Antioxidant Profiling by Two-dimensional Chromatography with Post-column ABTS: Example of Lignin

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Background

- Lignin is one of the most abundant biopolymers on earth. It can be found in, e.g., wooden plants.
- In the form of Kraft lignin, it is a byproduct of the pulp and paper industry. Worldwide around 100–150 million tonnes are produced every year. [2,3]
- Currently it is mainly used for energy production by incineration.
- As a renewable antioxidant it has great potential to be used as, e.g., food packaging material. [3]

Aim

Identification and profiling of lignin antioxidants by 2D chromatography with post-column antioxidant assay (ABTS).

Methods

Lignin Preparation

- Softwood black liquor precipitated by hydrochloric acid.

Chromatography

- **1st Dimension: SEC**
  2 × 300mm × 8 mm × 5 um PSS SUPREMA 100 Å * 60% ACN + 40% H2O + 0.2% HCOOH * 1 mL·min^{-1} * UV 280nm * 3 × 37 × 0.4 mL fractions.
- **2nd Dimension: RPLC**
  250 mm × 4.6 mm × 5 um Agilent ZORBAX SB-Phenyl * H2O + 0.1% HCOOH, ACN + 0.1% HCOOH * 1 mL·min^{-1} * UV 280 nm.
- **Post-Column-ABTS**
  13.7 m × 0.25 mm PEEK * 0.25 mM ABTS, 1 AU * 0.5 mL·min^{-1} * vis 734 nm.

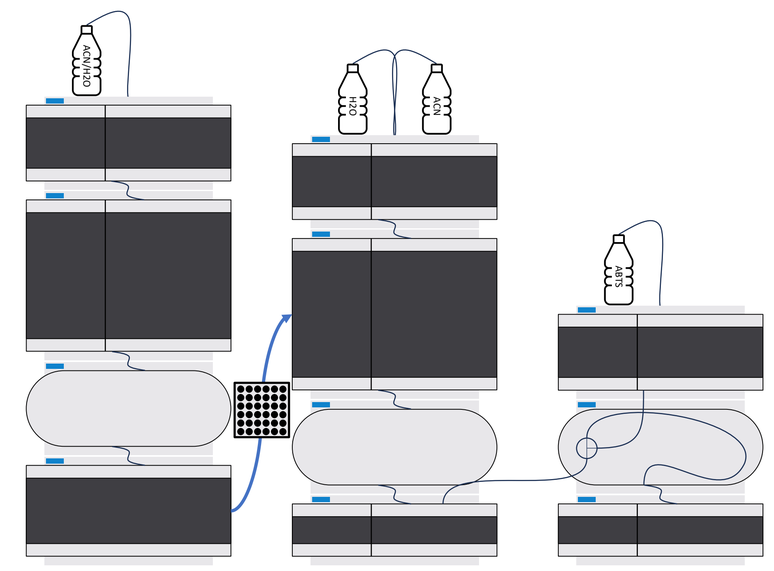

Figure 1. *2D chromatography set-up: 1st dimension: size-exclusion chromatography with fraction collector. 2nd dimension: reversed-phase liquid chromatography with post-column antioxidant assay.*

Results

- Size-exclusion chromatography alone does not have enough resolving power for lignin profiling but is acceptable as a first-dimension separation. It is useful to separate monomers from the polymeric fraction.
- Reversed-phase chromatography is able to separate monomer and oligomer species but suffers from superimposition by polymeric species.
- Combining SEC and RPLC allows for high resolution separation of the lower molecular weight fraction for profiling and further analysis by, e.g., mass spectrometry, or post-column reactions and assays.
- Adding ABTS to the effluent of the RPLC (post-column) allows for the quantification of the antioxidant activity for each species.
- Antioxidant activity was detected over the entire chromatogram (monomeric, oligomeric, and polymeric region).
- Vanillic acid, vanillin, acetovanillone, and guaiacol were identified within Kraft lignin.
- Guaiacol shows the highest specific activity followed by acetovanillone and vanillic acid. Vanillin's activity was lower than the detection limit.
- Also, high activity was detected in the oligomeric region, but compounds were not yet unidentified (uip1–uip4).
- Comparing the absorbance at 734 nm with the absorbance at 280 nm shows a drift in specific antioxidant activity in the polymeric region. Low-molecular-weight lignin polymer is more active than lager lignin polymer due to higher total phenol content.

Conclusion & Outlook

- Lignin is a complex and disperse polymer mixture.
  - Disperse molecular weight, monomer composition, chemical functionality, and linkages.
- One chromatographic dimension alone is not powerful enough.
  - SEC suffers co-elution of different chemical species with the same hydrodynamic volume.
  - RPLC not suitable for high molecular weight species.
- 2D chromatography combines the strengths of two separation mechanisms and is a very promising method for lignin profiling.
- Addition of post-column ABTS-assay enables antioxidant profiling of lignin.
- Next steps include alternative assays like DPPH and FRAP, and different types of lignins. Unidentified species will be subjected to mass spectrometric analysis for structure elucidation.

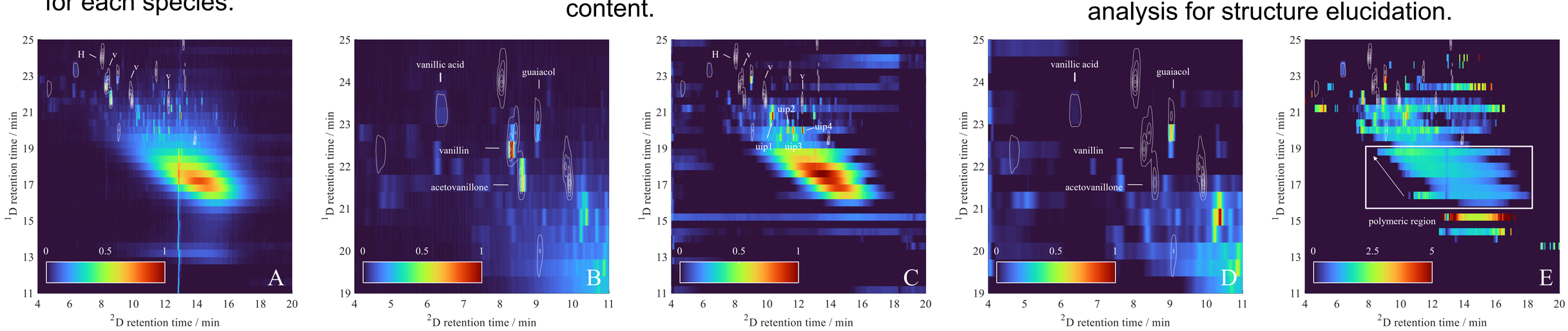

Figure 2. *SEC/ × /RPLC chromatogram of softwood Kraft lignin detected at 280 nm* ***(A)*** *and zoomed-in* ***(B)****, detected at 734 nm after post-column reaction with ABTS* ***(C)*** *and zoomed-in* ***(D)****. Chromatograms overlaid with contours of lignin model compounds. (H) indicates compounds derived from H-units, (v) indicates vinylic compounds and (uip) indicates unidentified peaks. Colour scale indicates normalized absorbance at corresponding wavelengths. Specific antioxidant activity map computed by dividing the absorbance at 734 nm after post-column reaction by the absorbance at 280 nm* ***(E)****. Rectangular box indicating region with polymeric lignin. Colour scale indicates ratio between absorbance at 734 nm and 280 nm.*

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