

Development of a 2D-offline chromatographic method for the classification of HULIS carbon in atmospheric particles

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Introduction

Motivation

Humic-like substances (HULIS) are a class of organic compounds found ubiquitous in fog, cloud water and atmospheric aerosols. They are named due to the similarity to terrestrial and aquatic humic substances (HAVERS et al. 1998). HULIS show a similar optical behavior and chemical complexity, but have a lower molecular weight, less aromatic properties and a higher surface activity (GRABER & RUDICH 2006). Currently HULIS is defined operationally by the applied extraction method (mostly solid phase extraction of aqueous particle extracts). Different established methods lead to different HULIS fractions (FAN et al. 2013). Together with the mostly unknown composition, results regarding HULIS are often hard to compare.

Goals

The goal of this work is to develop an offline 2D-chromatographic method combining reversed phase high performance liquid chromatography (RP-HPLC) and size-exclusion chromatography (SEC) for the fractionation of HULIS extracts. Figure 1 shows a general work schema of the used analytical protocol, starting with an aqueous extraction of quartz filters and an HULIS isolation protocol to get the HULIS-fraction, followed by the new 2D-offline chromatographic method leading to aliquots of different polarity and size, which are then analyzed and characterized by different analytical methods. The less complex composition of the aliquots will lead to more detailed information about HULIS.

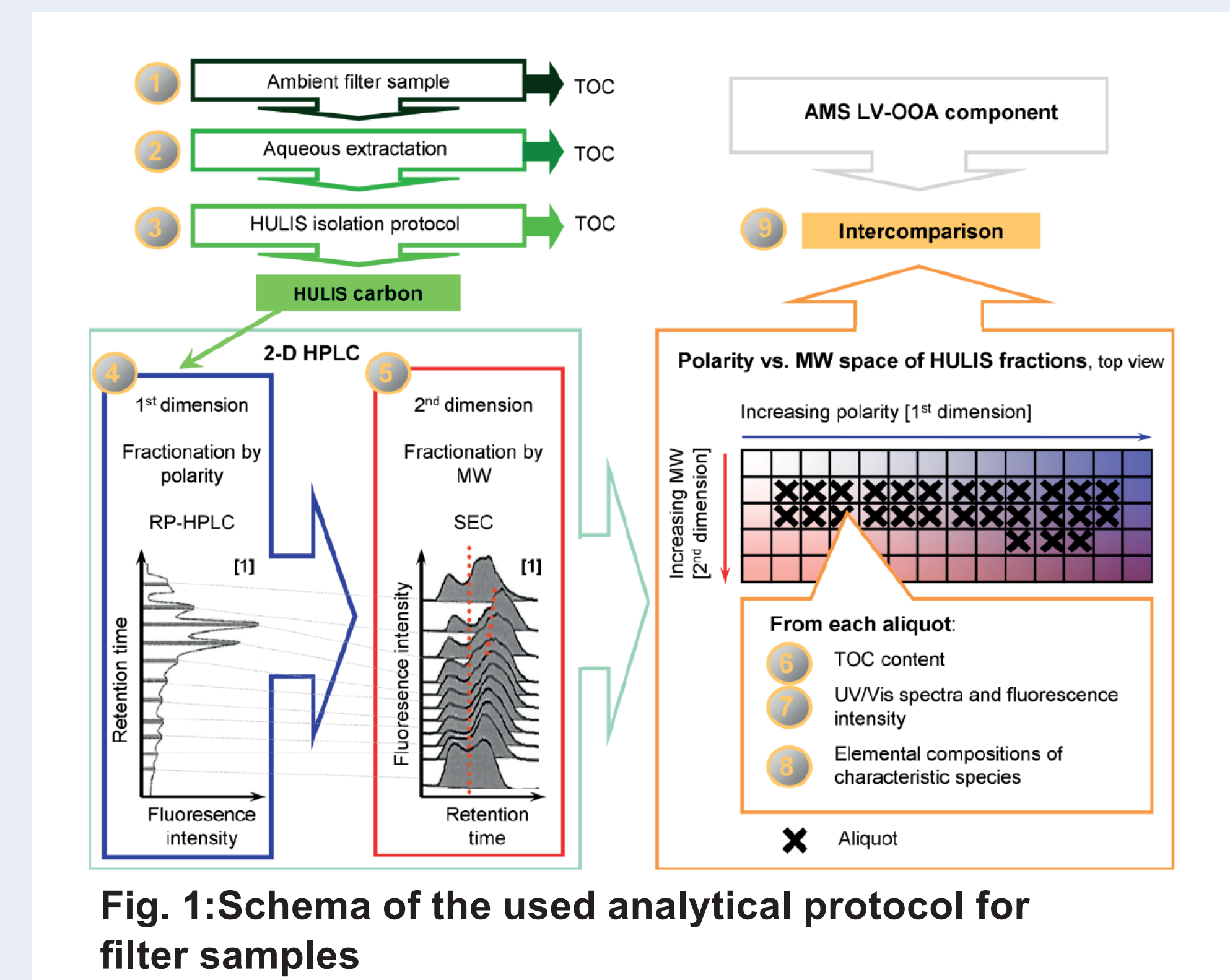


Fig. 1: Schema of the used analytical protocol for filter samples

Method development

RP-HPLC

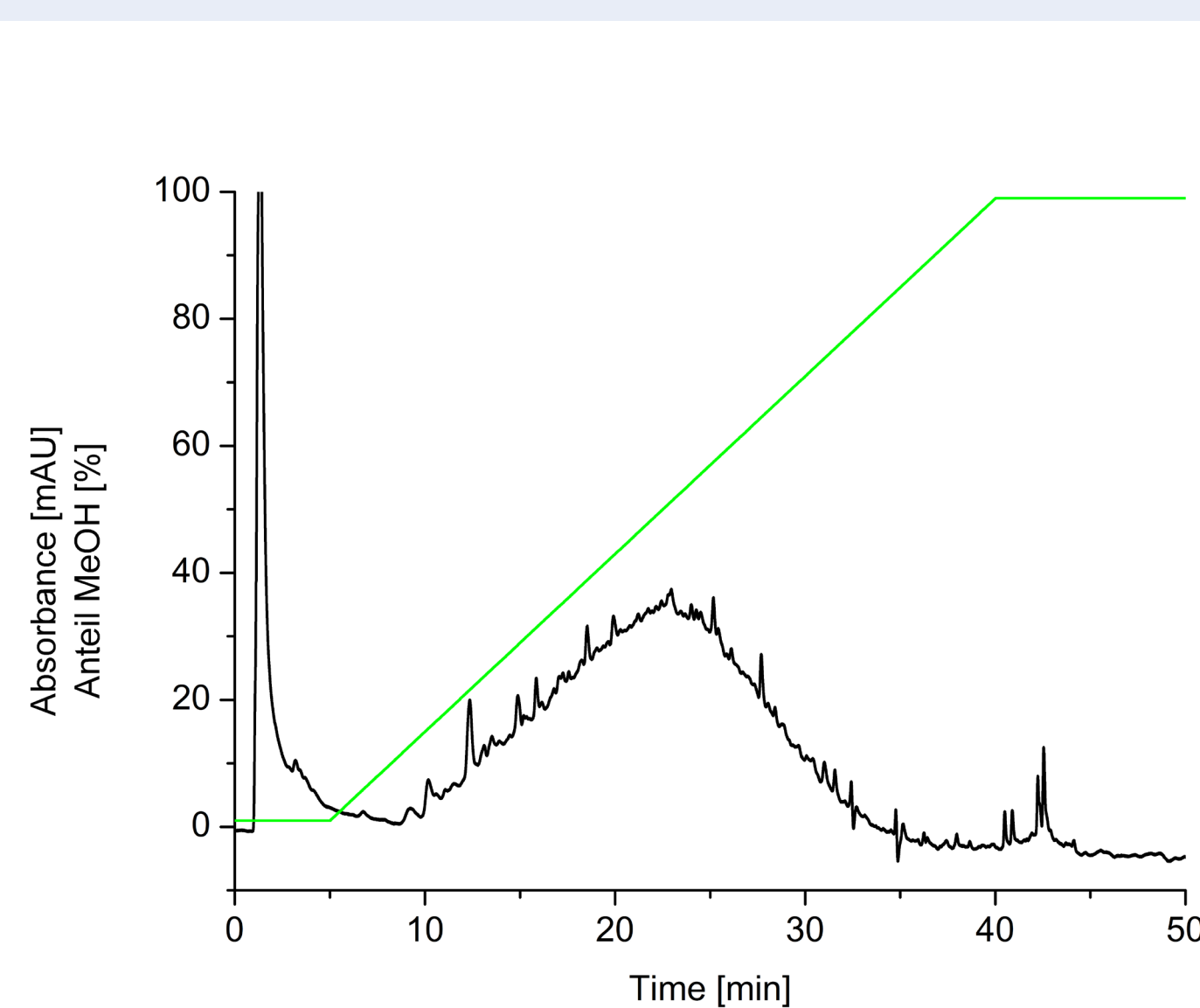


Fig. 2: HPLC separation with linear gradient

- High amount and chemical similarity of the HULIS components leads to an insufficient separation using a standard linear gradient (Fig. 2)
- No improvements with different organic solvents, pH-values of the aqueous phase, flowrates or slopes of the gradient achieved
- New gradient is needed for the RP-HPLC separation

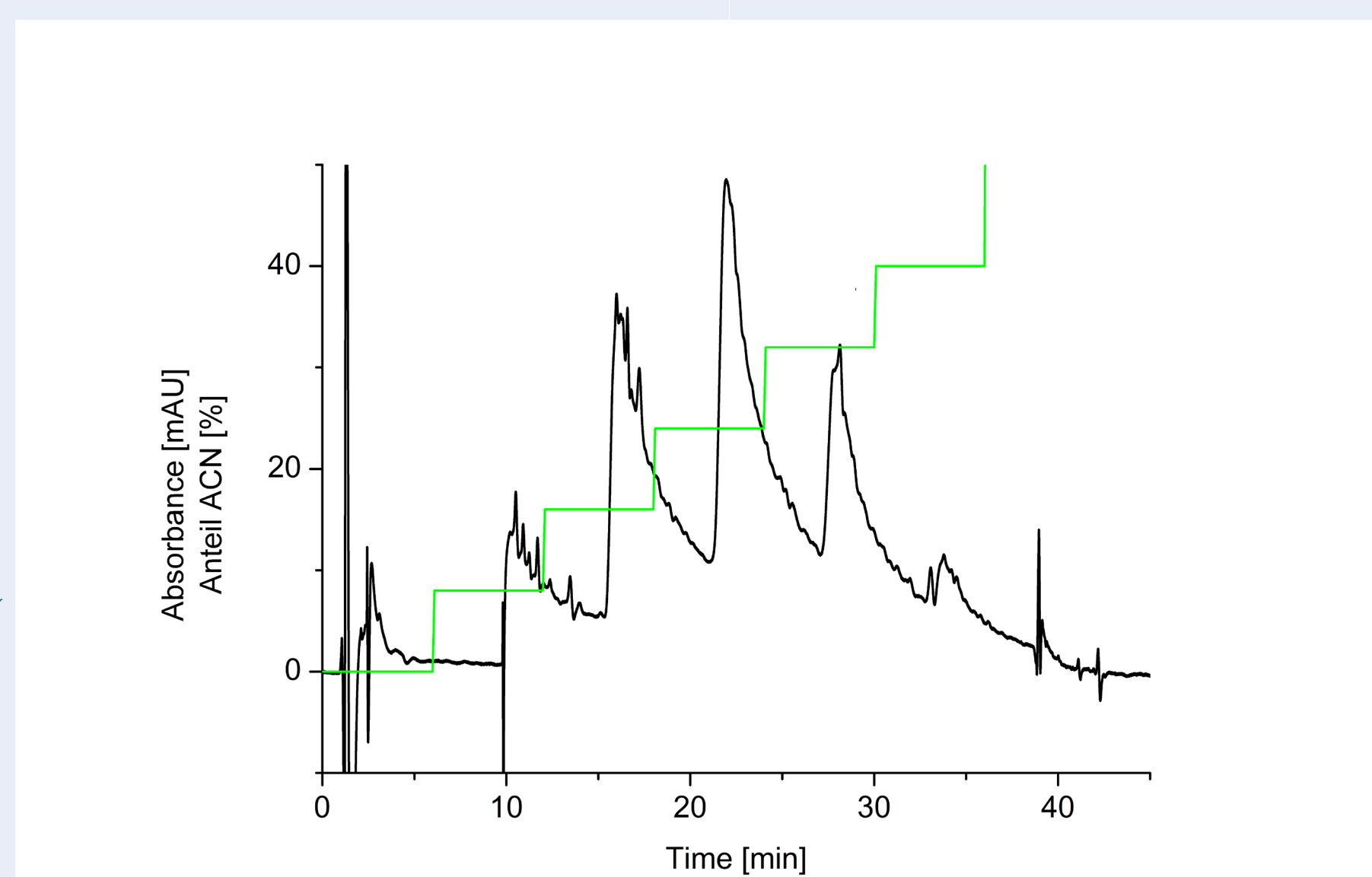


Fig. 3: HPLC separation with stepwise gradient

- Stepwise increasing gradient (similar to GORA et al. 2012) leads to one fraction of substances similar polarities per gradient step
- Methanol and acetonitrile are usable as organic solvents
- Acetonitrile should be favored due to a more stable baseline
- Relatively low purity of the fractions (70-80% for the 5-step gradient shown in Fig. 3)
- More fractions are possible, but purity decreases further

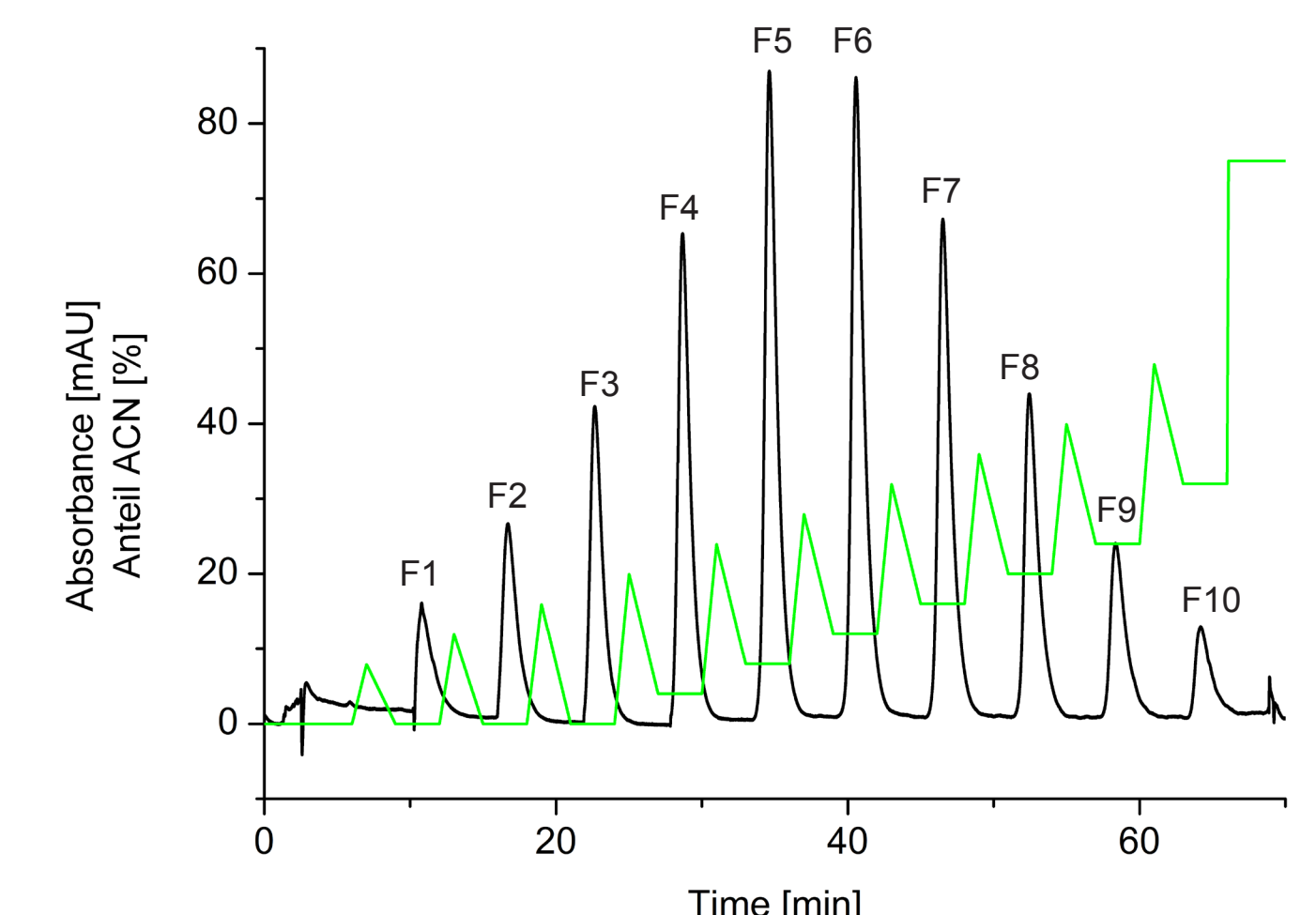


Fig. 4: HPLC separation with the new „spiked“-gradient

- Our newly developed gradient with short increase and immediately decrease („spikes“) of the organic portion leads to a heavily improved separation compared to the stepwise gradient (Fig. 4)
- Configurations with up to 20 fraction possible
- Purity for the 10-step gradient is between 90% (later fractions) and 99% (earlier fractions)
- Sharper peaks increase the intensity, thus lower concentrations are measurable

MS analysis

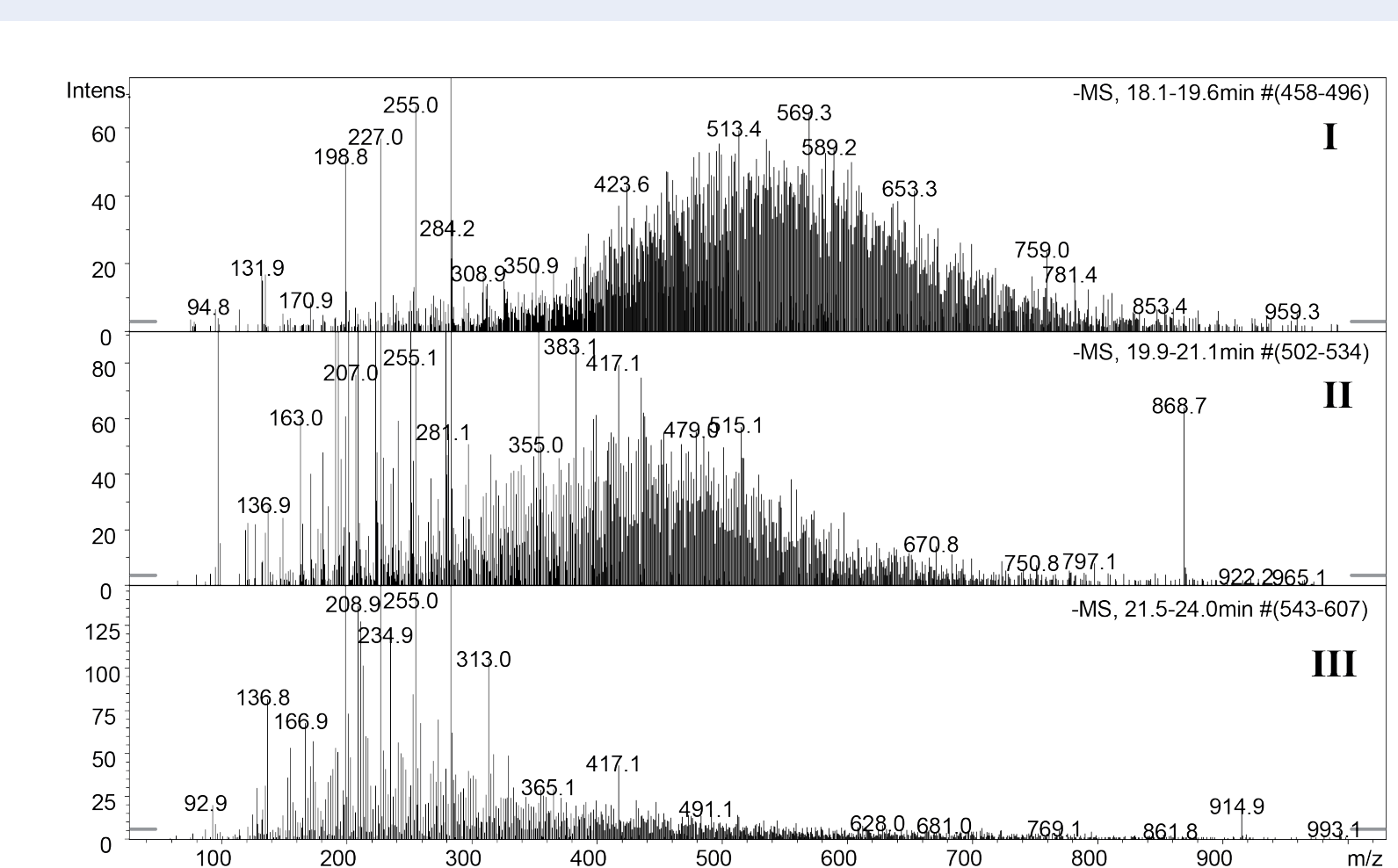


Fig. 7: Sum spectra for the individual peaks from the SEC separation of F3 (Fig. 6).

- Fig. 7 shows the sum spectra for the 3 peaks of Fig. 6
- Even after two separation dimensions still multitude of substances per fraction/ aliquot
- Average molar weight decreasing for later peaks is consistent with the separation principle of SEC

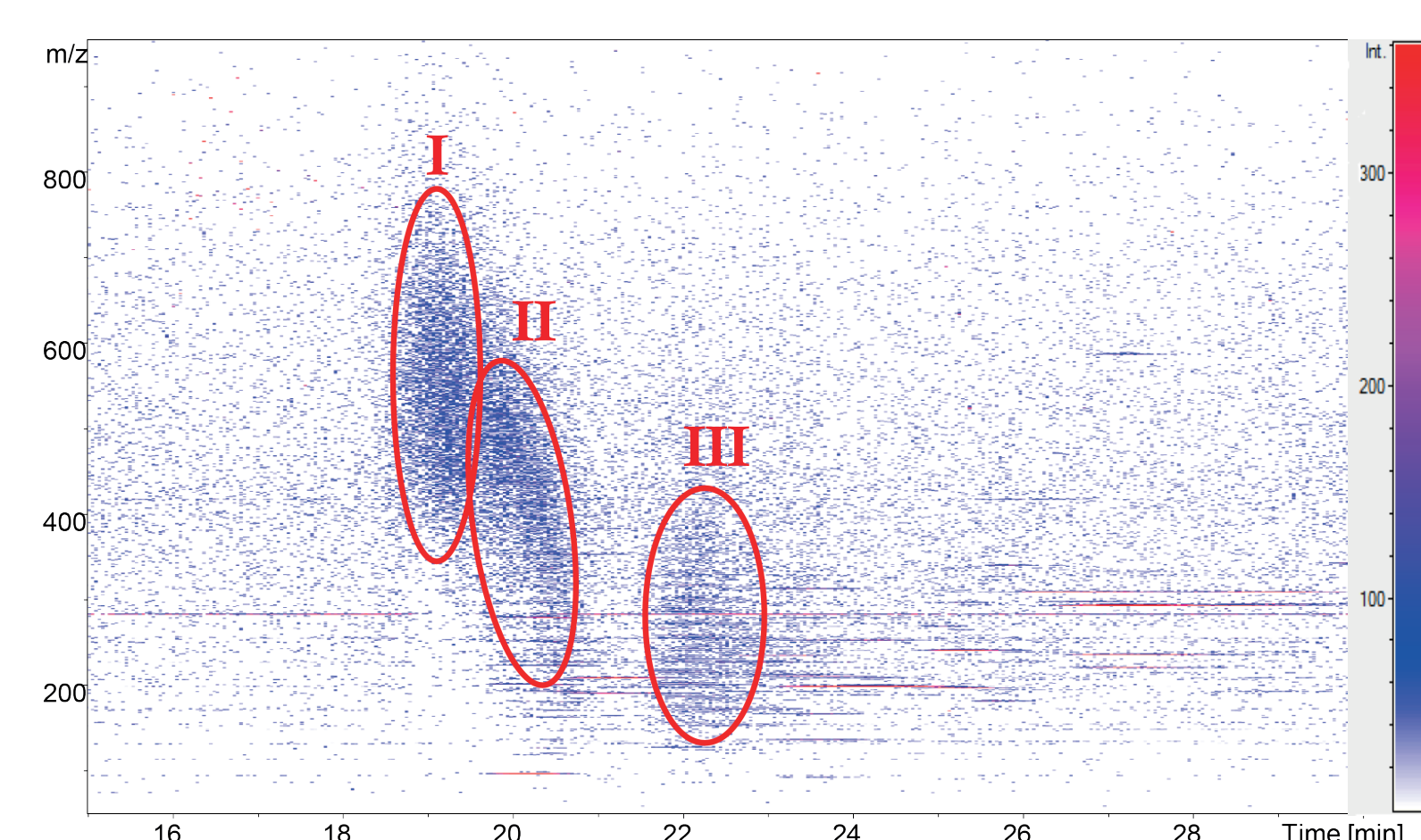


Fig. 8: 3D-MS-plot of the same chromatographic run like Fig. 6 and 7.

- 3D-MS-plot (Fig. 8) again shows 3 peaks with decreasing average molar weight for the later peaks
- Low signal to noise due to multitude of substances with very low concentrations each
- Intensive interferences below 300 m/z

SEC

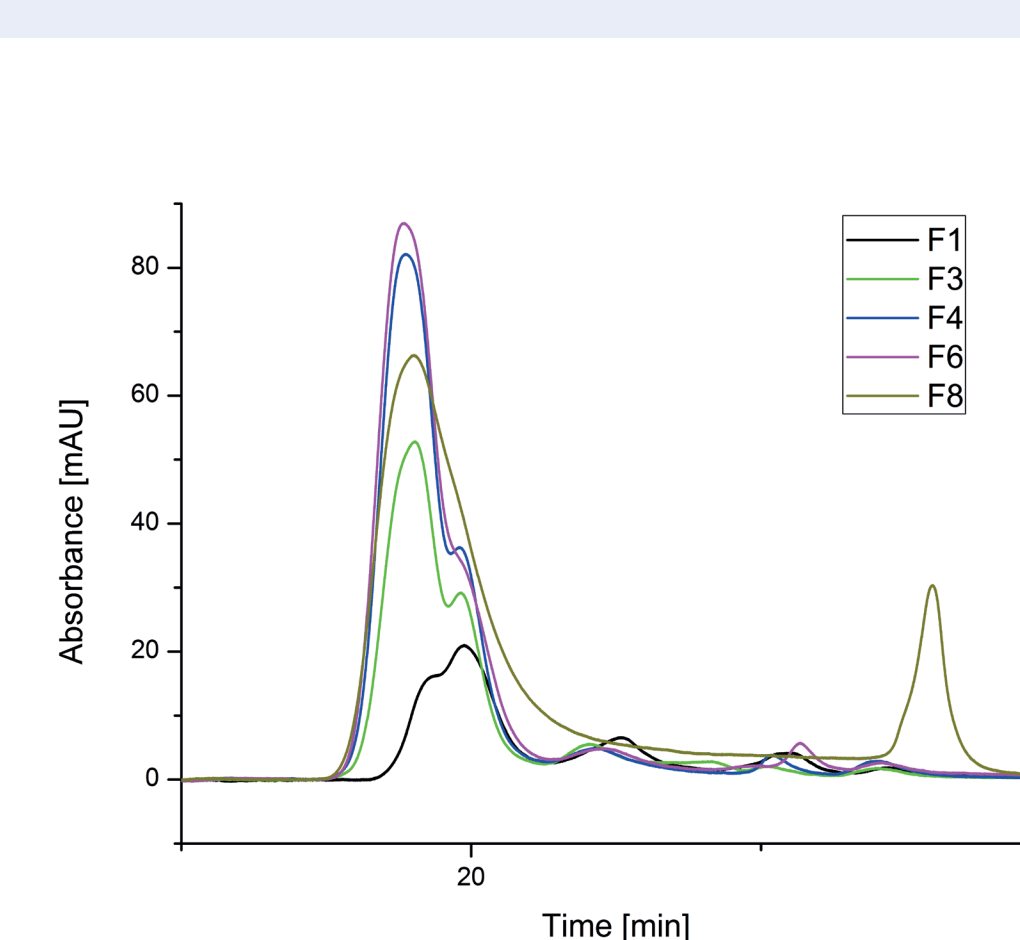


Fig. 5: SEC separation of five fractions from figure 4.

- Different solvent mixtures tested
- Solvent mix with the best separation: 80% 10mM NH_4HCO_3 ; 20% methanol
- SEC chromatograms for 5 out of the 10 fractionen (from the RP-HPLC in Fig. 4) are shown (Fig. 5)
- Two overlapping signals around 19.5 min
- At least one additional signal between 22-30min
- Online coupling with electrospray mass spectrometry (ESI-MS) shows a clear correlation between UV- and MS-signal (300-1000 m/z)
- Also showing two overlapping peaks around 19.5min and one more peak around 22min (Fig. 6)

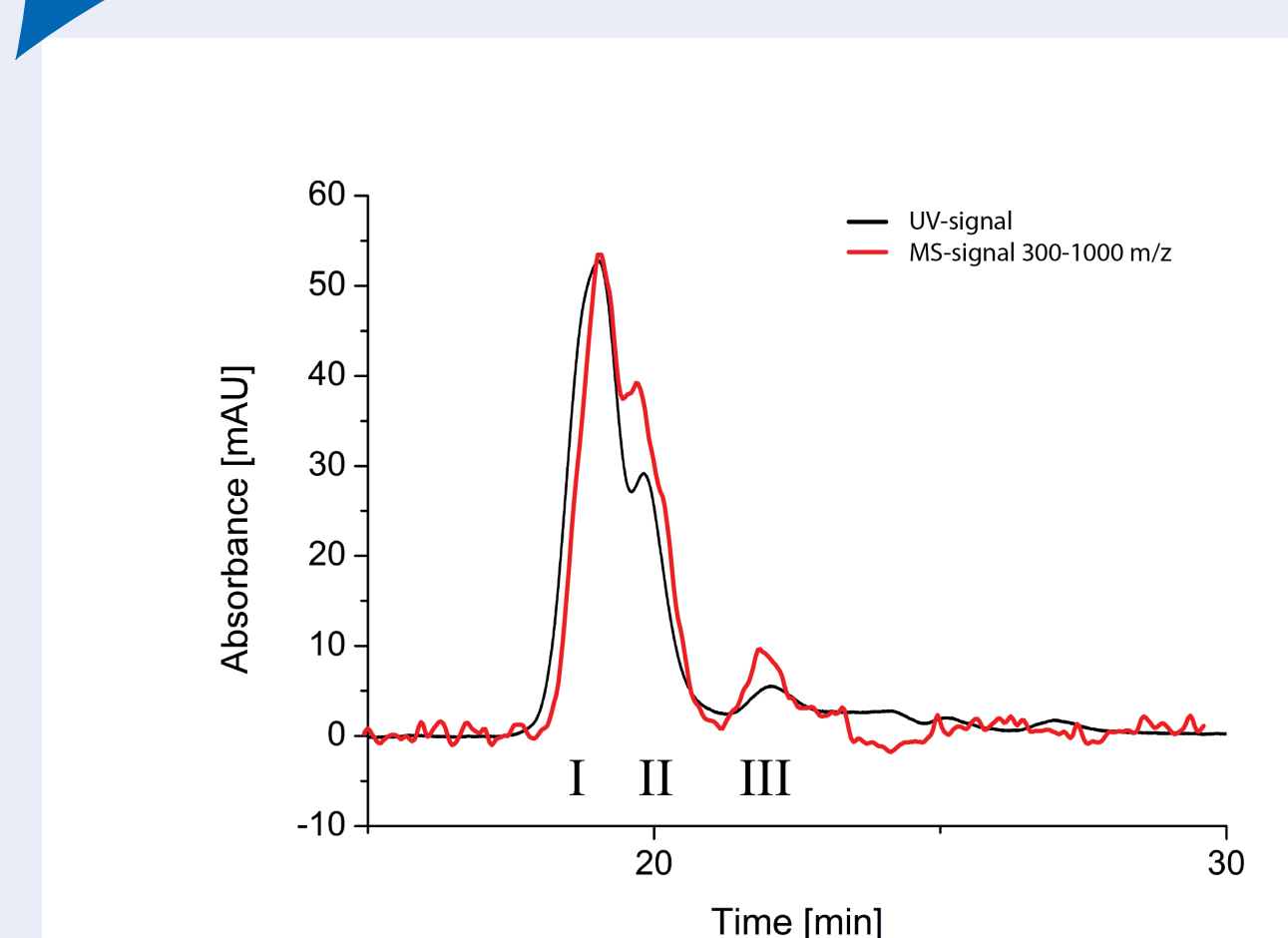


Fig. 6: SEC separation of fraction F3 with summed up MS-signal 300-1000 m/z.

Conclusion and outlook

A new 2-dimensional chromatographic method was developed, which is capable of separating HULIS extracts into groups of different polarity and sizes/ molar masses. The new spiked gradient allows for a baseline separated fractionation of HULIS extracts, while the SEC separation needs additional improvements. MS measurement with high-resolution mass spectrometers are planned to further analyze the fractions/aliquots after the 2D-separation in a more detailed way and identify possible tracer molecules for each fraction.

In the long run a variety of filter samples from different environments and seasons are analyzed by the 2D-chromatography to understand correlations between composition / distribution in the 2D-space (polarity, size) and the source of the particle as well as other atmospheric chemical data.

Acknowledgment and literature

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