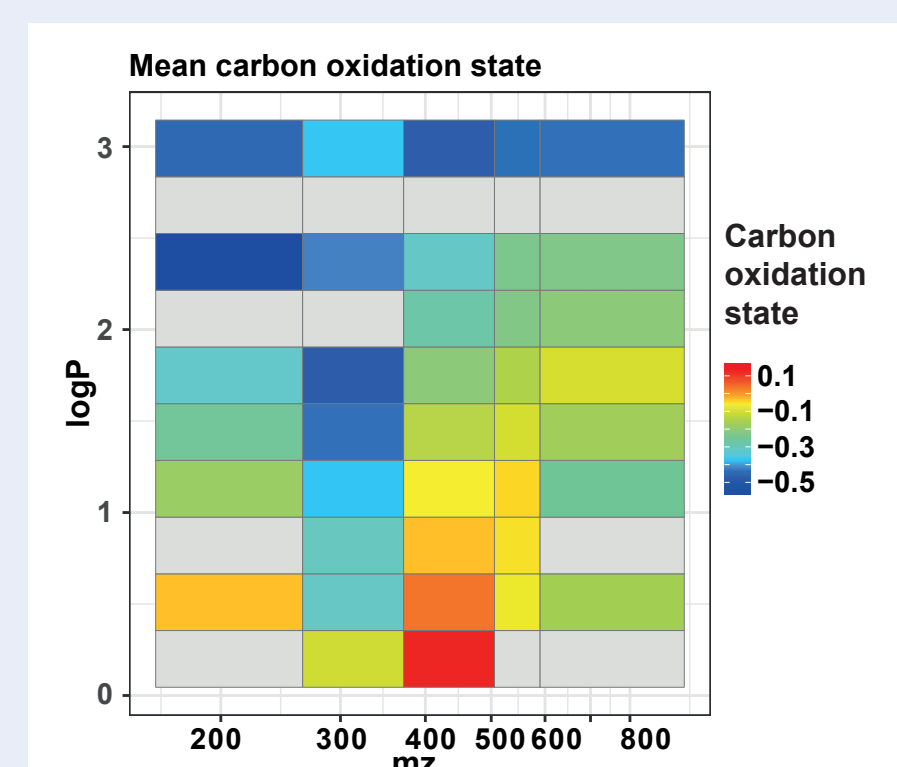


Fig. 7: 2D heat map of the mean carbon oxidation state

## Acknowledgment and literature

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- Kanakidou, M., et al. 2005. *Atmospheric Chemistry and Physics*, **5**: 1053-123.  
Kristensen, T. B., et al. 2012. *Journal of Geophysical Research-Atmospheres*, **117**.  
Mauderly, J. L., and J. C. Chow. 2008. *Inhalation Toxicology*, **20**: 257-88.  
Fuzzi, S., et al. 2006. *Atmospheric Chemistry and Physics*, **6**: 2017-38.  
Spranger, T., et al. 2017. *Environ. Sci. Technol.*, **51**, 9, 5061-5070.  
Noziere, B., et al. 2015. *Chemical Reviews*, **115**: 3919-83.  
Zheng, G. J., et al. 2013. *Environmental Pollution*, **181**: 301-14.  
Yassine, M. M., et al. 2014. *Rapid Commun. Mass Spectrom.*, **28**, 2445-2454.