

Profiling phenolics compounds
in shea by 2D LC (RPLC × HILIC)
– cyclic ion mobility –
quadrupole time-of-flight
Valorizing sustainable shea

Nikoline J Nielsen, Oskar M Kronik, Romina
A Forte Neran, Tore K Ravn, Jan H
Christensen, André de Villiers

University of Copenhagen, AAK Denmark, Stellenbosch University

KØBENHAVNS UNIVERSITET



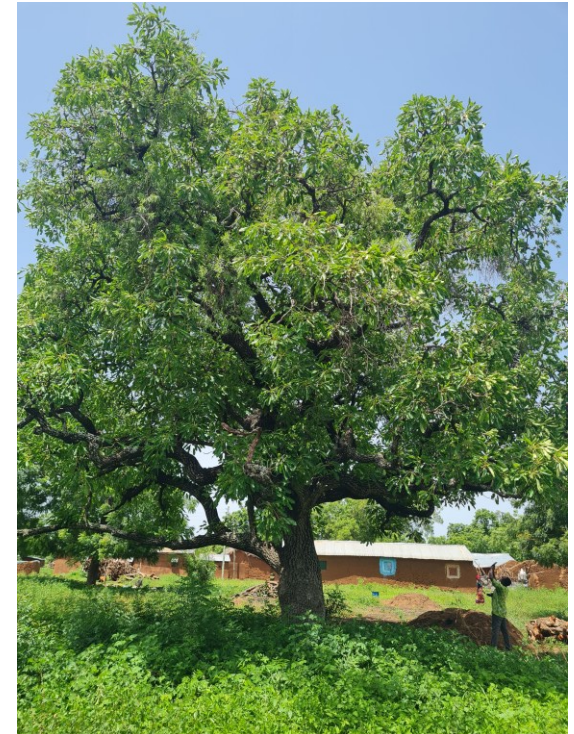
Shea

- Industrially, the triglyceride fraction is used as a cocoa butter replacement, in confectionary and other foods or as ingredient in personal care products



The shea kernel supply chain

- The shea tree (*Vitellaria paradoxa*) belonging to the *Sapotaceae* family
- Native to sub-Saharan Africa, distributed across 5000 km from Senegal to Ethiopia
- ~2 billion shea trees support ~18 million women
- Shea kernels are collected from these wild growing trees, and processed for preservation
- For each unit of kernels produced, 1.04 CO₂ equivalents are captured
- Shea is considered a sustainable alternative to palm-oil



Aim

- Link traditional shea quality markers (content of **triglycerides** and **free fatty acids**, oil extractability) to supply chain parameters
- Map secondary metabolites with beneficial properties that can be conveyed to kernels and shea products, or recovered from waste-streams

This study:

- Shea was recently shown to contain high levels of phenolics (>140 mg/100 g)
- To profile semi-polar secondary metabolites, primarily phenolics, in shea kernels and shea nut shells
- Investigate how the 4D platform: RPLC × HILIC × cIMS × HRMS could contribute to resolving and identifying metabolites from complex samples when using a DIA-strategy

RPLC and (RPLC × HILIC)

1D RPLC



Zorbax SB-C18 (2.1 mm x 100 mm, 1.8 µm)

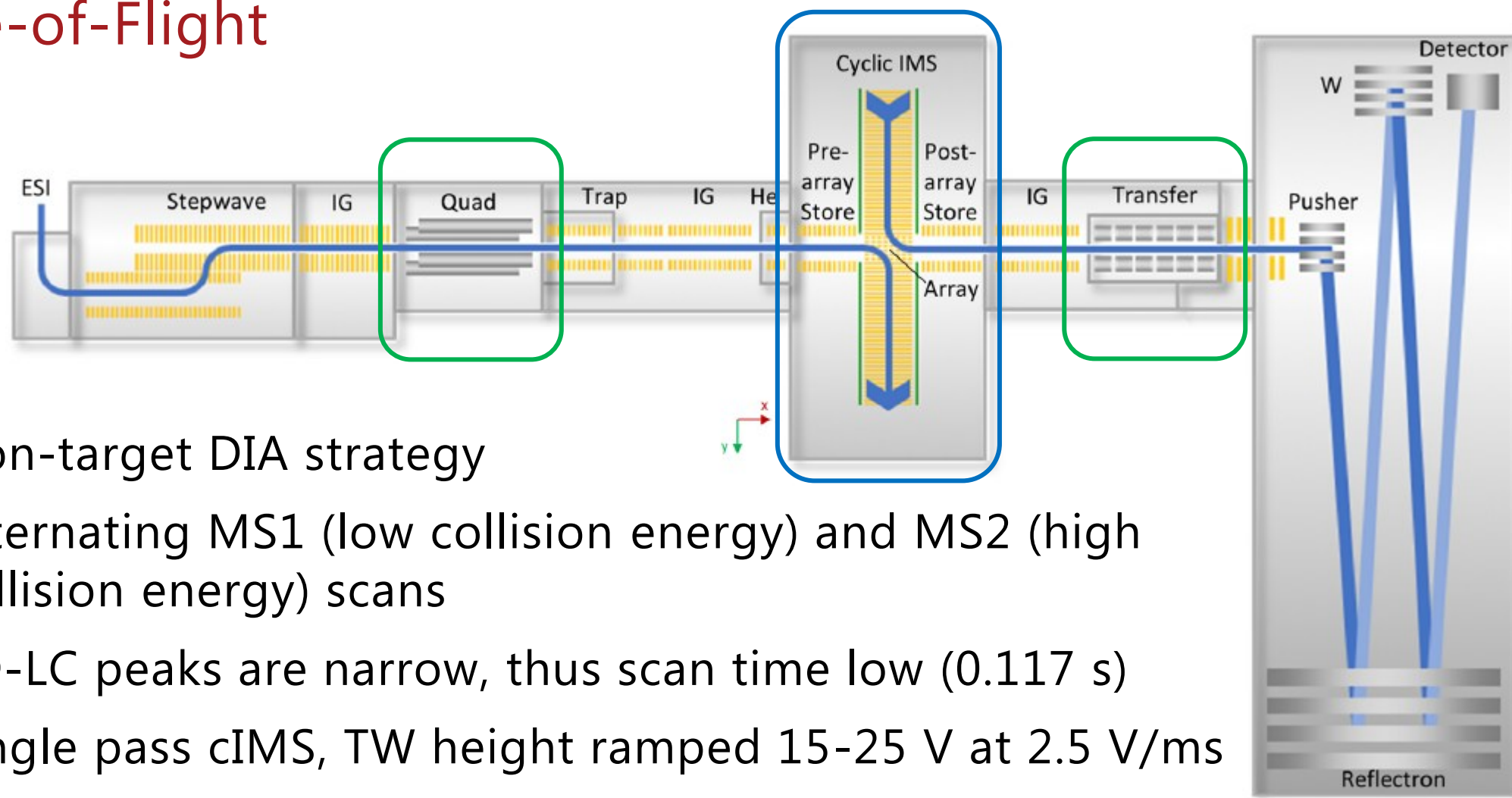
Flow 0.3 mL/min; Temperature 35°C

MPs: 0.1% formic acid in water and acetonitrile

1D optimized using
the predictive kinetic
optimization tool

Muller et al. (2018). DOI:
<https://doi.org/10.1016/j.chroma.2018.08.004>.

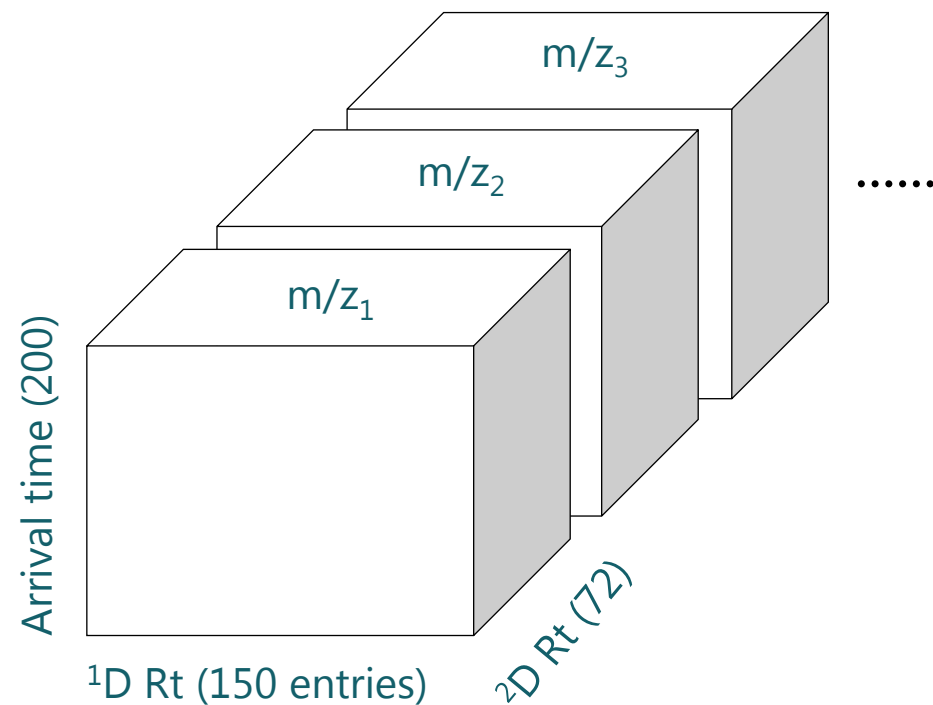
Quadrupole – cyclic Ion Mobility Spectrometry – quadrupole – Time-of-Flight



- Non-target DIA strategy
- Alternating MS1 (low collision energy) and MS2 (high collision energy) scans
- 2D-LC peaks are narrow, thus scan time low (0.117 s)
- Single pass cIMS, TW height ramped 15-25 V at 2.5 V/ms

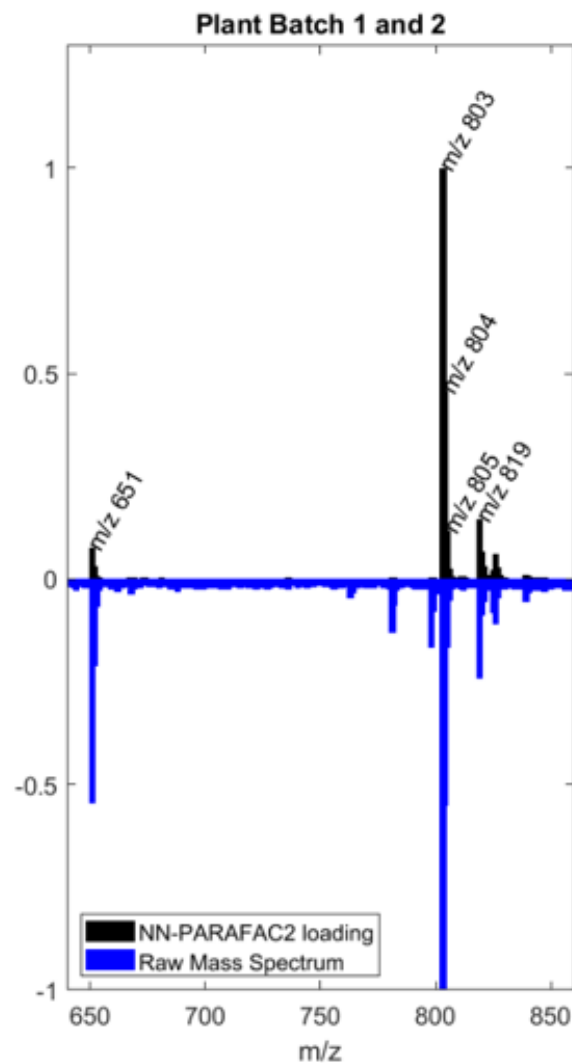
RPLC × HILIC × IMS × HRMS data

MS1: Low collision energy in the transfer cell (4 V)



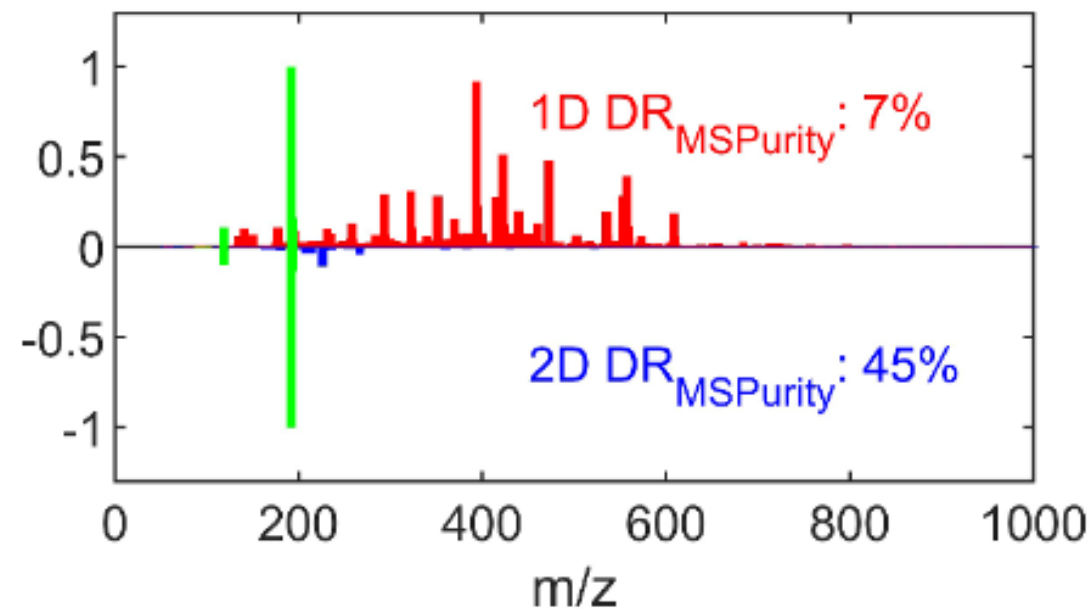
Current means to improve DIA mass spectral purity

- Curve resolution



Kronik et al. (2022). DOI: 10.1016/j.chroma.2022.463501

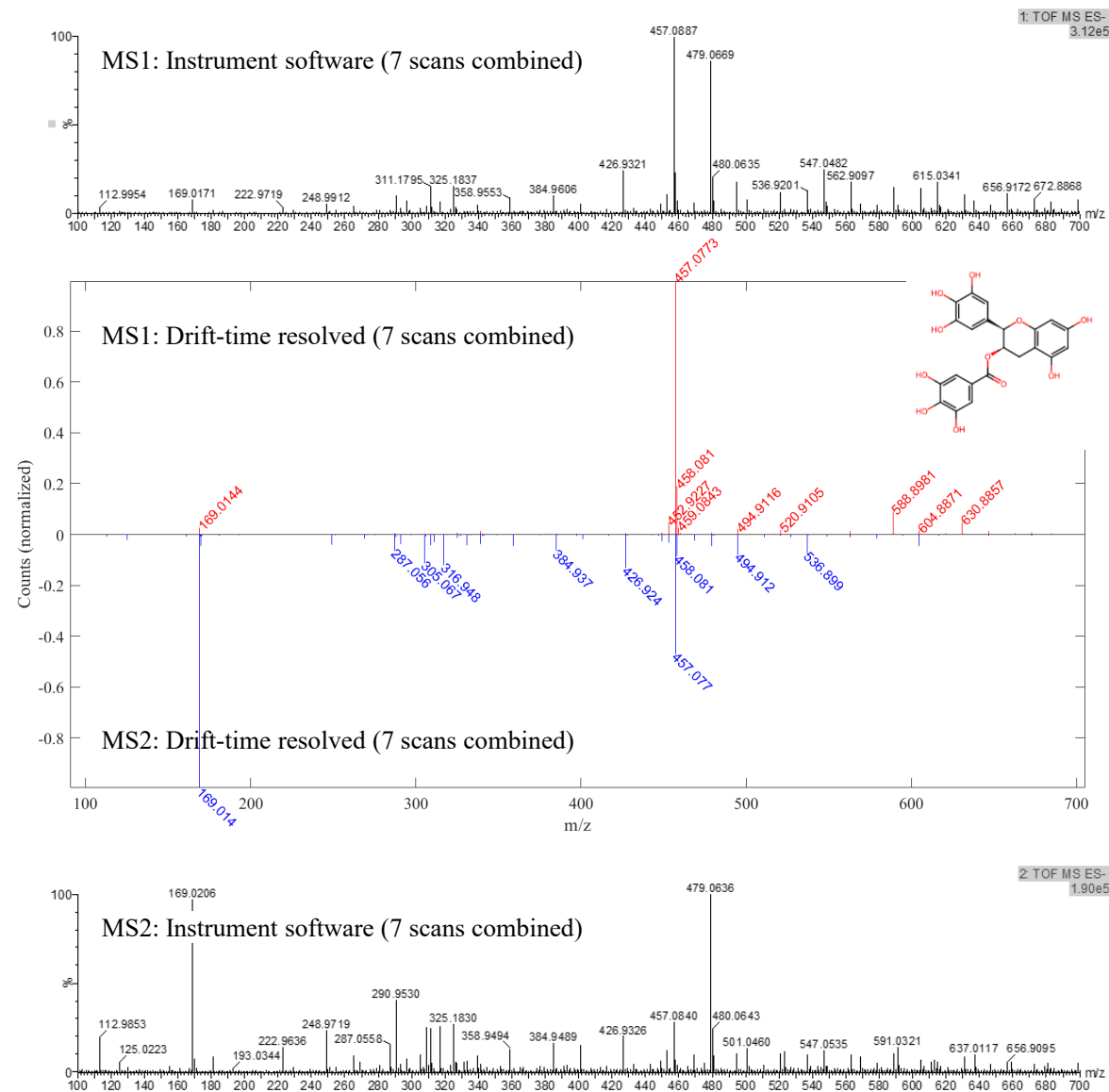
- Improve chromatographic resolution



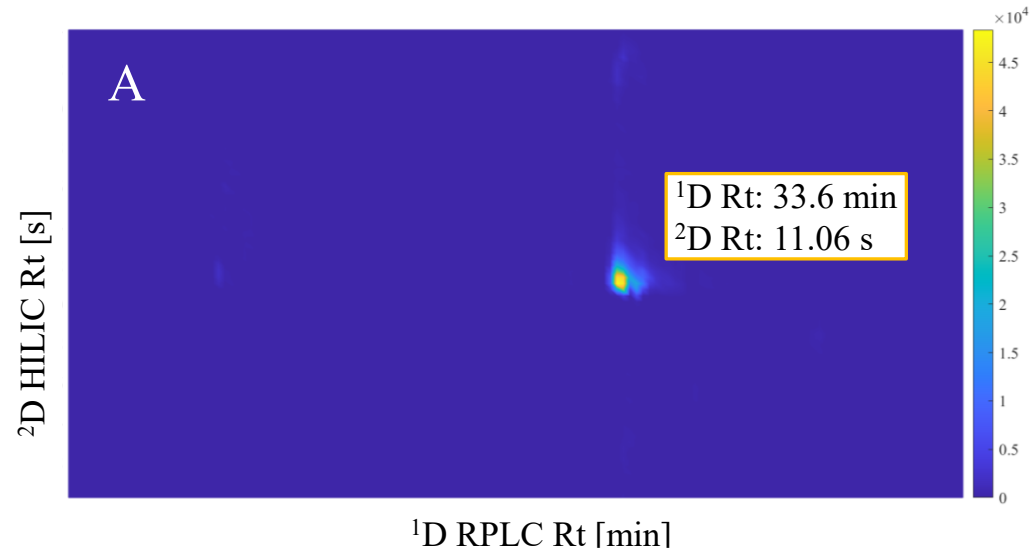
Kronik et al. (2024). DOI: 10.1016/j.chroma.2024.465079

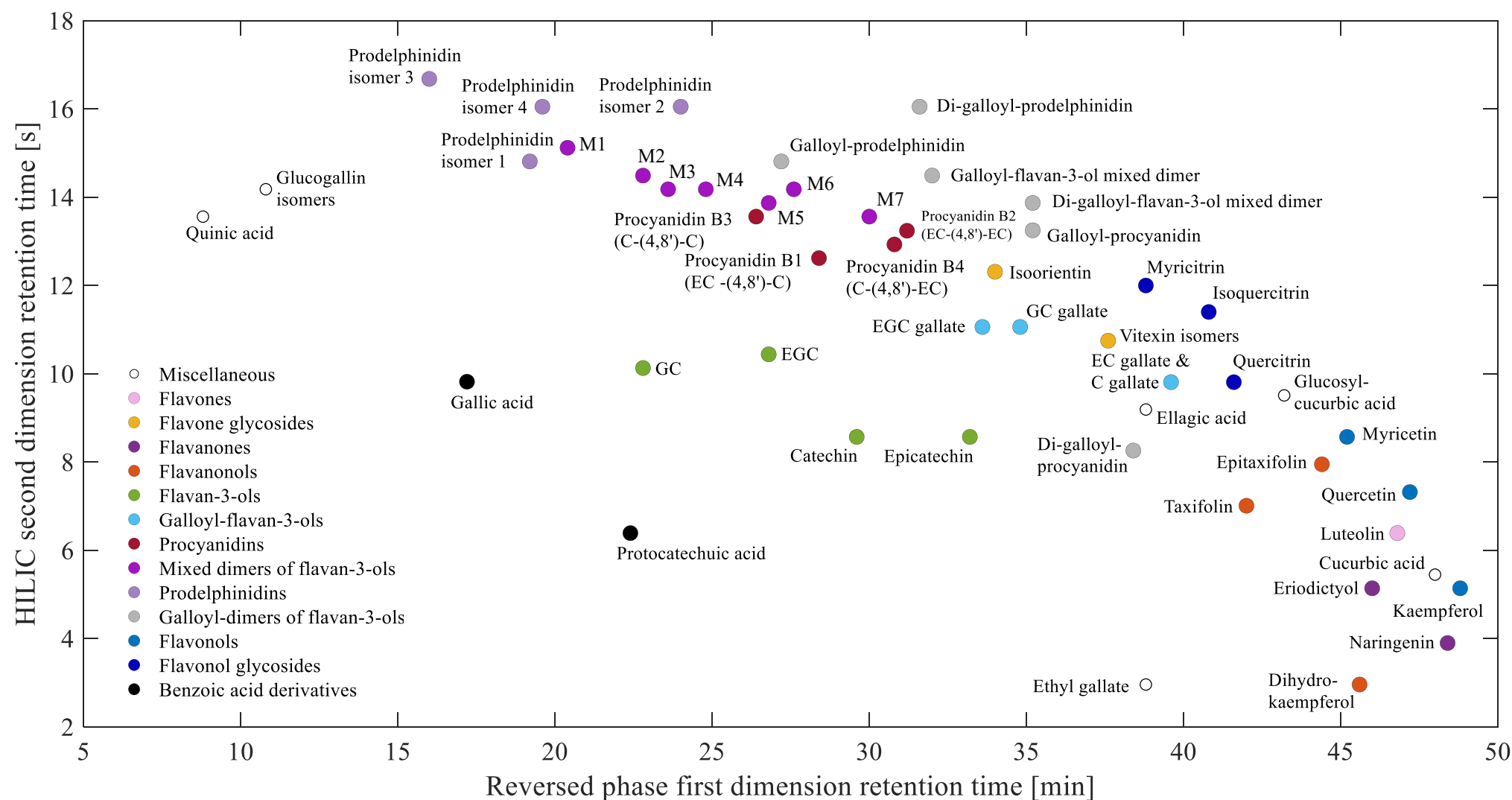
Can IMS improve the mass spectral purity of DIA data?

- Even single pass cIMS is powerful
- But instrument software forces a collapse of either the IMS dimension or the chromatographic dimension when extracting mass spectra, so we would never see that
- When we extract mass spectra from the chromatographically- and arrival-time resolved data, then DIA spectra are unusually clean!



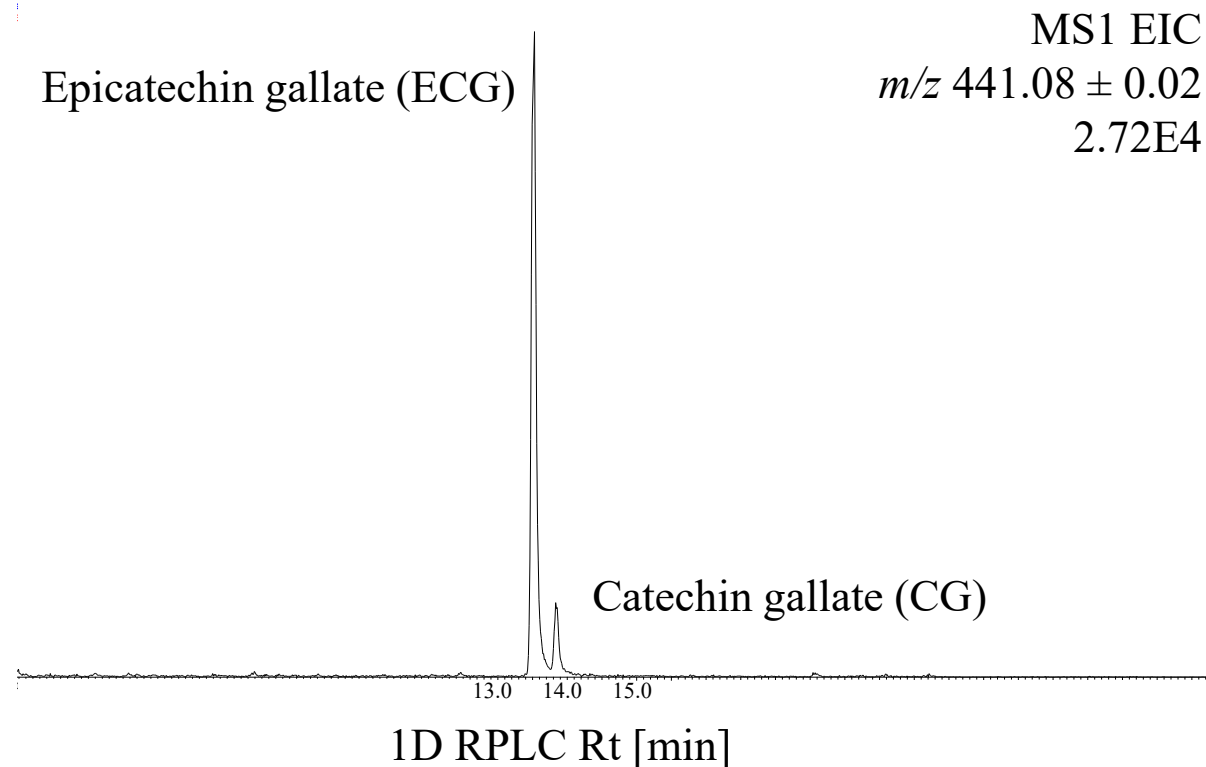
Output from the customized tool to process 4D data



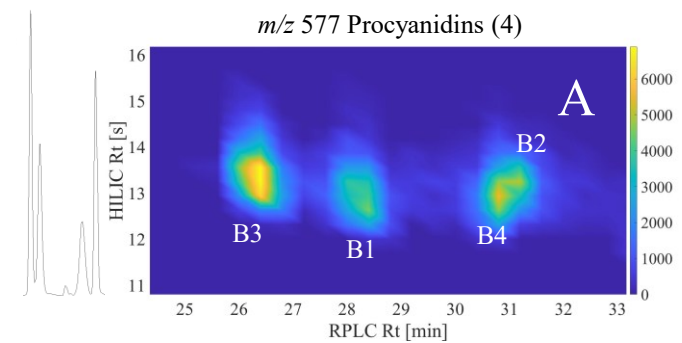


RPLC separation was better in 1D

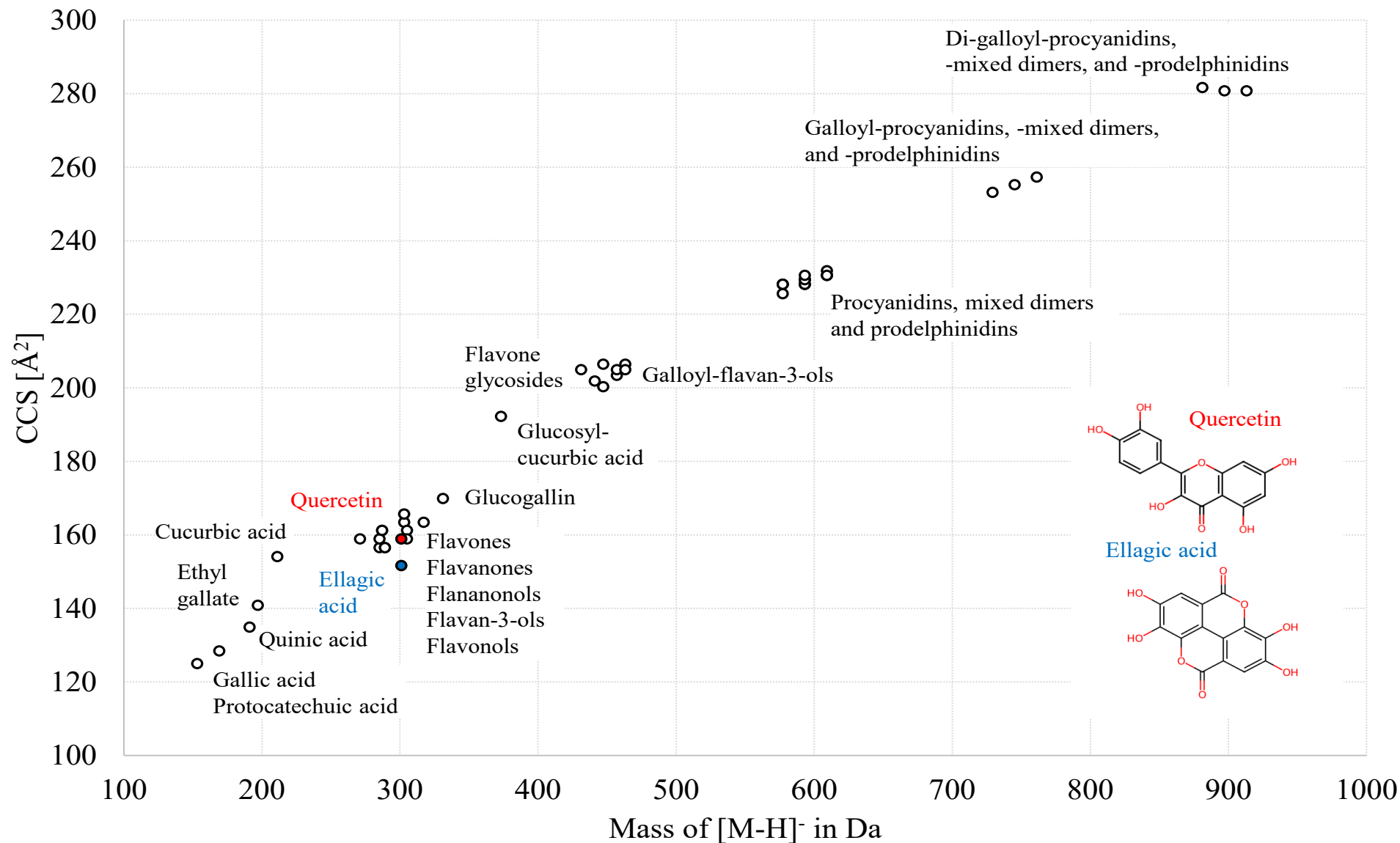
- **No modulation, so no remixing of closely eluting peaks from RPLC**
- Less extra column peak broadening in this 1D set-up



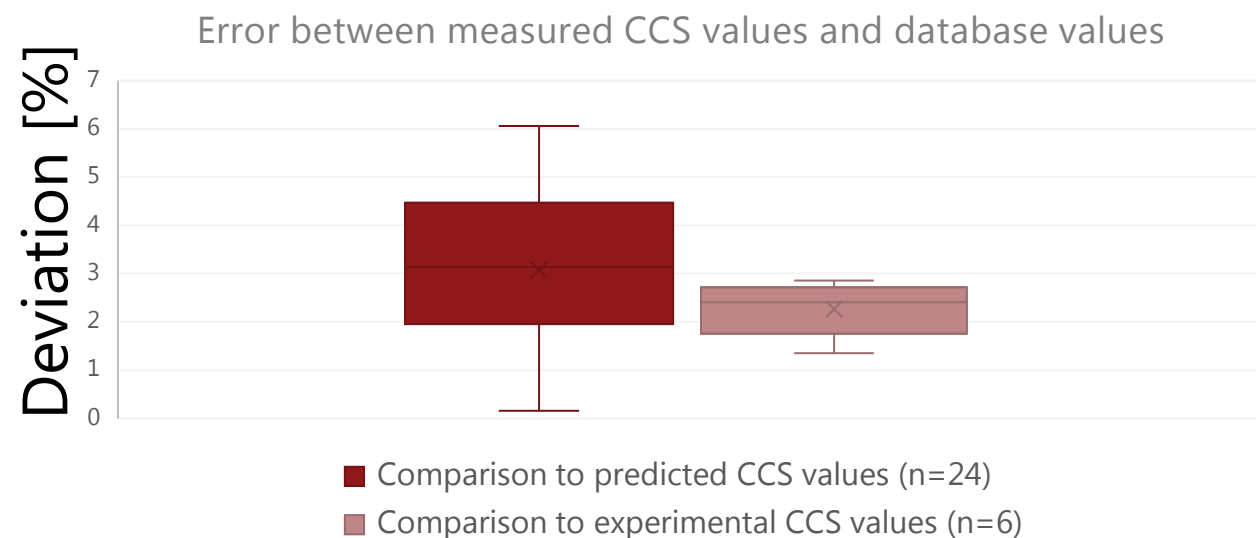
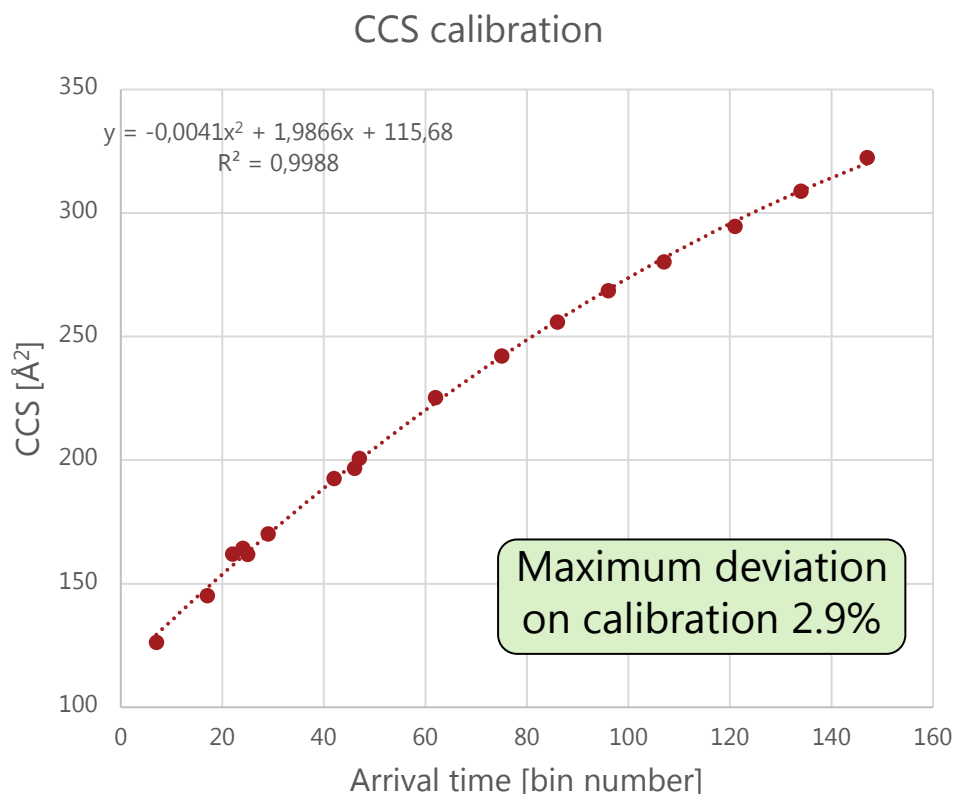
Proanthocyanidins



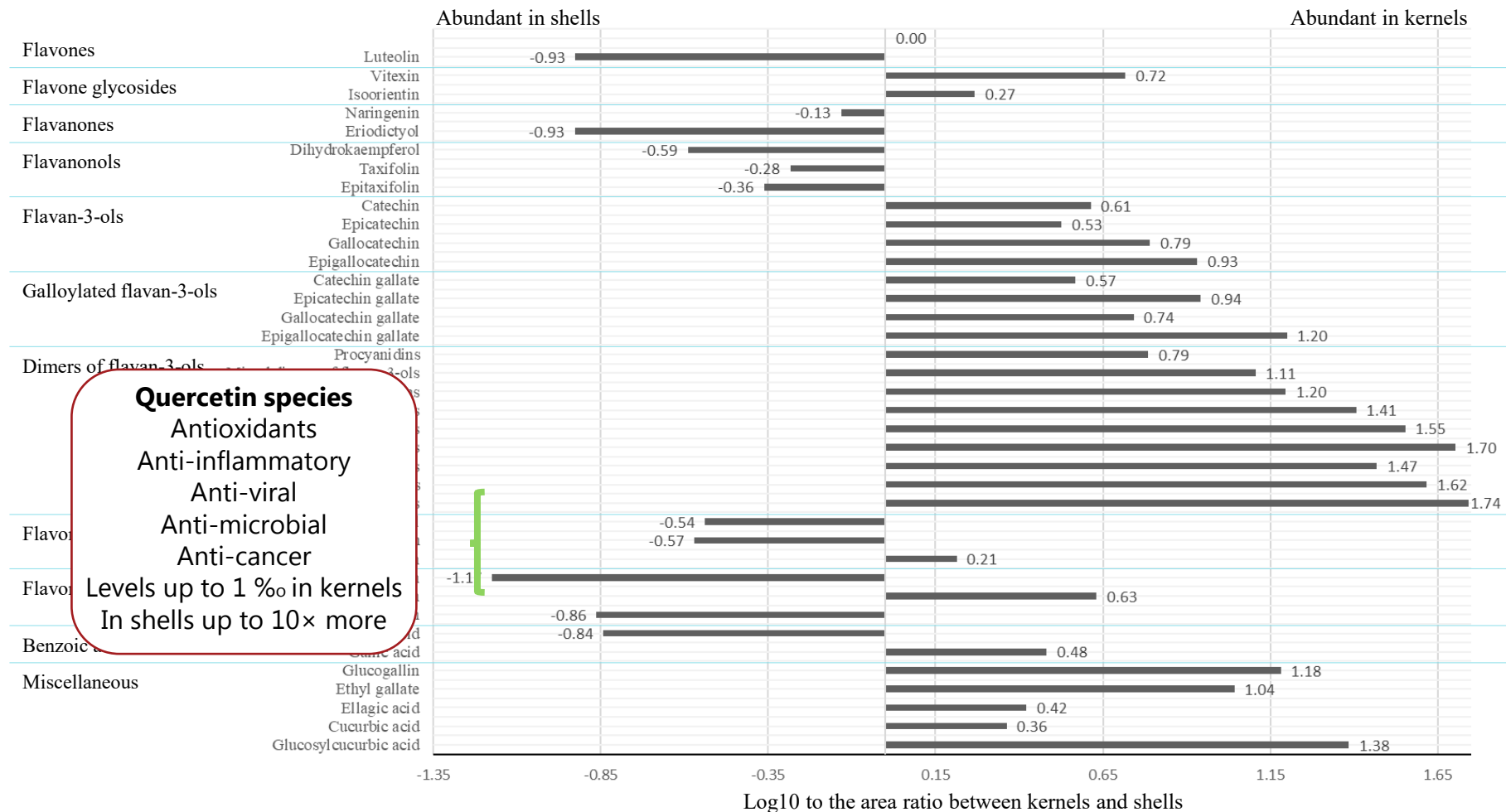
Collisional cross sections (CCS) as confirmatory evidence



CCS calibration and errors when matching against experimental and predicted CCS values from AllCCS database



Differences in relative response between kernels and shells



Flavan-3-ols and proanthocyanidins
 Antioxidants
 Cardio-protective
 Neuro-protective
 Immuno-modulatory
 Lipid-lowering
 Anti-obesity
 Anti-cancer
 Anti-microbial

Quercetin species
 Antioxidants
 Anti-inflammatory
 Anti-viral
 Anti-microbial
 Anti-cancer
 Levels up to 1 ‰ in kernels
 In shells up to 10× more

Conclusions

- Samples were separated in 4 dimensions (RPLC × HILIC × IMS × HRMS)
- A customized tool was necessary to access the fully resolved data i.e. the high purity mass spectra
- 2D-LC combined with single pass cyclic IMS significantly improved DIA mass spectral purity
- Single pass cIMS could resolve near-isobars and isobars in certain cases
- CCS values matched experimental CCS values from a database well (< 2.9 % error), larger errors observed when matching toward predicted CCS values (up to 6.0 % error)
- 50 metabolites identified in shea using RPLC × HILIC-ESI-Q-cIMS-qTOF and a DIA strategy
- Epitaxifolin, 8 proanthocyanidins, isoorientin and isoquercitrin identified in shea for the first time

Acknowledgements



André de Villiers

Oskar M Kronik Tore K Ravn



Romina A Forte Neran



Jan Christensen

Ella Walbeogo



Stellenbosch
UNIVERSITY
IYUNIVESITHI
UNIVERSITEIT





Tuesday Feb 4th

01:30-01:40

PL10 Oskar Munk Kronik: Data processing workflows for non-target screening on LC×LC-HRMS data: Ready to go?

Poster:

P-37: Nadine Gawlitta: The evaluation of GC×GC-QTOF data by pixel-based analysis: A tutorial

Customized data processing

From instrument software (MassLynx) to flexible computing environment

ProteoWizard (import raw)	Convert format from .raw to .mzML using the MSConvert GUI
---------------------------	---

Converting continuum mode data to centroid data to reduce size of data

R (import mzML)	Using functions 'peaks' (mzR package), 'createspectrum' and 'detectpeaks' (MALDIquant package)
-----------------	--

Lock mass correct centroids across each mass spectrum

Matlab (import NetCDF)	$m/z_{corrected} = \left(1 + \frac{m/z_{lock\ mass,\ true} - m/z_{lock\ mass,\ exp}}{m/z_{lock\ mass,\ true}} \right) \times m/z_{uncorrected}$
------------------------	--

Detect Regions of Interests (ROIs)

Matlab	analytical and bioanalytical chemistry https://doi.org/10.1007/s00216-024-05718-7 RESEARCH PAPER A fast region of interest algorithm for efficient data compression and improved peak detection in high-resolution mass spectrometry Oskar Munk Kronik¹ · Jan H. Christensen¹ · Nikoline Juul Nielsen¹ · Selina Tisler¹ · Giorgio Tomasi¹ Modified to detect peak behaviour in the arrival time distribution
--------	---