Off-line LC×SFC-HRMS/MS method for the non-target analysis of depolymerized lignin

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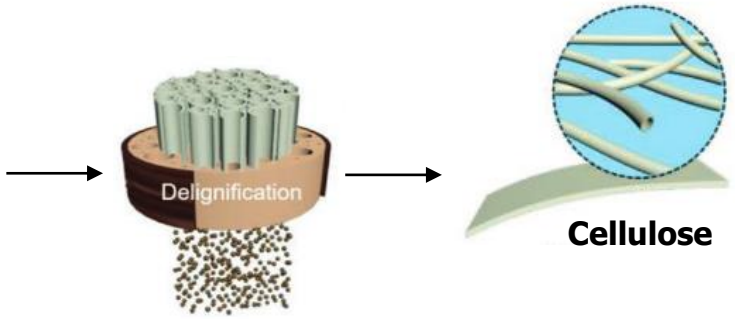
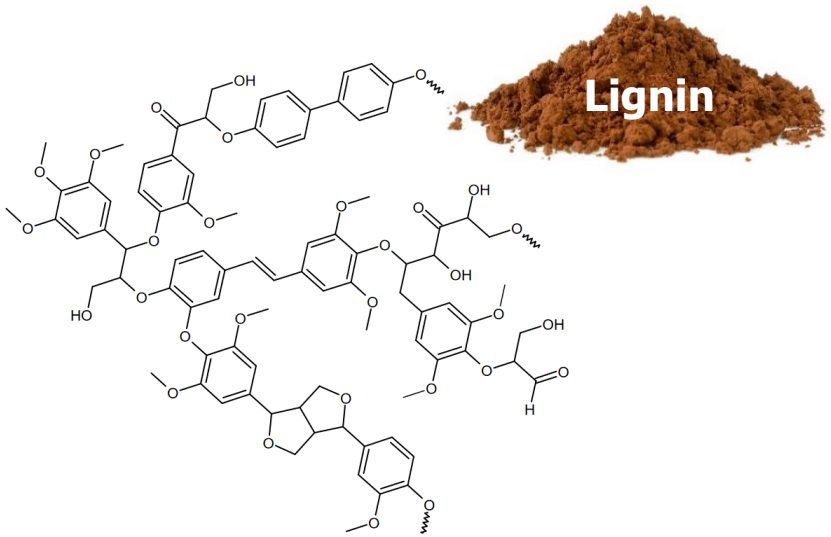
Background – what is lignin?



Isolation of chemicals



Biofuels

Novel biomaterials

Lignin ...

- is a **by-product** of the paper industry; only 2% is commercialized
- should be **valorized more for circular economy**
- needs thorough **structural characterization** for efficient valorization

Scheme adapted from: Jiang *et al.* Adv. Funct. Mater. **2020**, *30* (4), 1906307

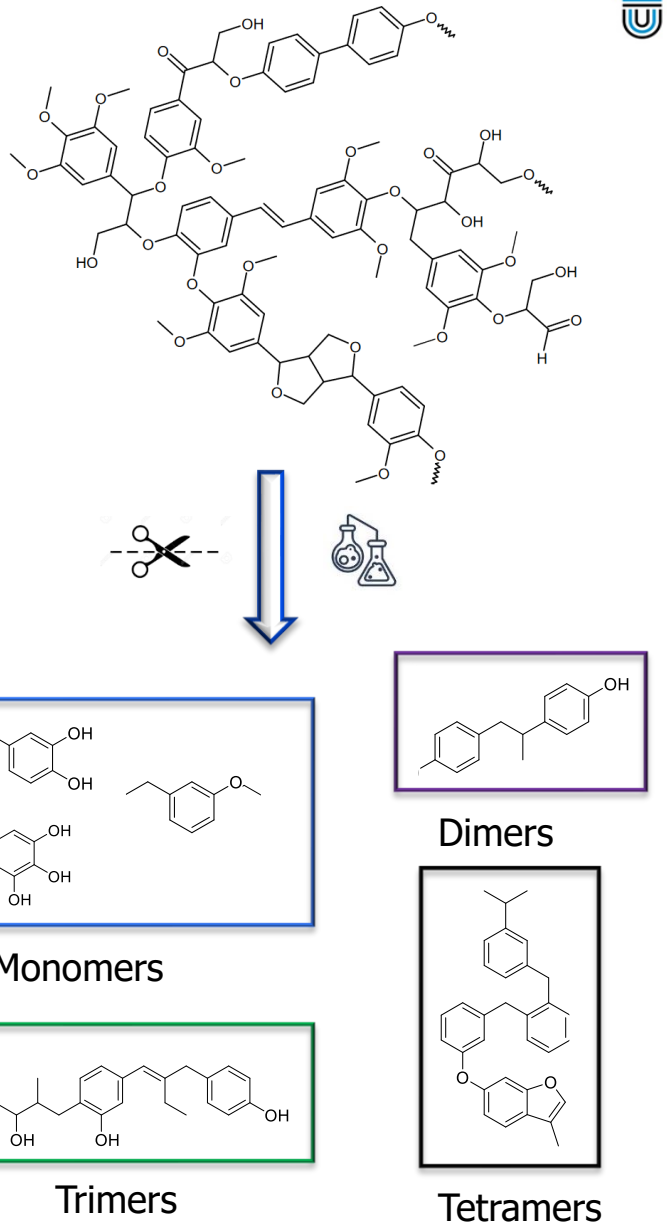
Analysis of depolymerized lignin – obstacles

Depolymerized lignin is a **complex mixture,** consisting of:

- numerous compounds
- compounds with similar structures, including isomers
- mostly neutral compounds

Possible solution: Two-dimensional RPLC×SFC

SFC (supercritical fluid chromatography) – can be used under normal phase conditions with supercritical fluid (here sCO_2) as the less polar eluent.

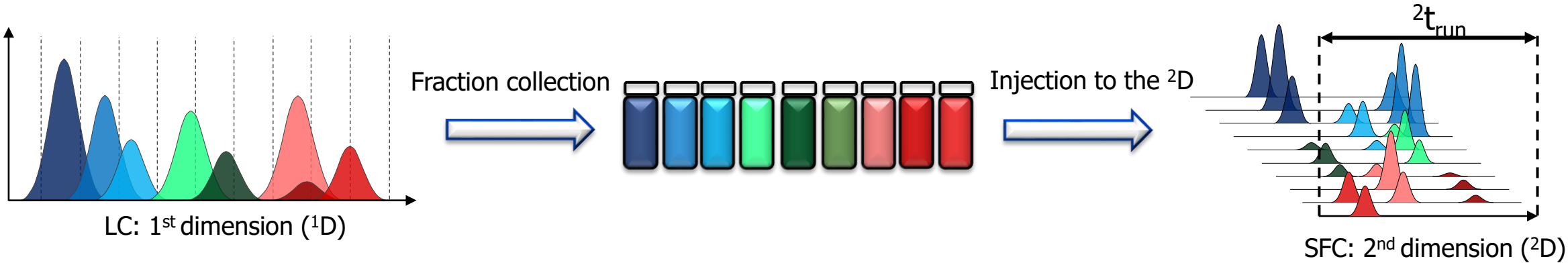


Lack of commercial standards

- monomers – quite a lot of commercial standards
- dimers and other oligomers – only a few dimers are commercially available

Possible solution: non-target analysis with HRMS/MS detection

Off-line two-dimensional LC×SFC method



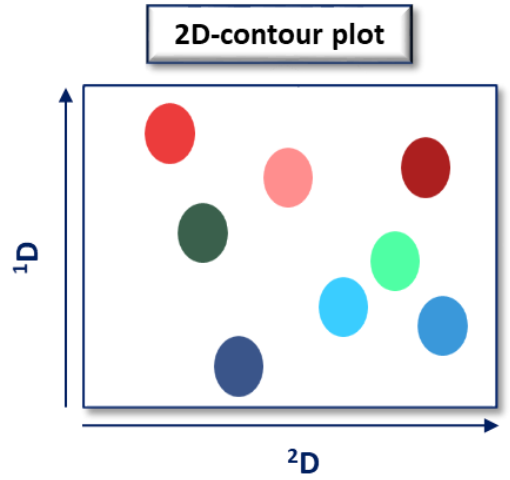
Off-line 2D method compared to on-line 2D:

Advantages

- Possibility to dissolve the fractions in a solvent suitable for ^{2}D
- Easier to obtain good quality MS^2 data
- No need for „complex" on-line LC×SFC system
  - *O-19 Margaux Sanchez*

Disadvantages

- Long analysis time
- Higher risk of contamination



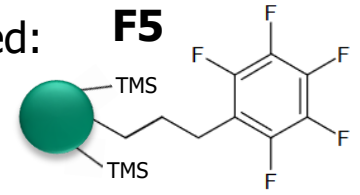
Original scheme: Soraya Chapel

Selection of columns and optimization of conditions

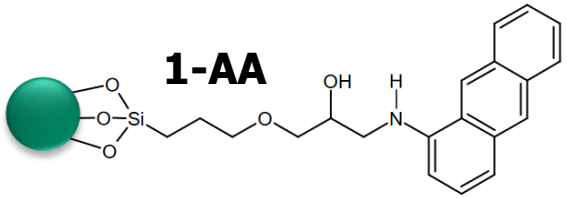
Various columns were screened using **33 monomer standards**. The following columns were selected:

- RPLC: **Kinetex F5** (pentafluorophenylpropyl)
  - *Hydrophobic, dipole-dipole, aromatic, steric, and hydrogen bonding interactions*
- SFC: **Torus 1-AA** (1-aminoanthracene)
  - *Aromatic, steric, and hydrogen bonding interactions*

F5

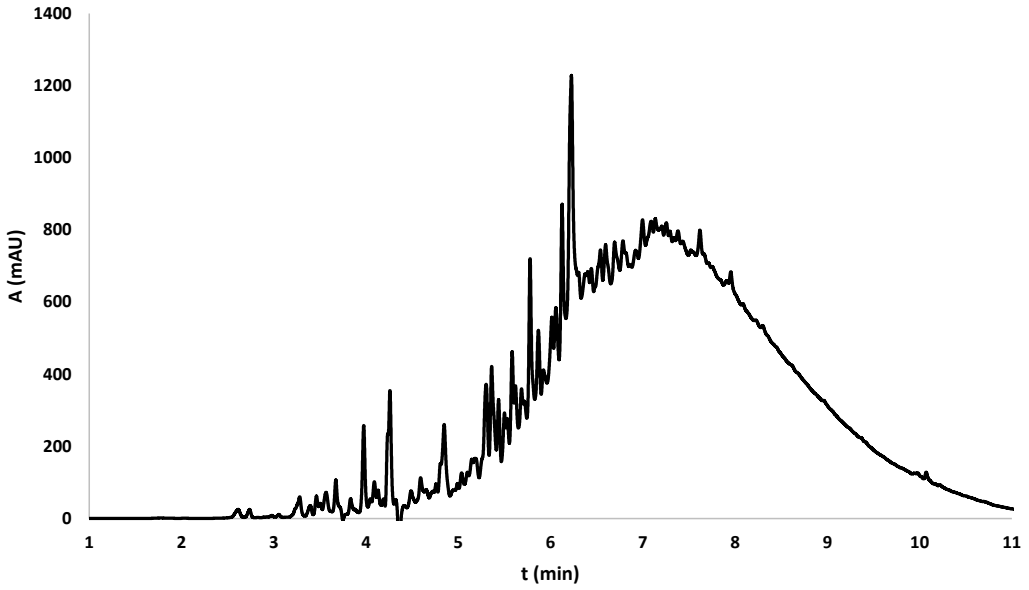


1-AA



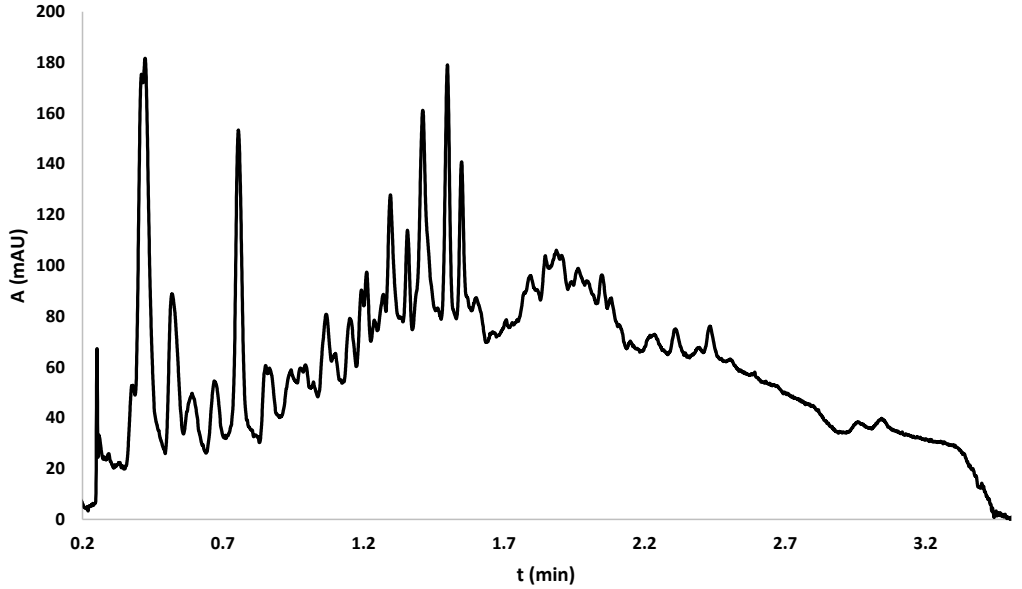
The two dimensions were separately optimized using the **depolymerized lignin** sample:

LC-UV with Kinetex F5



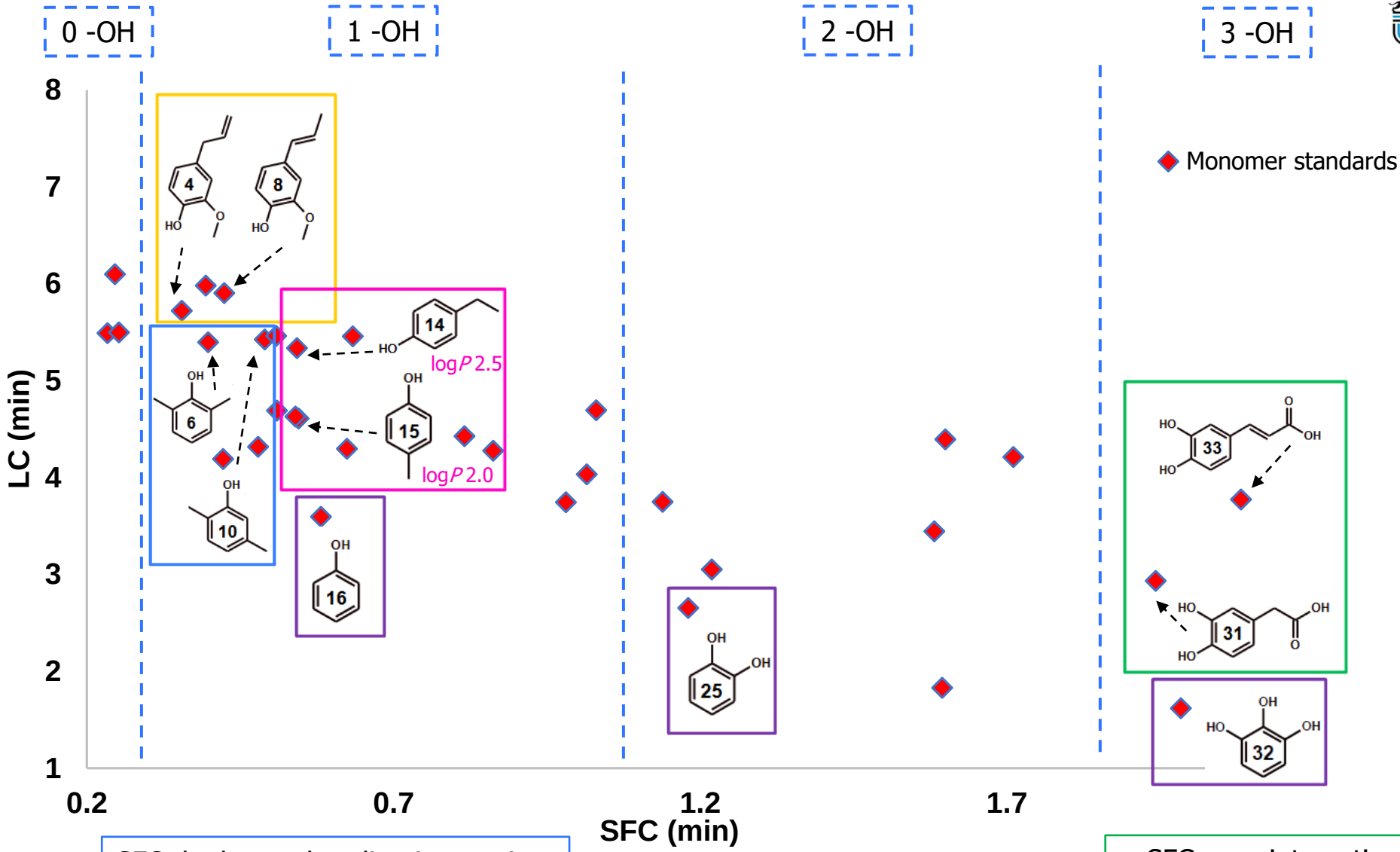
Kinetex F5, 150 mm x 4.6 mm, 5 µm; depolymerized lignin 30 mg/mL in MeOH; A: H_2O + 0.1% FA; B: ACN + 0.1% FA, T = 30 °C, gradient: 1-90 % B in 9 min, F = 2.1 mL/min, V_{inj} = 10 µL, UV (254 nm)

SFC-UV with Torus 1-AA



Torus 1-AA, 50 mm x 3 mm, 1.7 µm; depolymerized lignin 5 mg/mL in MtBE, T = 40 °C, BPR = 140 bar, gradient: 5-50% MeOH in 2.95 min, F = 1.7 mL/min (= F_{max}), V_{inj} = 7 µL, UV (210 nm)

LC×SFC separation mechanism – 33 monomer standards



RPLC: hydrophobicity

SFC: π-π interactions, conjugation

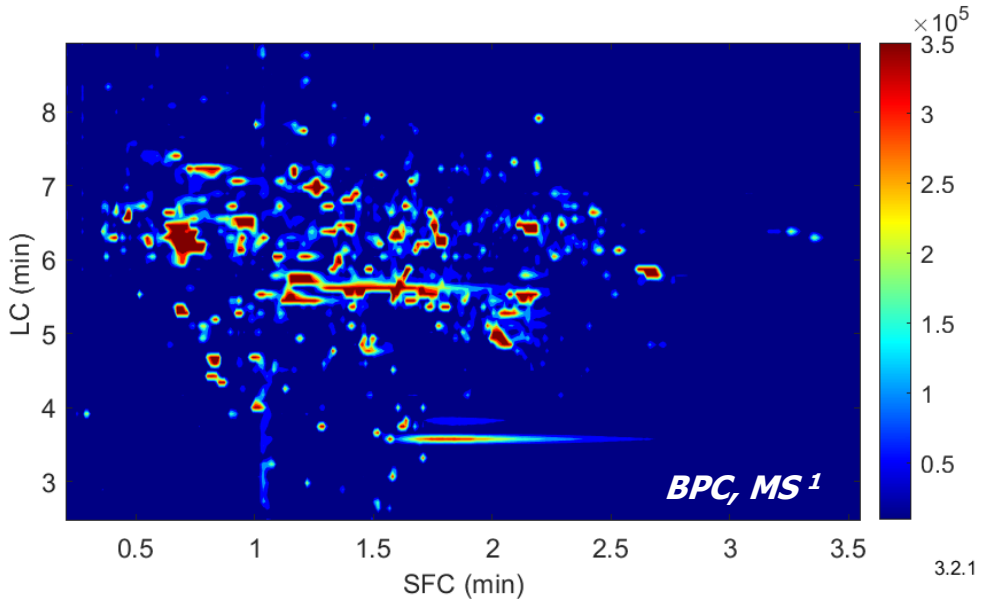
SFC: hydrogen bonding interaction, steric hindrance

SFC: hydrogen bonding interaction, number of –OH groups

SFC: π-π interactions

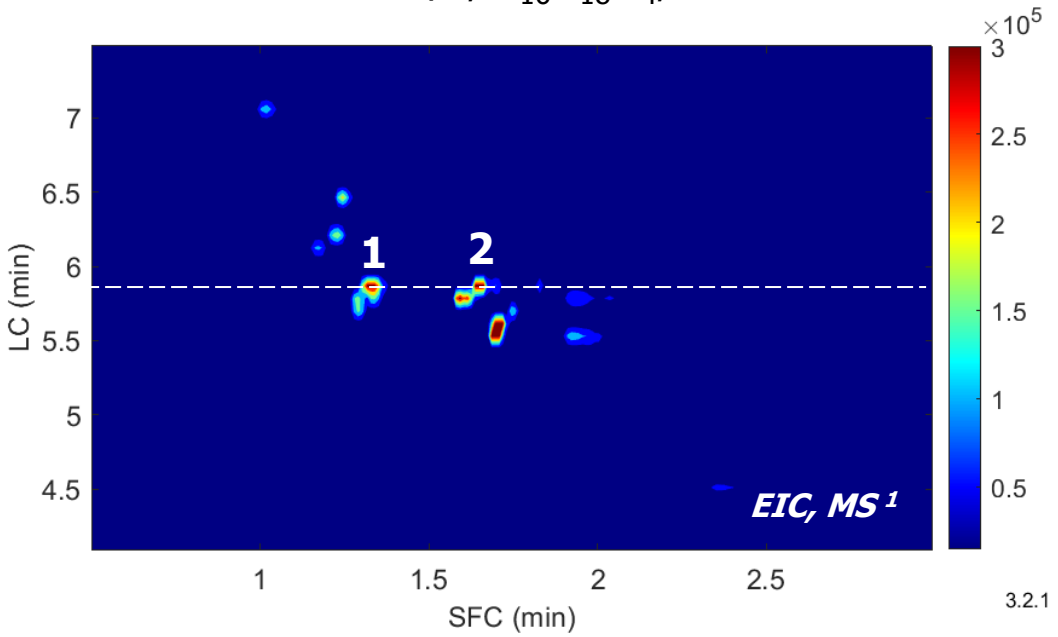
2D LC×SFC-HRMS/MS analysis of depolymerized lignin

Corrected peak capacity: **1995**
Time of analysis: 430 min



Good separation of depolymerized lignin compounds

273.11279 *m/z*, $C_{16}H_{18}O_4$, DBE 8



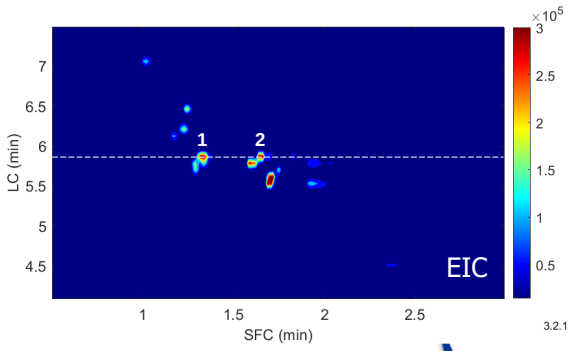
Good separation of isomers

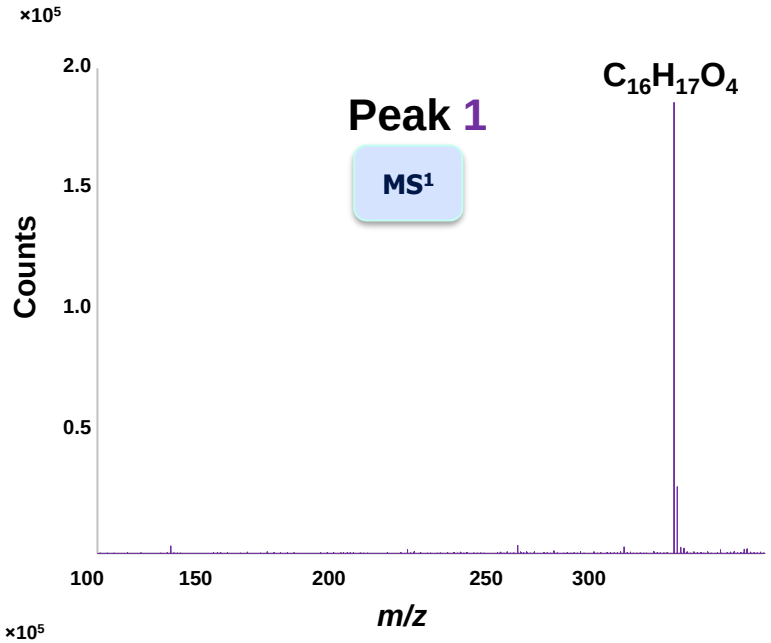
LC×SFC conditions same as on slide 5

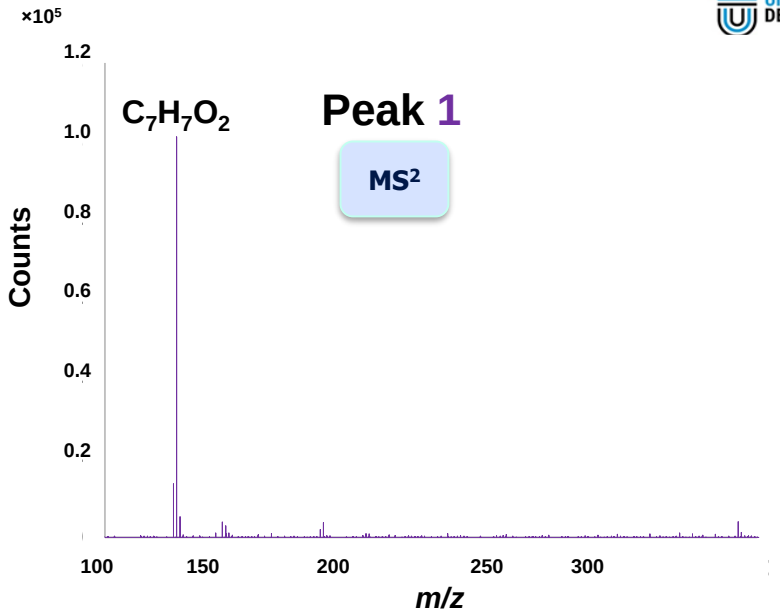
Q-ToF detection for both: ESI(-) mode, 90-1700 *m/z*, acquisition rate 3 spectra/sec, capillary 3.5 kV, fragmentor 185 V, make-up solvent MeOH, collison energy for MS^2 20V

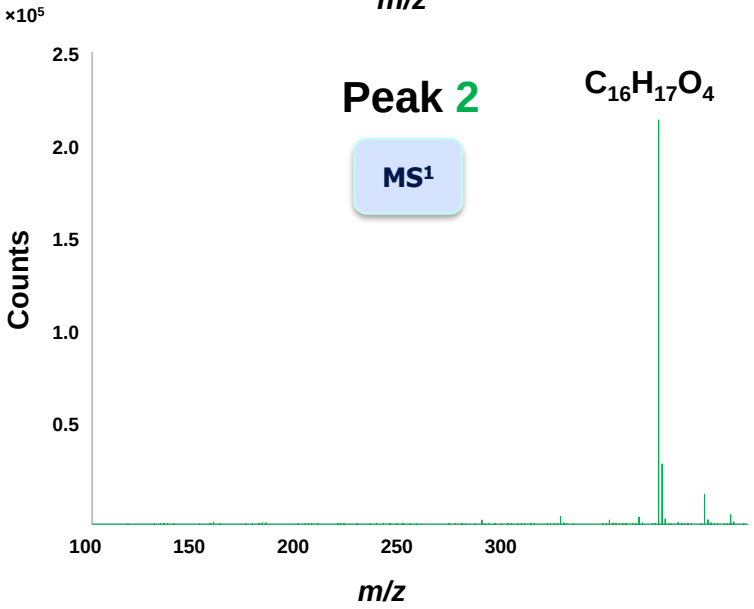
Good quality MS¹ and MS² spectra

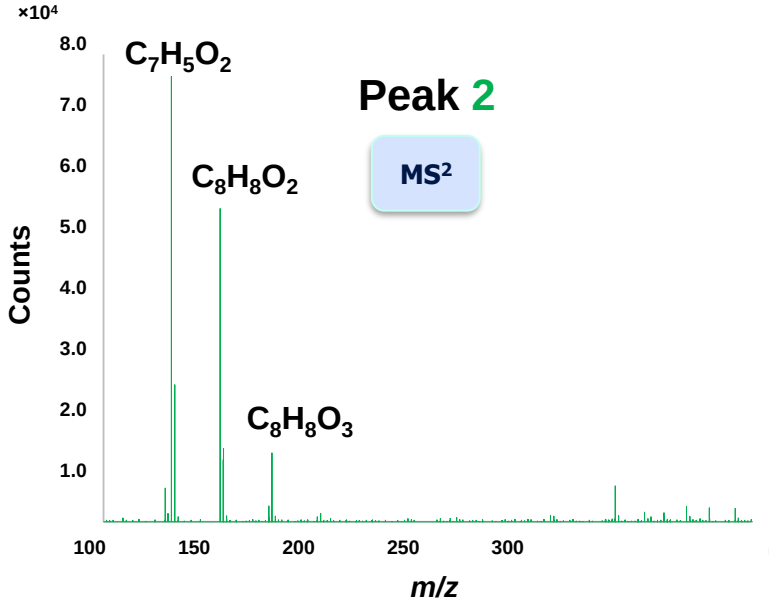
273.11279 *m/z*, $C_{16}H_{18}O_4$, DBE 8











- ✓ **Clean MS¹ and MS² spectra**
- ✓ **Isomers of a dimer**

Grouping of analytes based on their DBE values

- MS-DIAL software
  - peak picking
  - MS/MS deconvolution

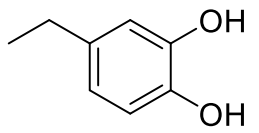
MS-DIAL

DBE: double bond equivalent

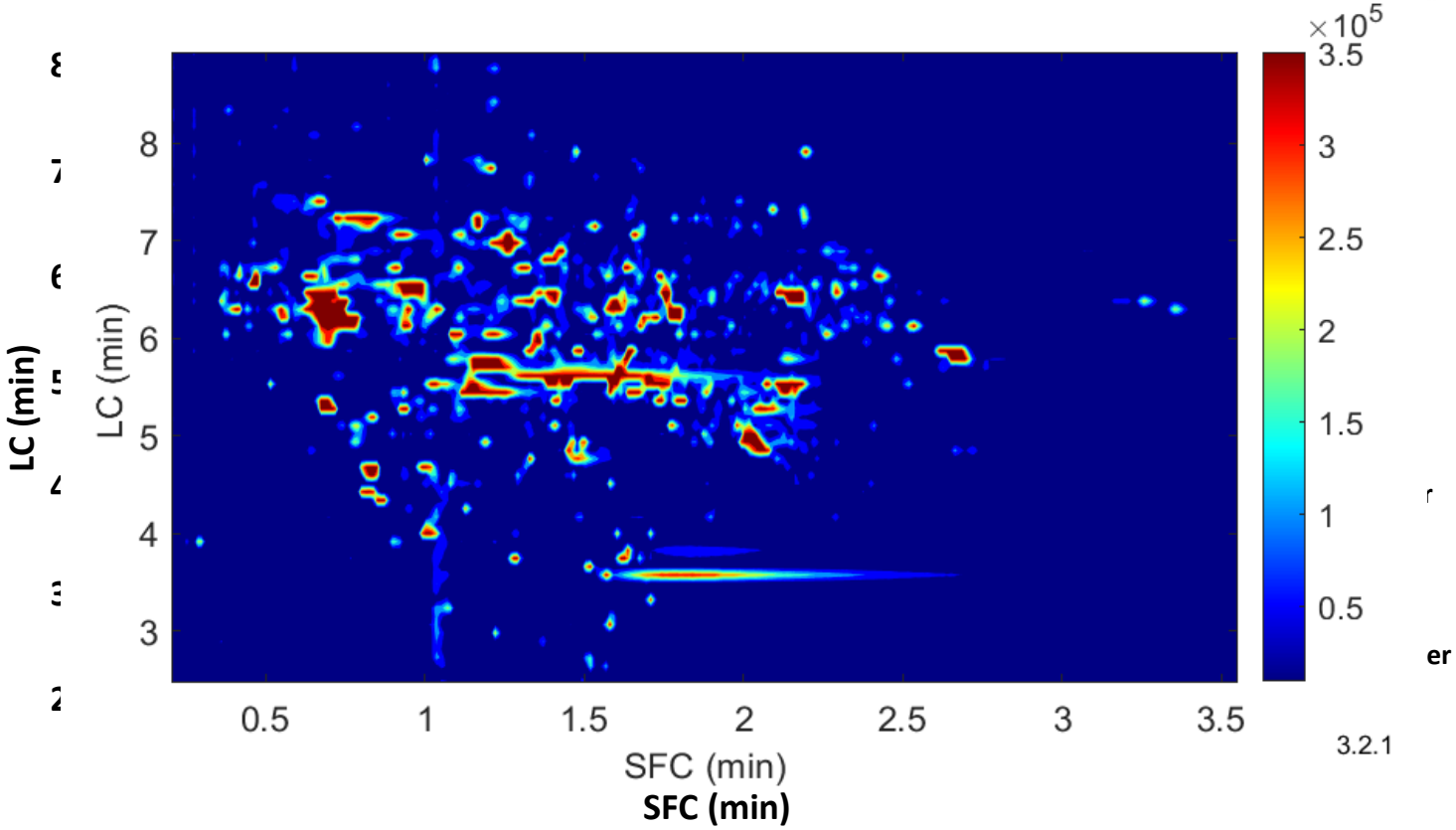
$$DBE = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2}$$

For $C_xH_yO_z$ compounds:

$$DBE = C + 1 - \frac{H}{2}$$

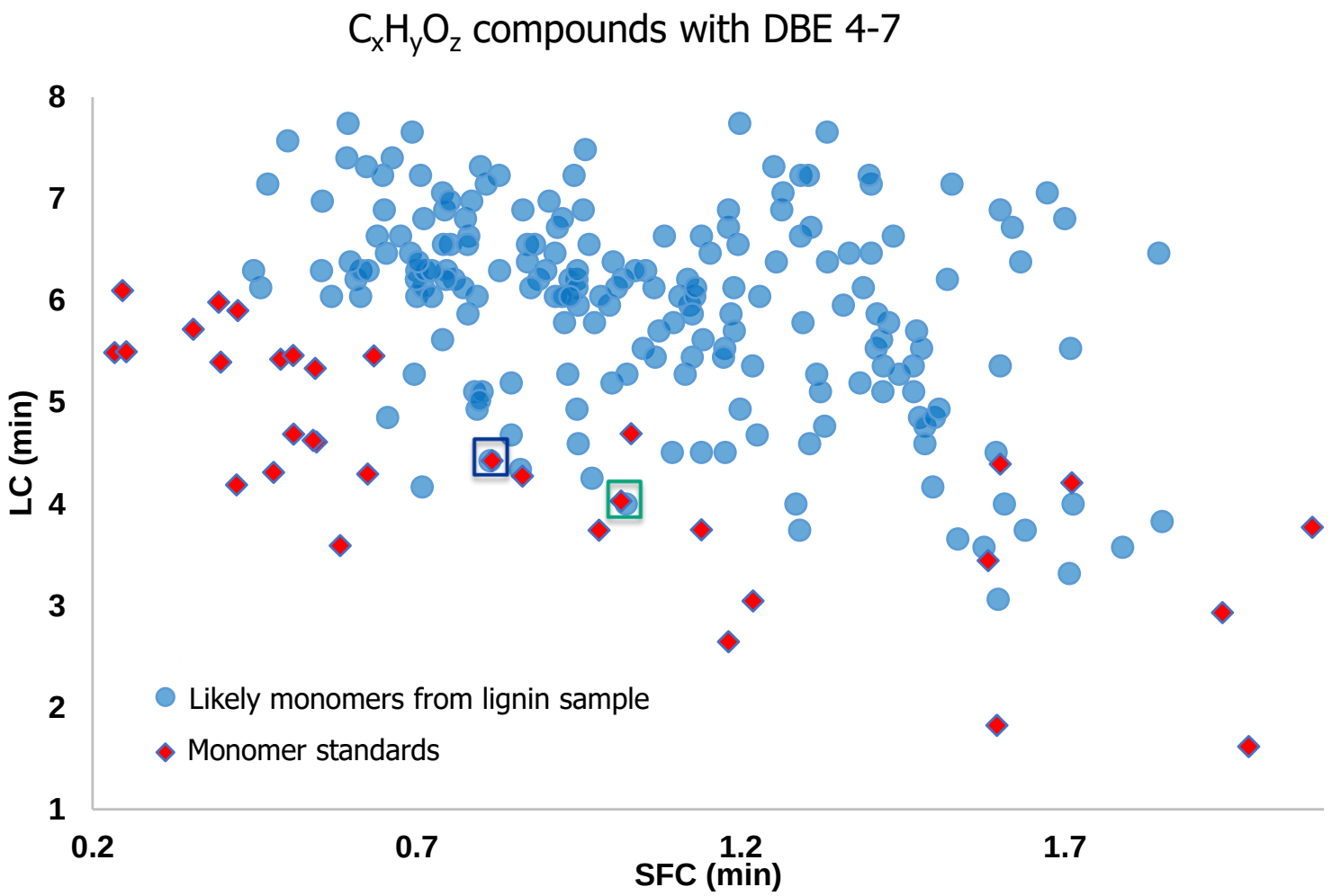


DBE = 8 + 1 – 10/2 = 4



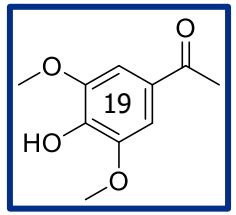
471 $C_xH_yO_z$ compounds with DBE ≥ 4

Annotation of the detected compounds

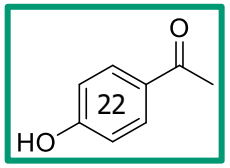


Identification based on 4 criteria:

I. t_R in LC (± 0.085 min)
II. t_R in SFC (± 0.012 min)
III. Accurate mass (± 3 ppm)
IV. MS/MS (match above 90%)



Acetosyringone



p-Hydroxyacetophenone

For the structural characterization of the other detected compounds MS/MS is needed

Feature-Based Molecular Network – FBMN

Molecular Network (MN)

Connects together features with similiar MS^2 pattern

- The **more similar the MS^2** (= structurally similar), the **more close the features** are on the Molecular Network

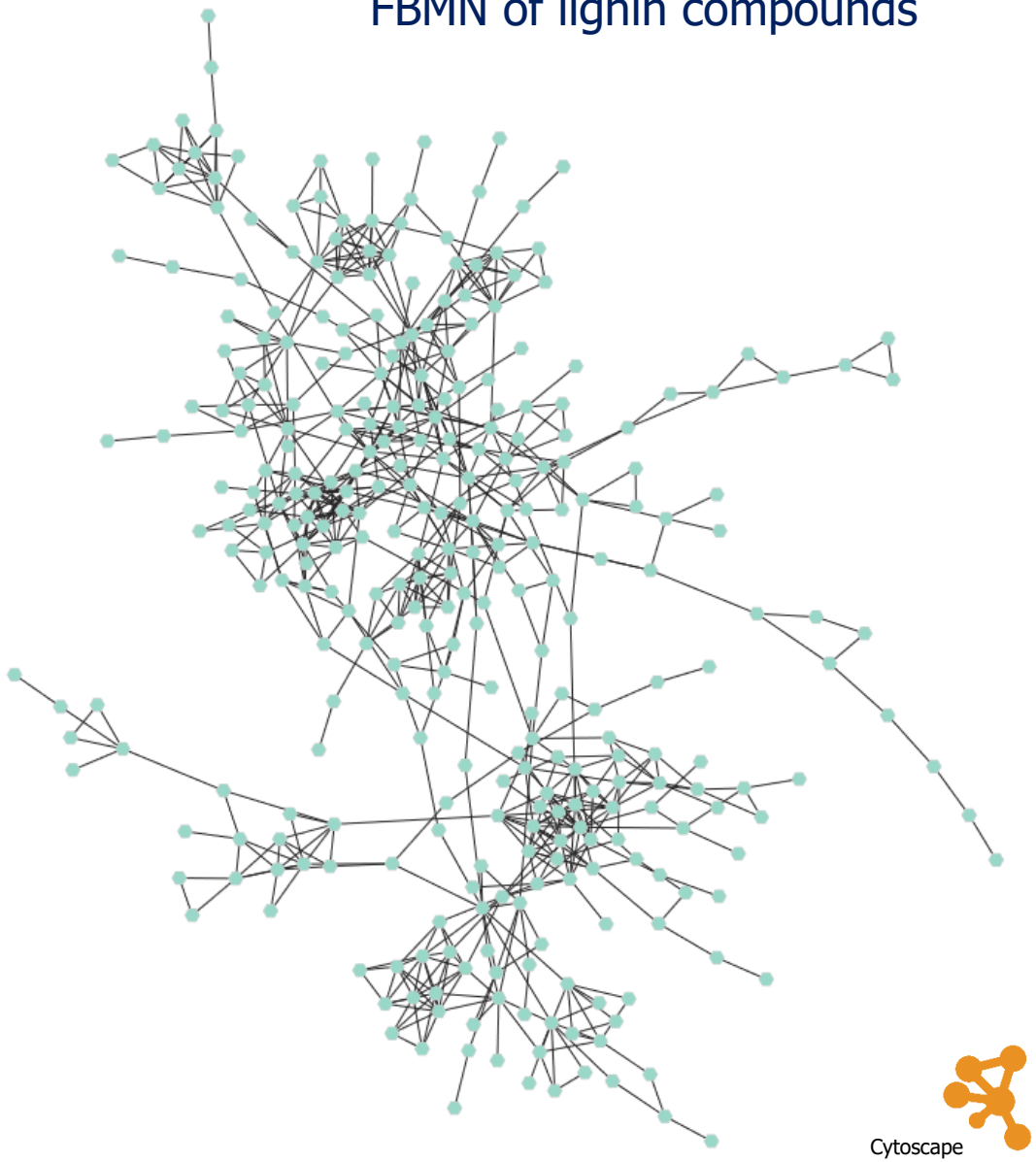
Feature-Based Molecular Network (FBMN)

MN that enables the visualization of features with:

- the **same monoisotopic mass**
- **different retention times**

Sample with a high number of isomers –
77% of compounds had at least one isomer

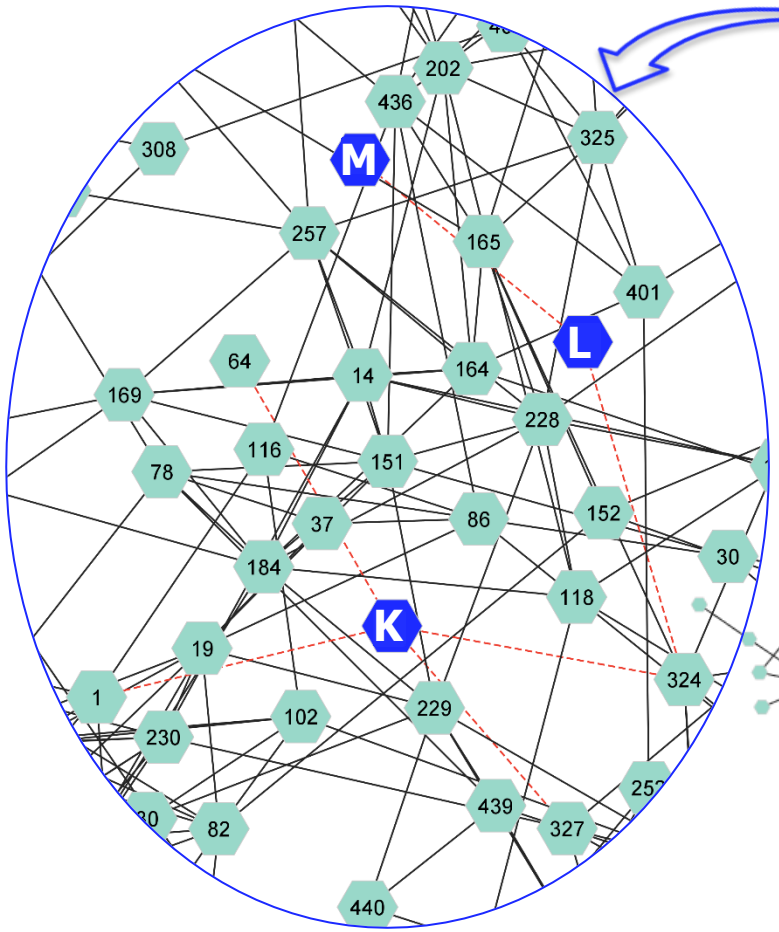
FBMN of lignin compounds

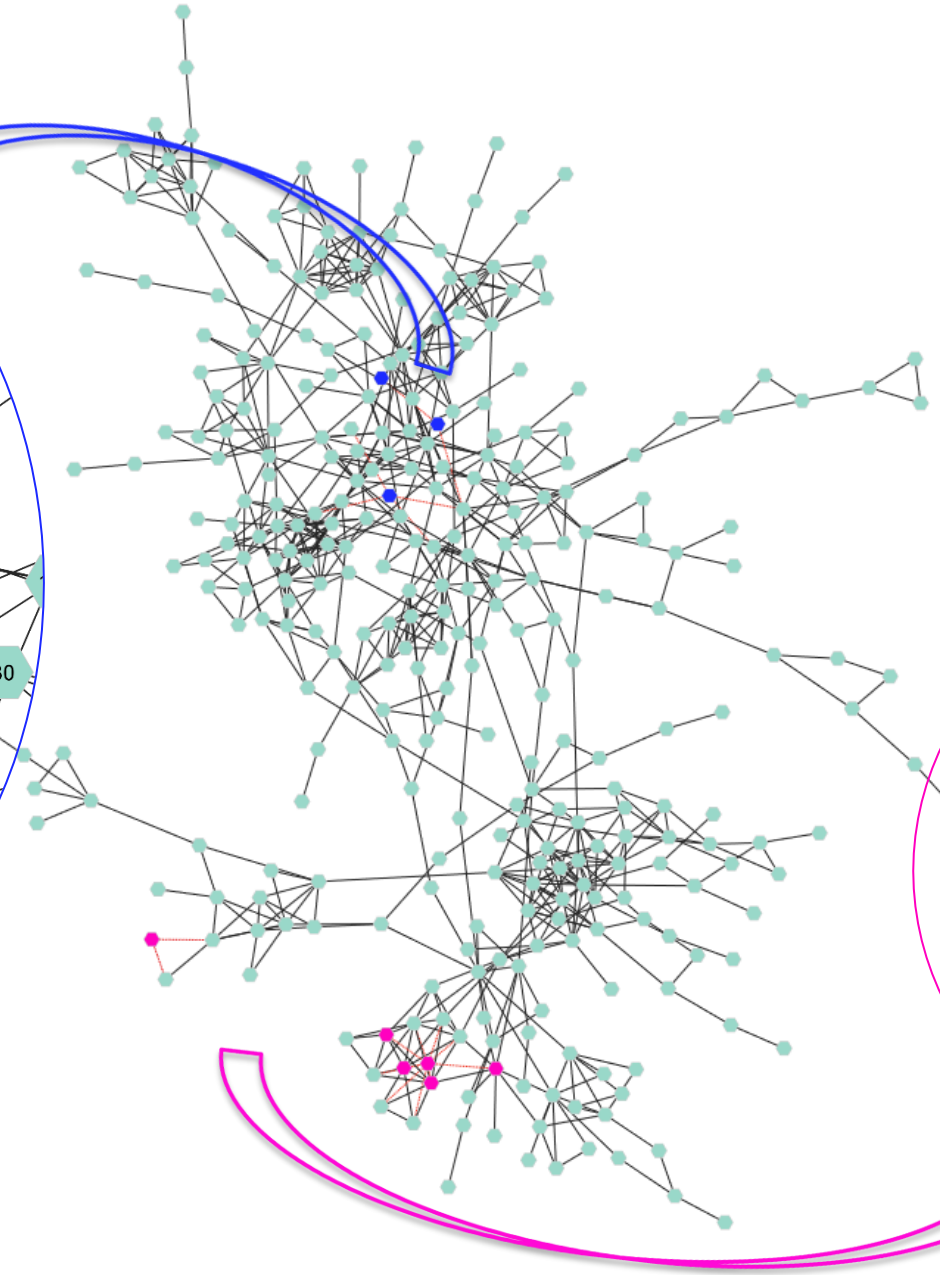


Cytoscape

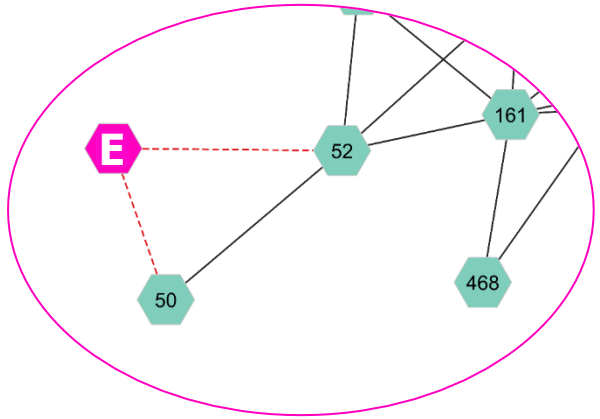
Feature-Based Molecular Network – FBMN

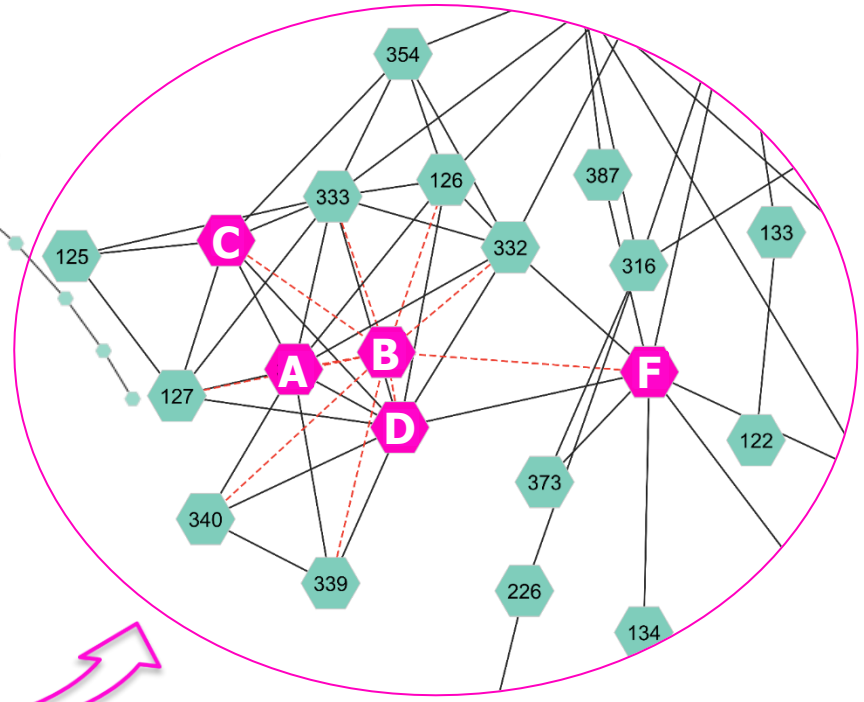
Isomers of $C_9H_{10}O_4$



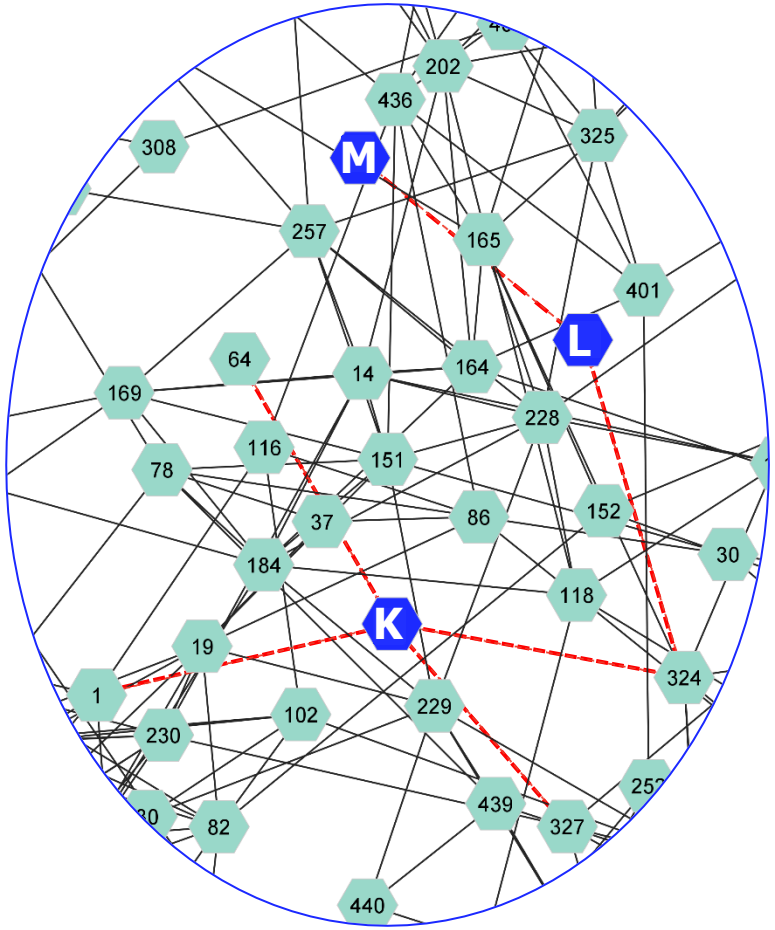


Isomers of $C_{19}H_{18}O_6$

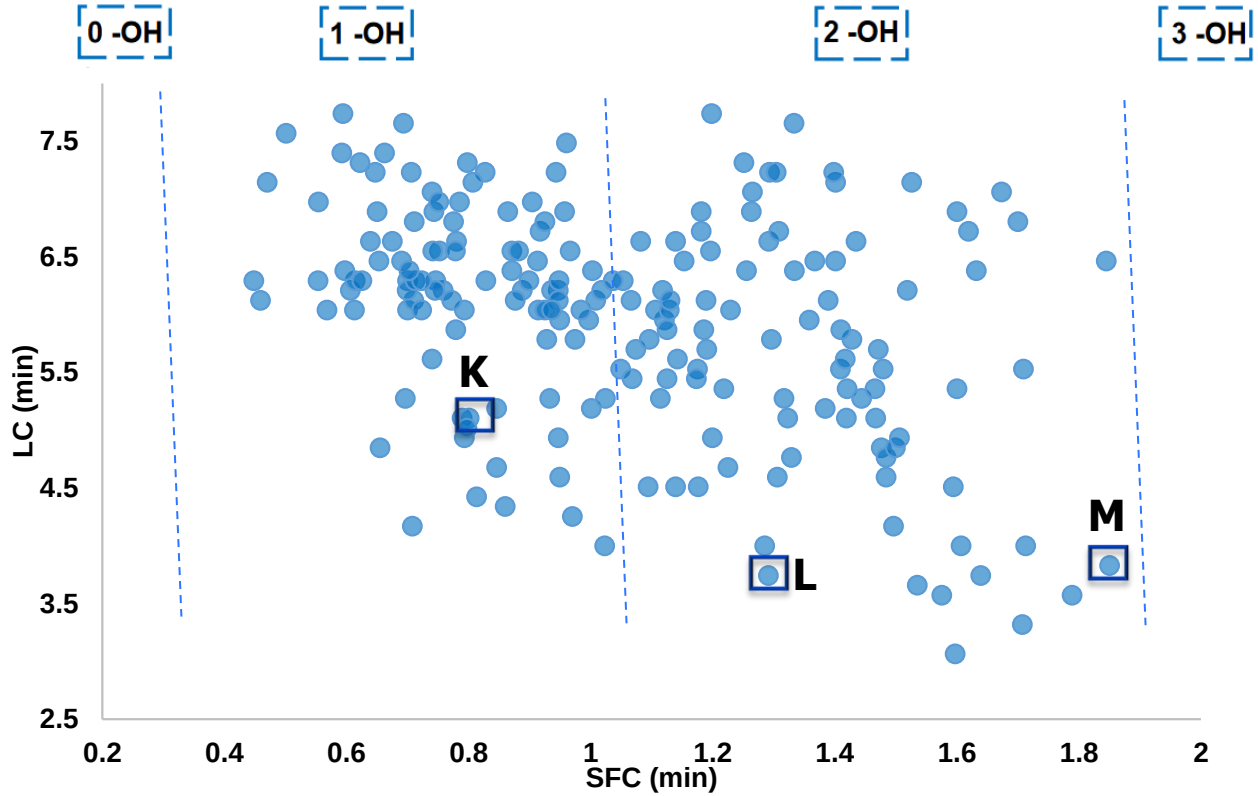




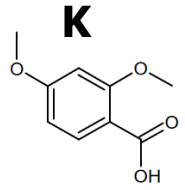
Feature-Based Molecular Network – FBMN

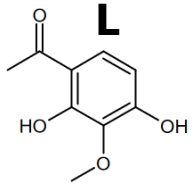


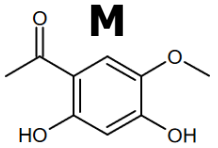
181.05009 *m/z*, $C_9H_{10}O_4$, DBE 5



SIRIUS



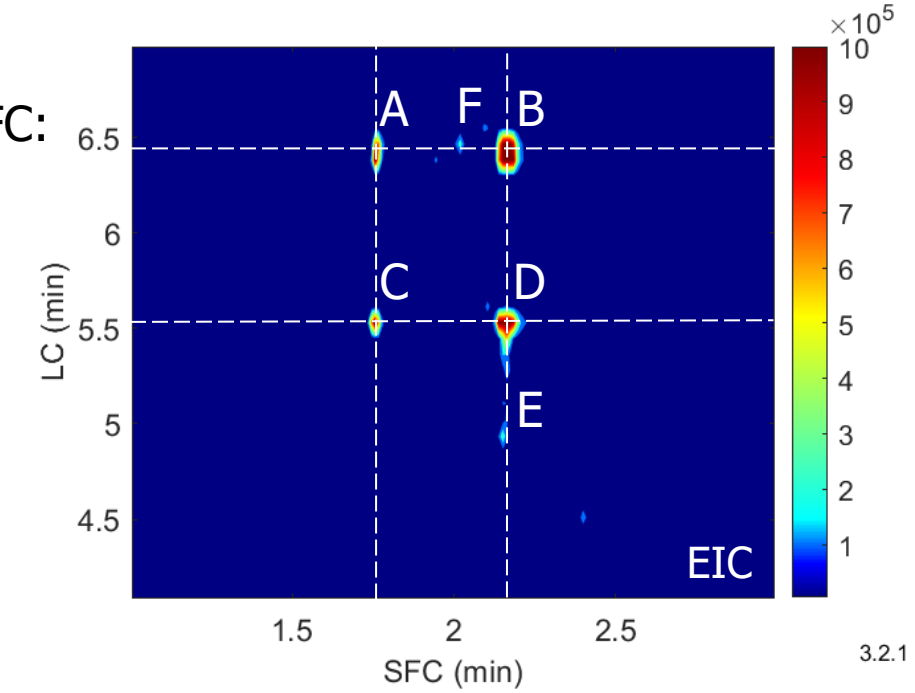




Potential structural candidates using SIRIUS annotation software

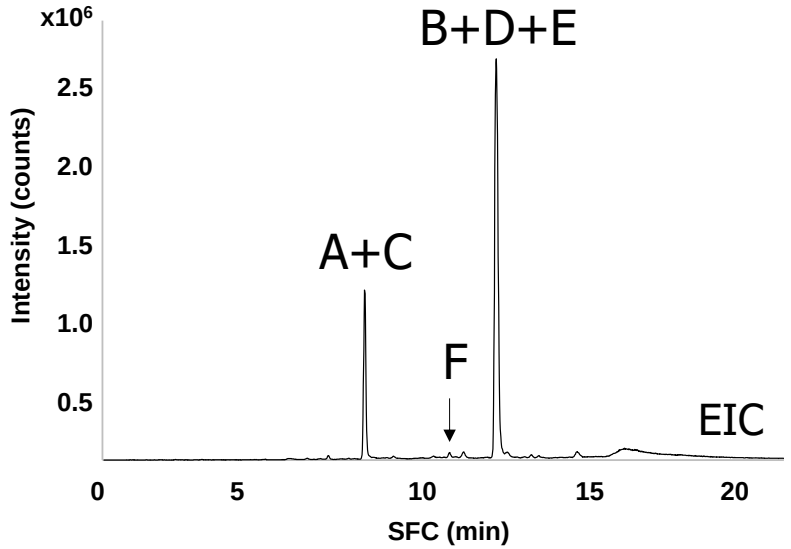
Feature-Based Molecular Network – FBMN

LC×SFC:

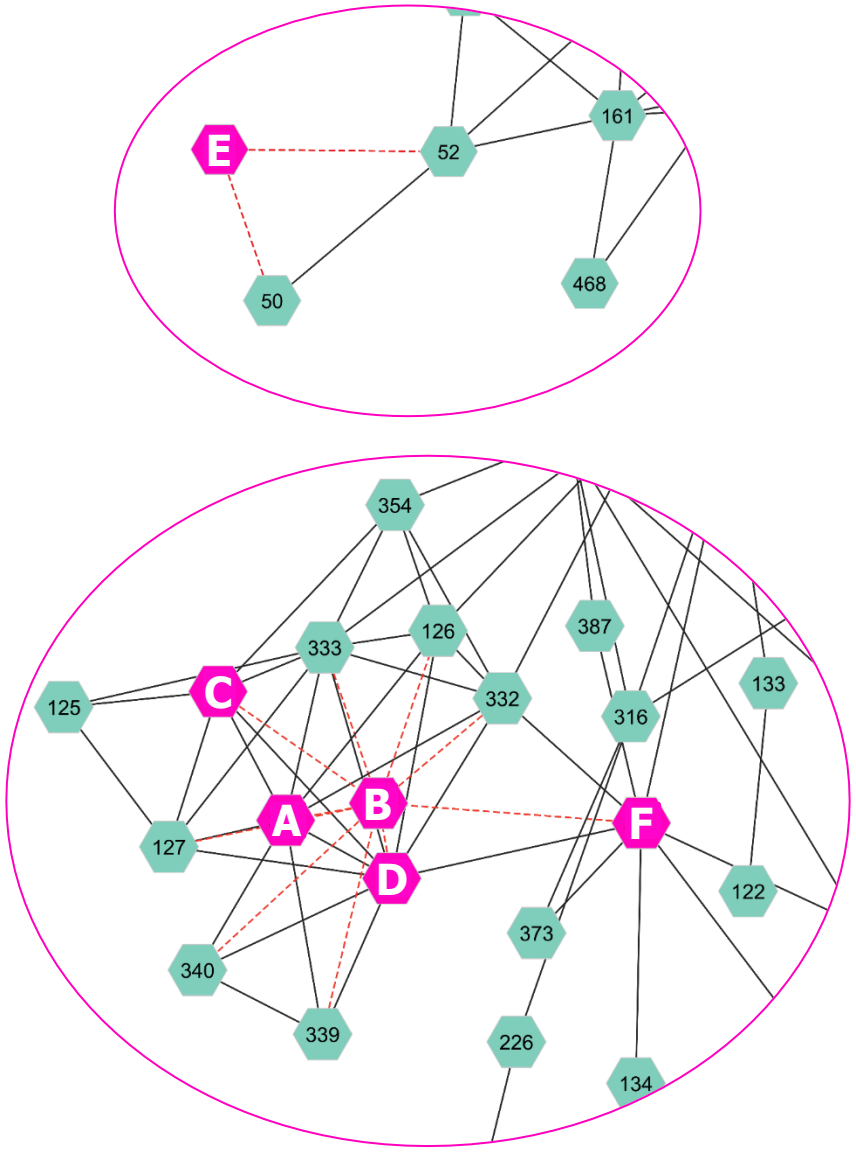


Number of major peaks: 4

SFC:



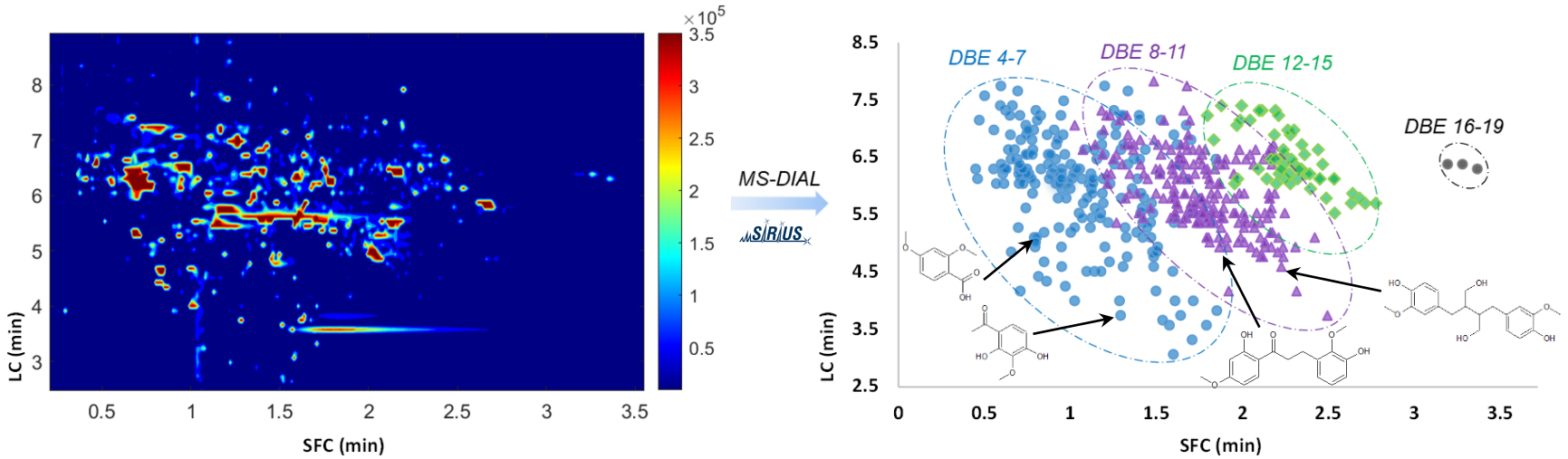
Number of major peaks: 2



341.10263 *m/z*, $C_{19}H_{18}O_6$, DBE 11

Off-line LC×SFC-HRMS/MS is an efficient method for the non-target analysis of lignin

- ✓ Compound **annotation** based on **4 criteria**
  - I. t_R in LC
  - II. t_R in SFC
  - III. Accurate mass
  - IV. Fragmentation pattern
- ✓ Good **separation of isomers**
- ✓ **Grouping of lignin compounds** on the **2D plots** based on their **DBE value**



A powerful two-dimensional chromatography method for the non-target analysis of depolymerised lignin. *Analytica Chimica Acta* **2023**, 342157
Tammekivi, E.; Batteau, M.; Laurenti, D.; Lilti, H.; Faure, K.

Thank you for your attention!

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