Development of a MULTI-2D LC×LC-ESI/TPI-DUAL SOURCE-QTOF-MS for the analysis of complex samples

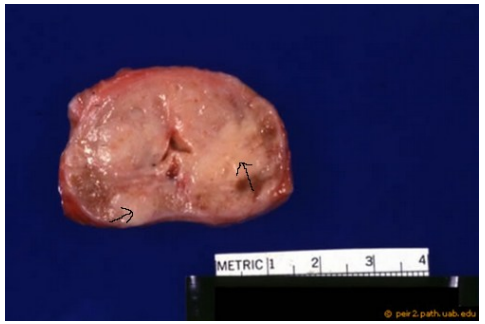
Oliver J. Schmitz

Labs of the AAC group at University of Duisburg-Essen

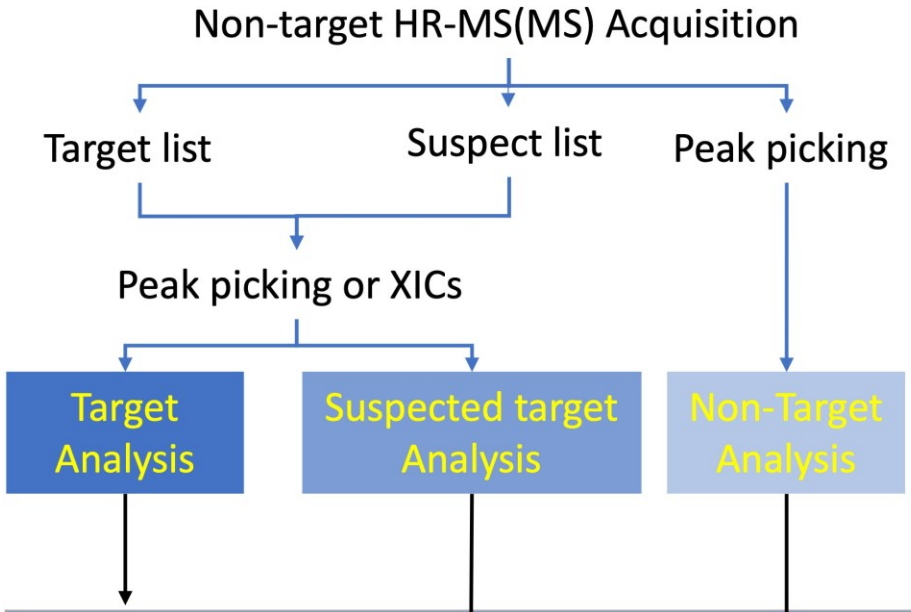
Agilent

Members 2024

Complex samples



Workflow of non-targeted analysis (NTA) at AAC: Identification level

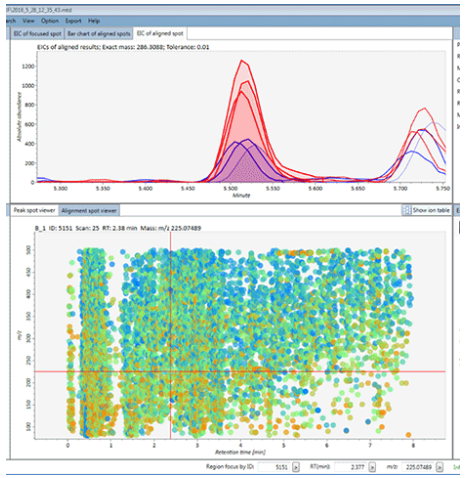


How can we reach these levels?

Start	**Level 1**	**Confirmed Structure** (by reference standard)
	Level 2	**Probable Structure** (by library/diagnostic evidence)
Start	**Level 3**	**Tentative Cancidate** (suspect, substructure, class)
	Level 4	**Unequivocal Molecular Formula** (insufficient structural evidence)
Start	**Level 5**	**Mass of Interest** (multiple detection, trends …)

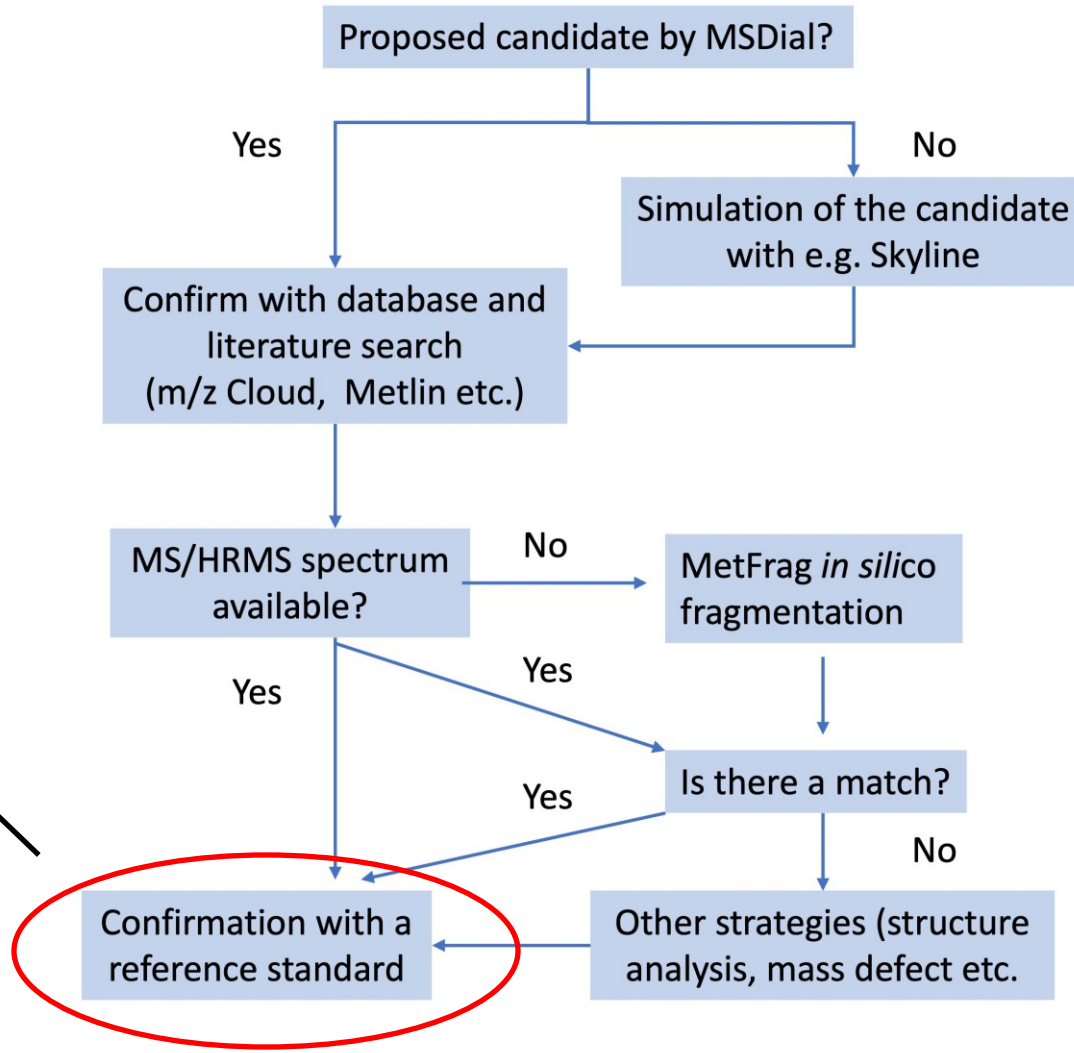
E. L. Schymanski et al. **Anal Bioanal Chem** (2015) 407:6237–6255

Workflow of a non-targeted analysis



But we need an idea of what compound it is to get the right standard!

I believe that first of all we need a good separation so that we can also separate isomeric compounds and thus differentiate between them. And secondly, we need an idea of what kind of substance it might be.

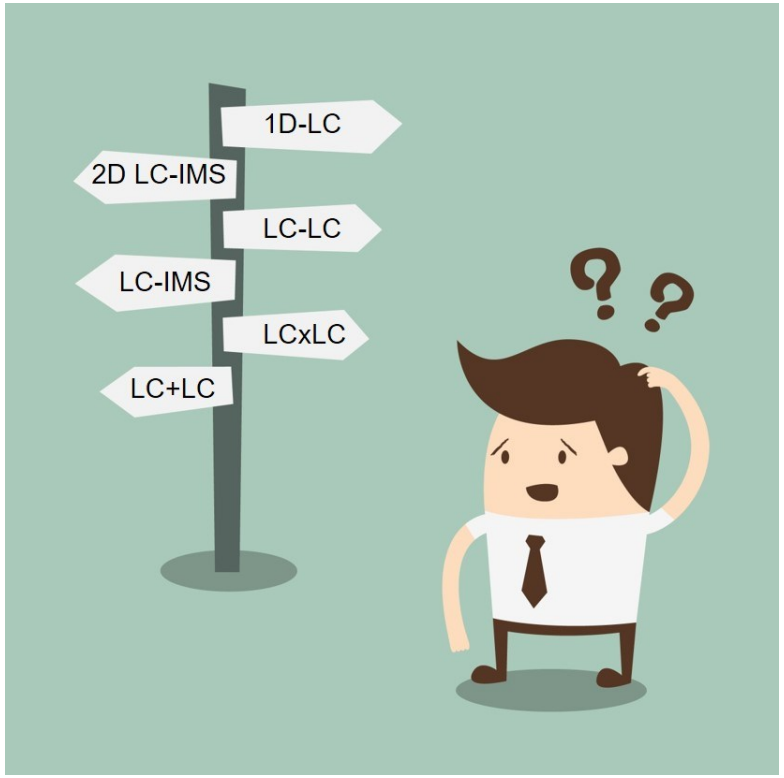


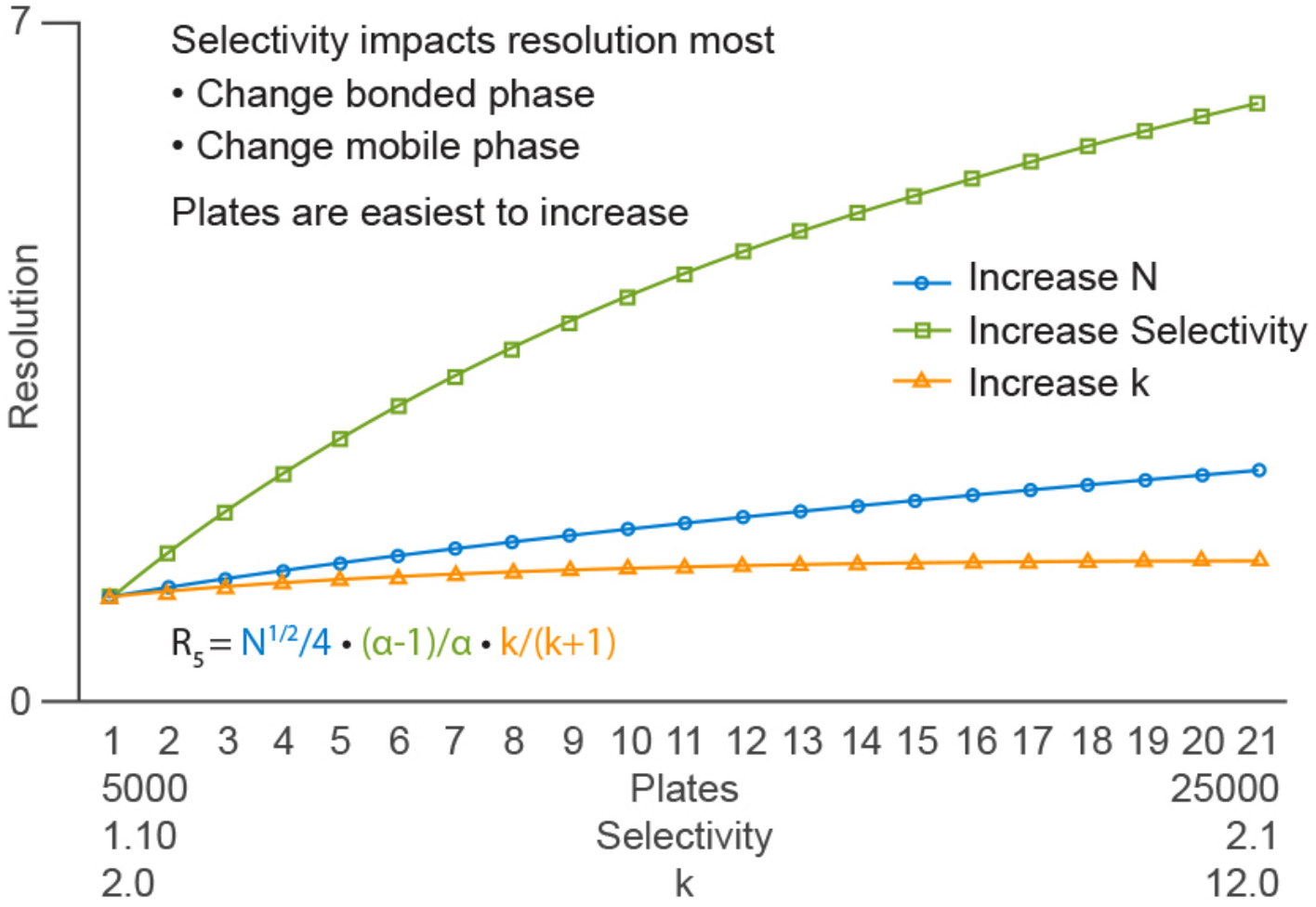
Sven Meckelmann

How can we get a good separation?

$$R_s = \frac{1}{4} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k}{k+1}\right) \cdot \sqrt{N}$$

Selectivity Retention Efficiency



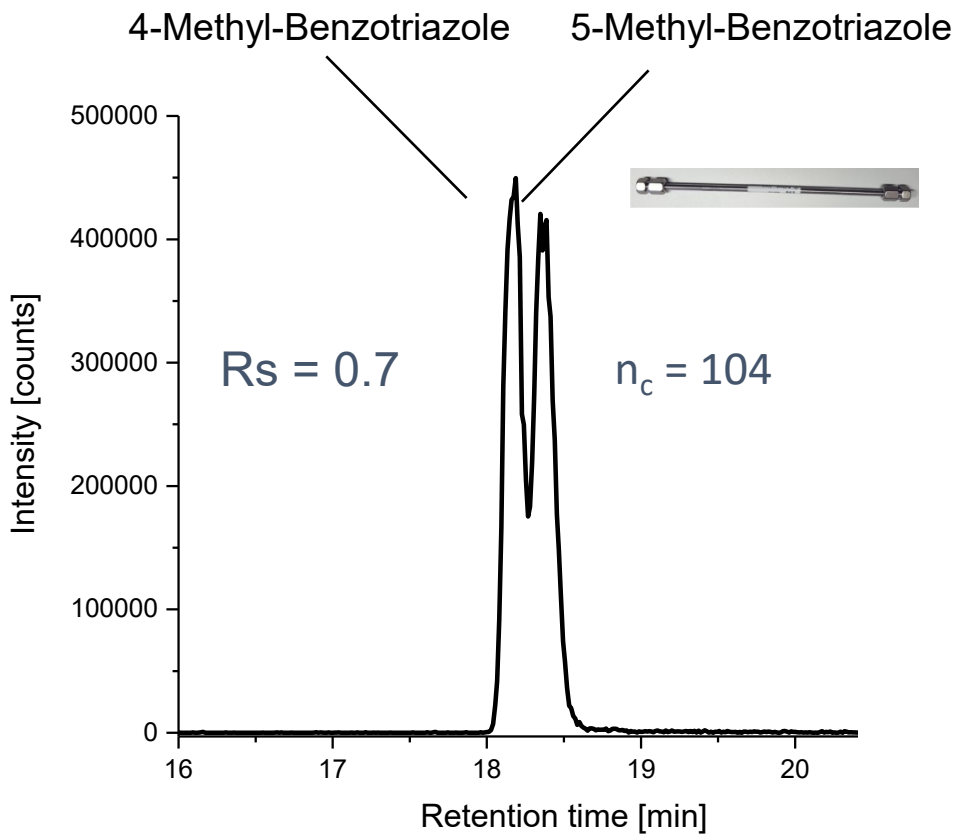


P. Sandra, **Journal of High Resolution Chromatography** (1989) 12:82-86

How can we solve the problem?

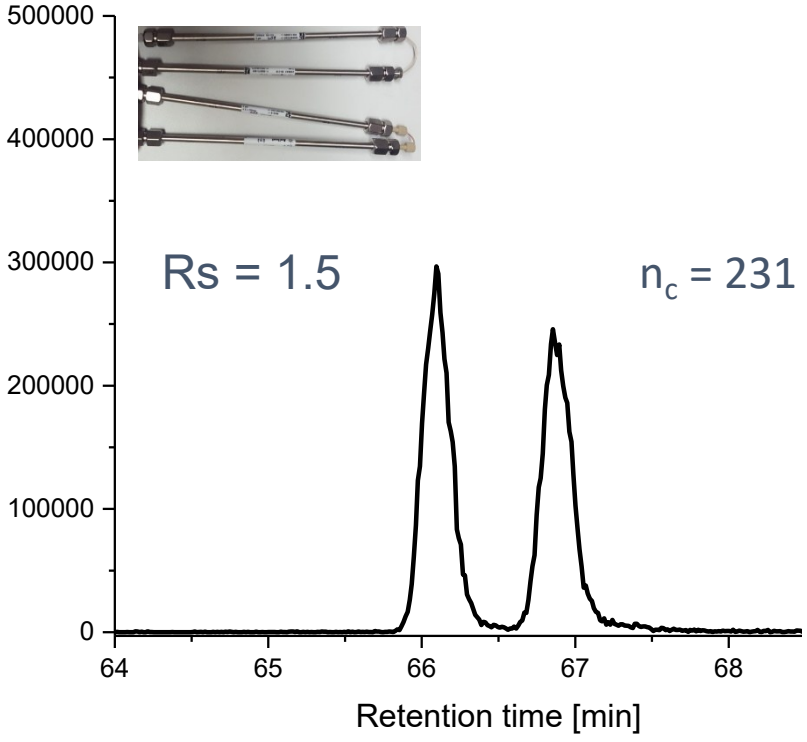
$$n_c = 1 + \frac{t_G}{w}$$

30 Min Gradient, 250 x 4.6 mm; 5 µm



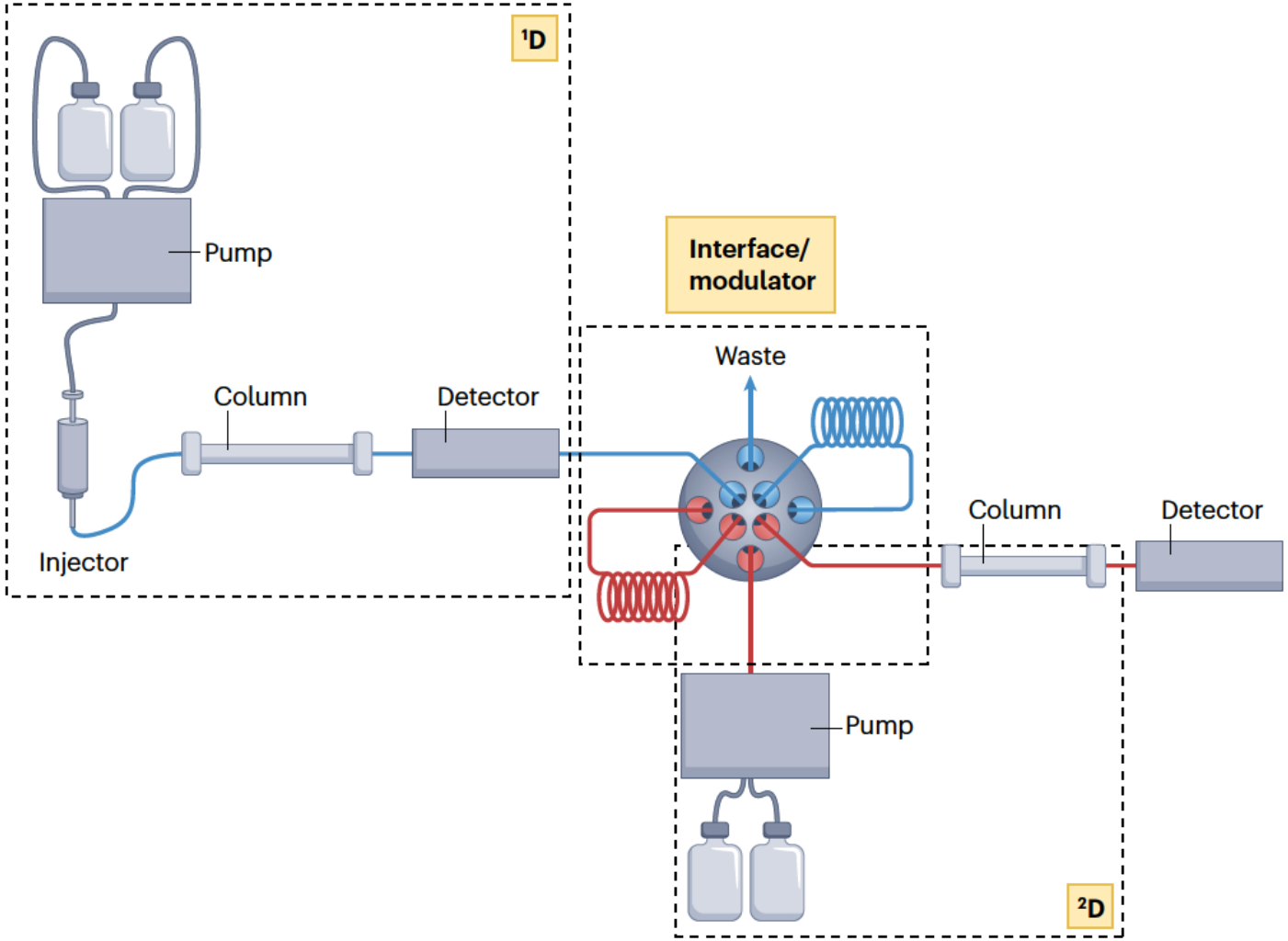
$w_{0,5}$ = 0.11/ 0.12 min

120 Min Gradient, 1000 x 4.6 mm; 5 µm



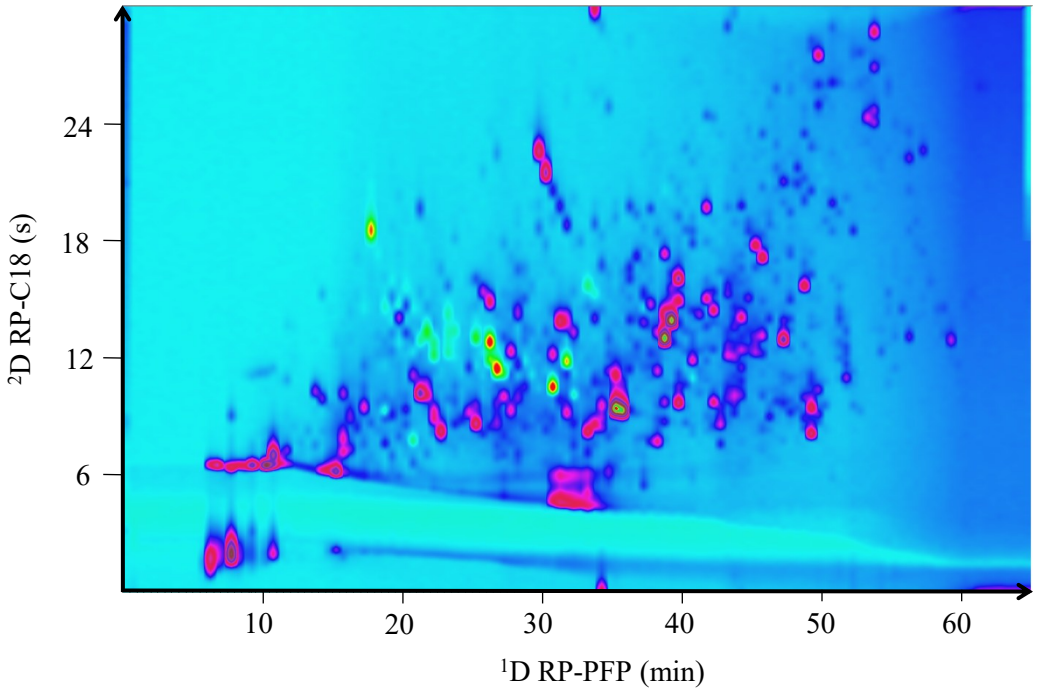
$w_{0,5}$ = 0.20/ 0.19 min

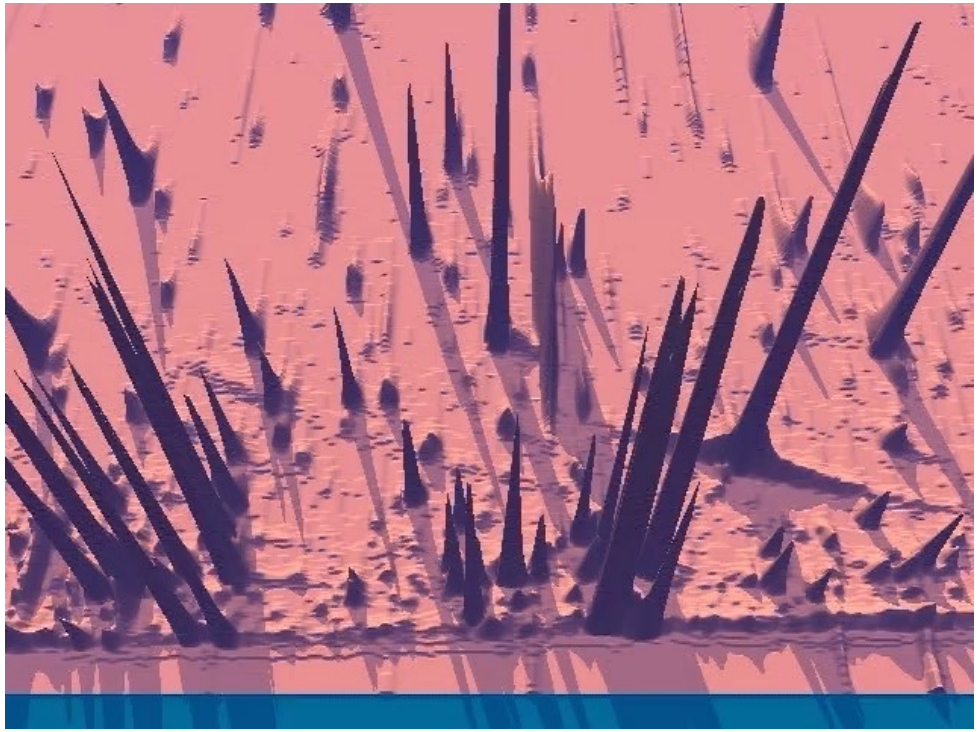
Principle of comprehensive two-dimensional LC (LC × LC)



L. Mondello, P. Dugo, P. Donato, M. Herrero, L. Montero, O. J. Schmitz, **Nature Reviews Methods Primers** (2023) 3:86

Exemplary contour plot of LC × LC and GC × GC





L. Montero, S. Meckelmann, H. Kim, J. F. Ayala-Cabrera, O. J. Schmitz, **Anal Bioanal Chem** (2020) 414:5445-5459; video from J. Dallüge (in reality it is a GCxGC-contour plot)

Bayesian optimization of separation gradients of LC × LC and Multi-^{2}D LC × LC analysis

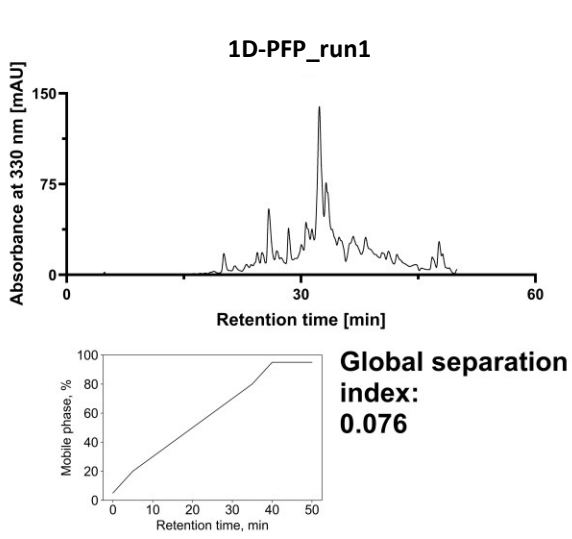
- 2D-LC method development as a multi-variate approach requires in-depth knowledge and experience
- Demonstrating the applicability of this beginner friendly tool to optimize elution gradients
- Column screening and optimization for the ^{1}D and ^{2}D before coupling to LC × LC

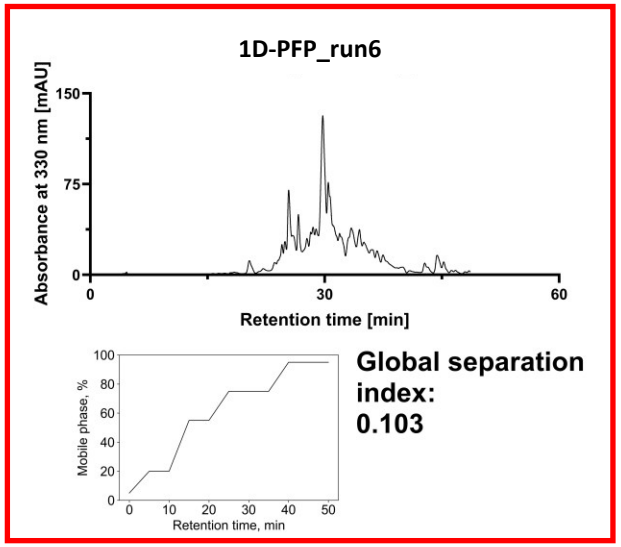
Tao Huan, UBC

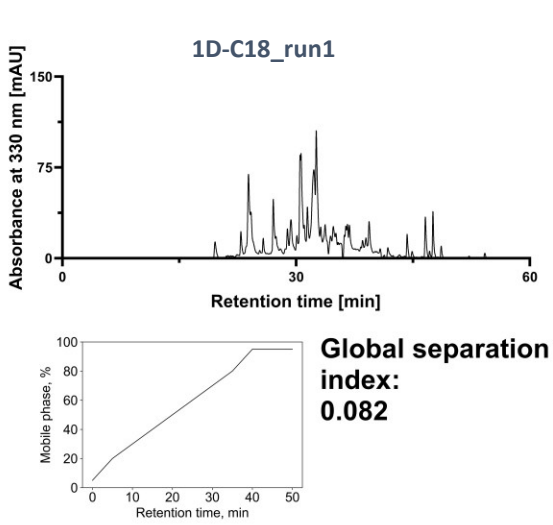
^{1}D columns	**^{2}D columns**
C18 (150 x 2.1 mm, 1.7 µm)	C18 (50 x 4.6 mm, 2.6 µm)
PFP (150 x 2.1 mm, 1.7 µm)	Polar C18 (50 x 4.6 mm, 2.6 µm)
Phenyl-hexyl (150 x 2.1 mm, 1.7 µm)	C8 (50 x 4.6 mm, 2.6 µm)
Cyano (150 x 2 mm, 3 µm)	HILIC (50 x 4.6 mm, 2.6 µm)
Amino (150 x 2 mm, 3 µm)	ZIC-HILIC (50 x 4.6 mm, 5 µm)
HILIC (150 x 2.1 mm, 2.6 µm)	

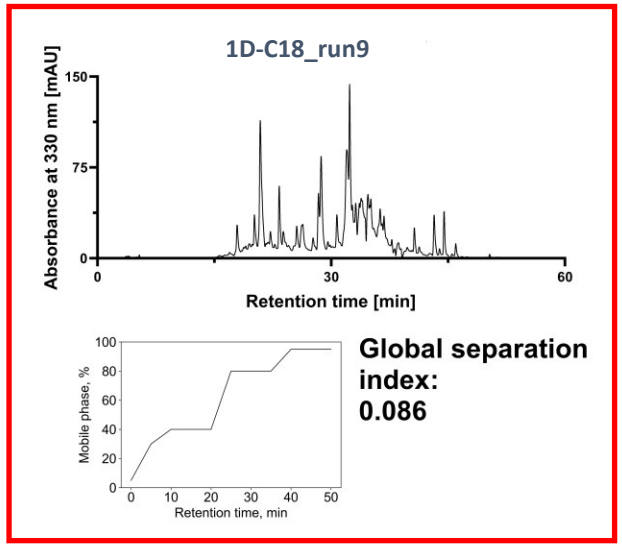
The algorithm tries to separate the *n* (e.g. 100-500) most abundant features as best as possible.

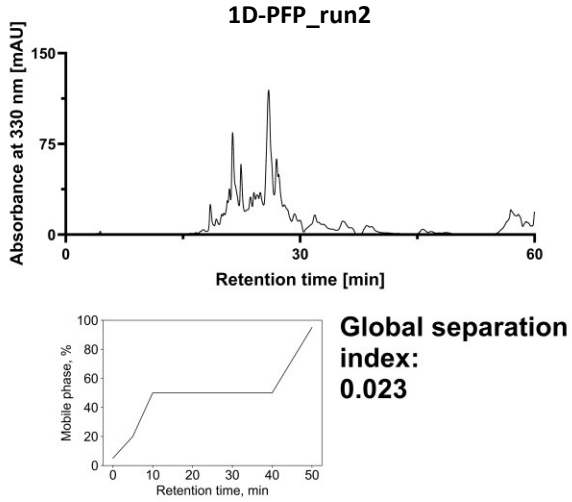
Column optimization for the ^{1}D - PFP and C18

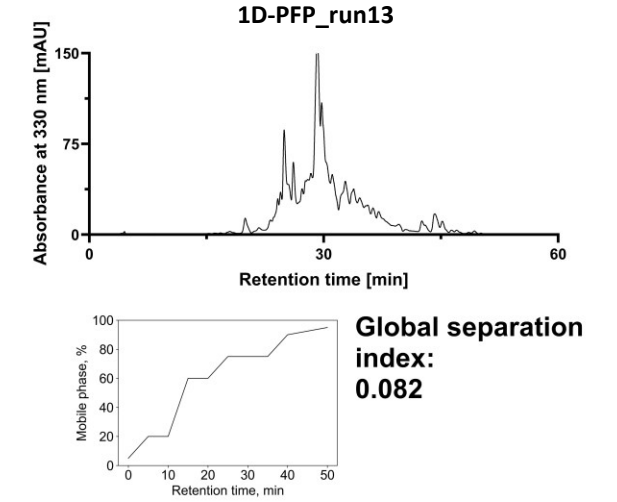


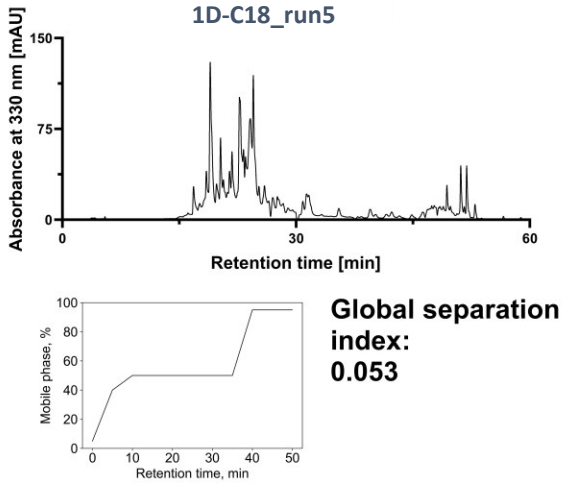


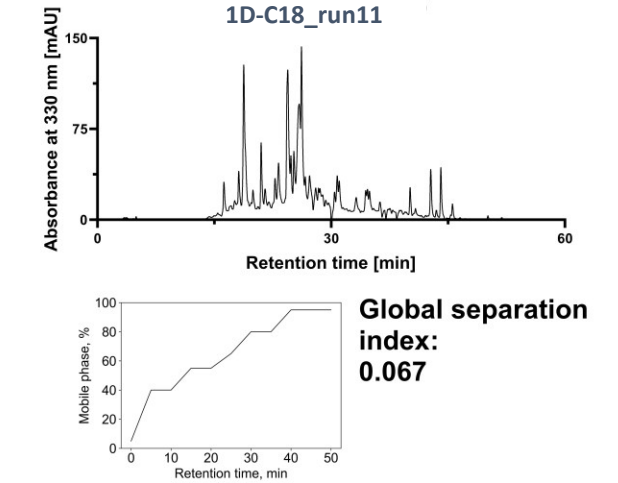








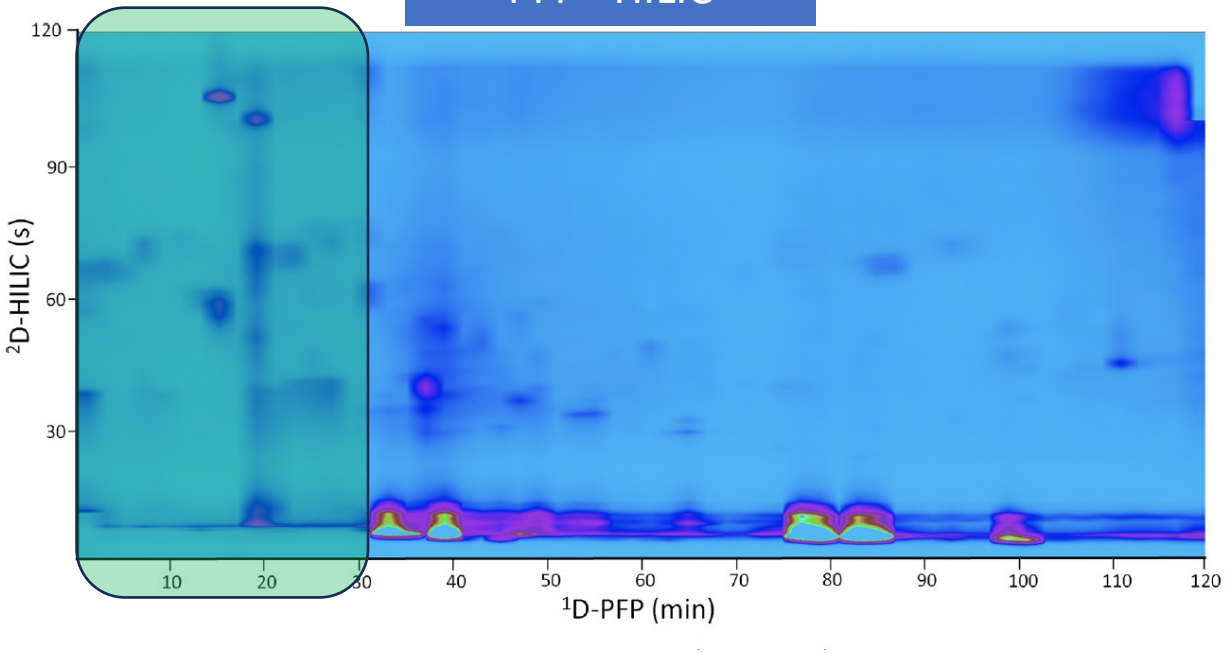




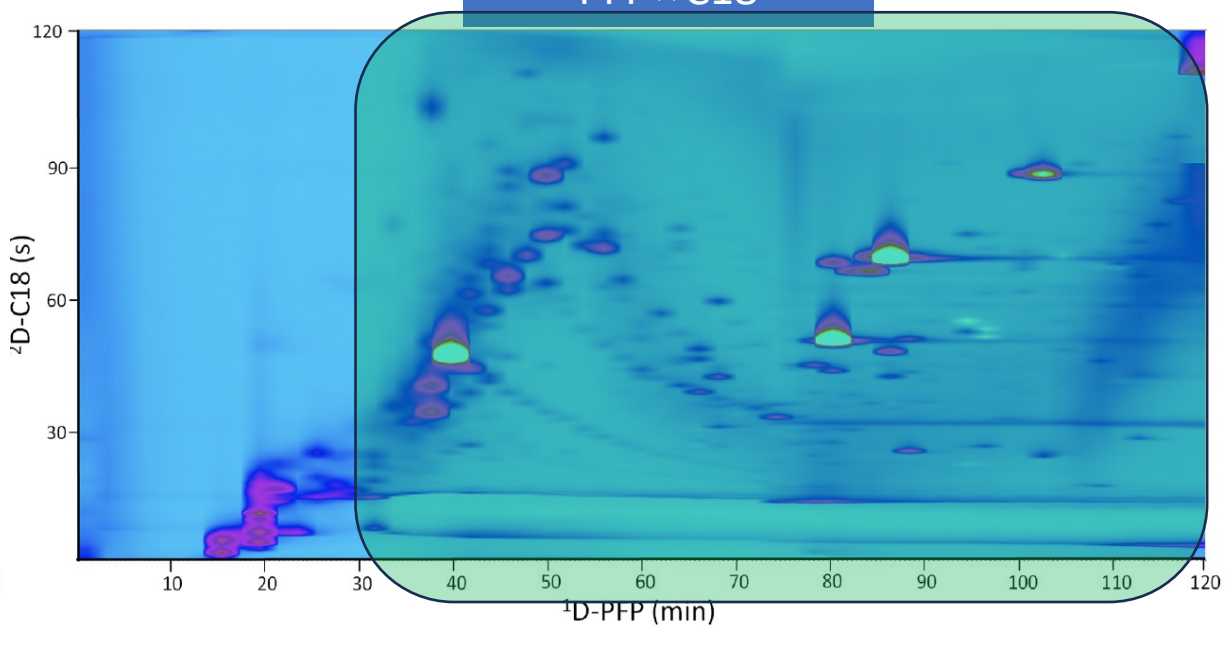
Optimization works with MS detection but here are only shown the UV detection for a better overview.

Analysis of a vermouth with LC × LC-DAD

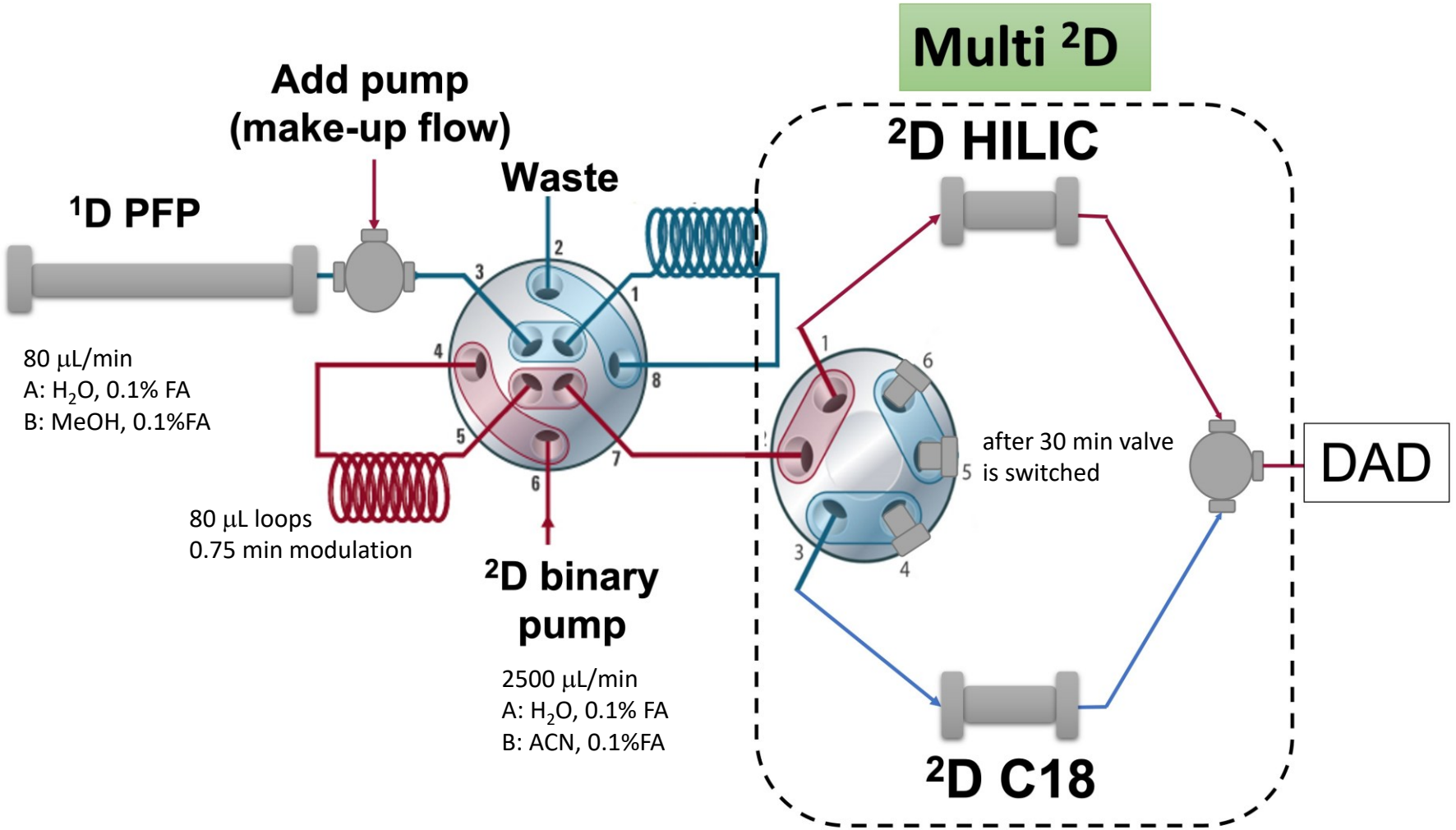
PFP×HILIC



PFP×C18



Multi-^{2}D LC × LC-DAD

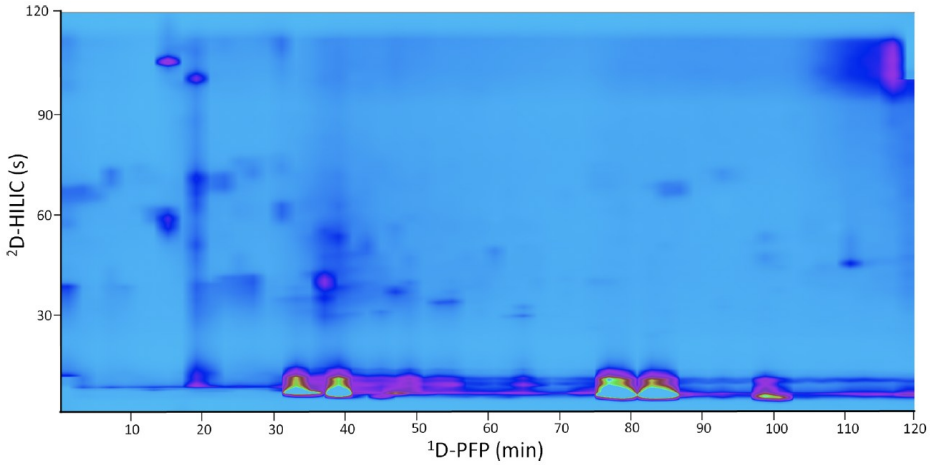


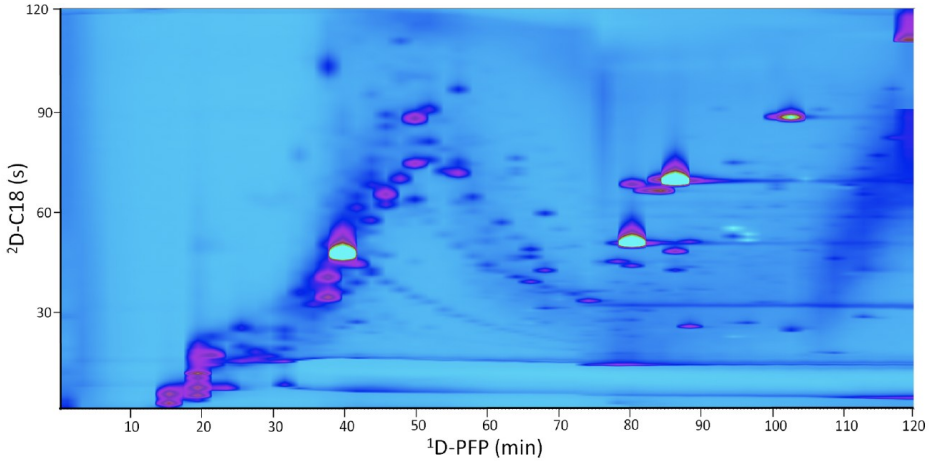
Katharina Wetzel

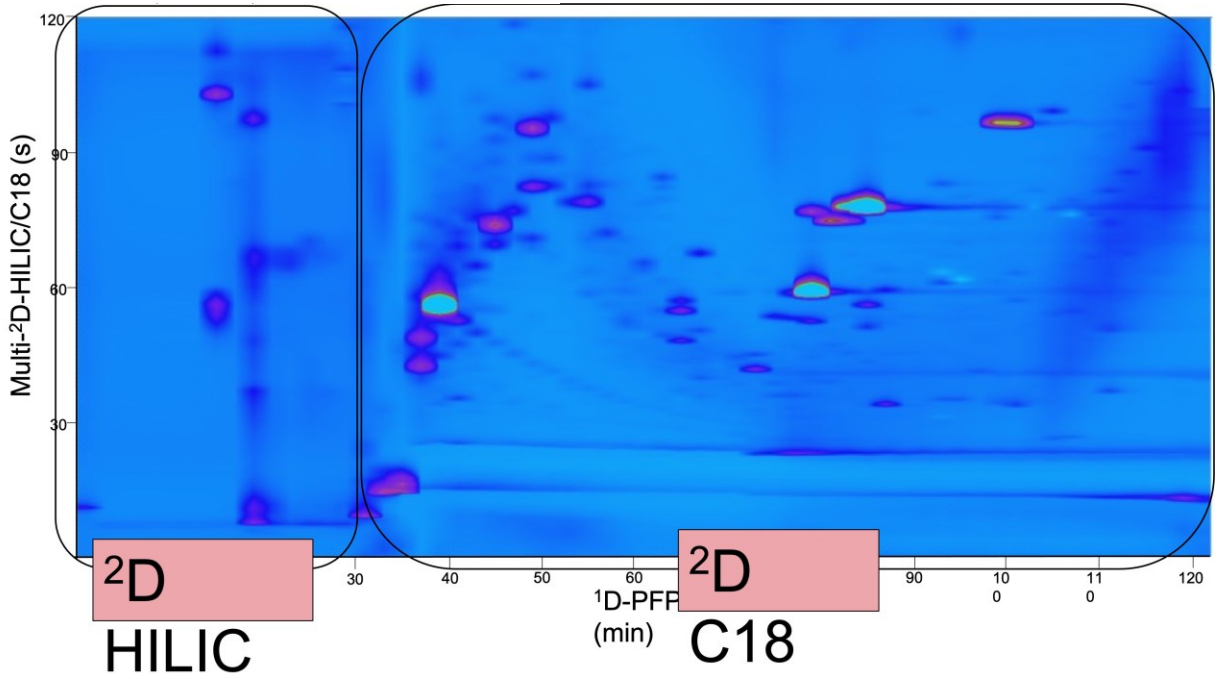
Lidia Montero

L. Montero, J. F. Ayala-Cabrera, F. F. Bristy, O. J. Schmitz, **Analytical Chemistry** (2023) 95:3398-3405

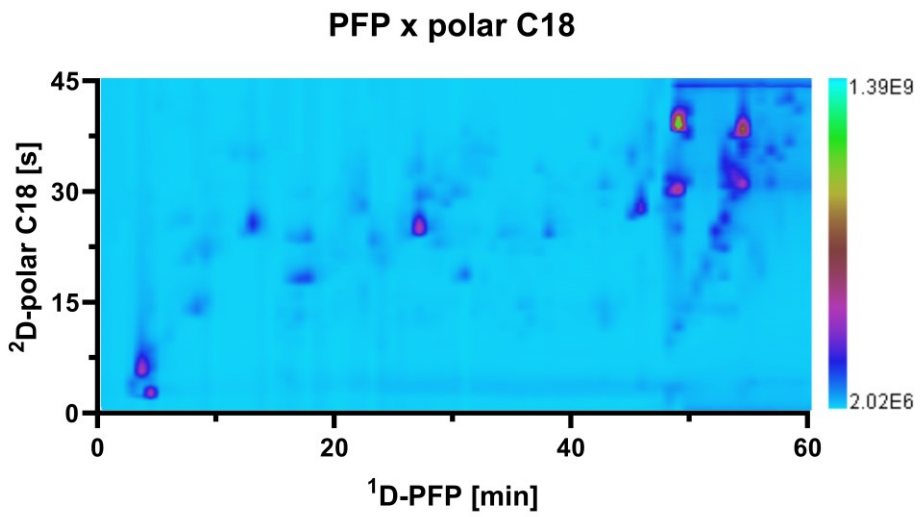
Analysis of a vermouth with Multi-^{2}D LC × LC-DAD

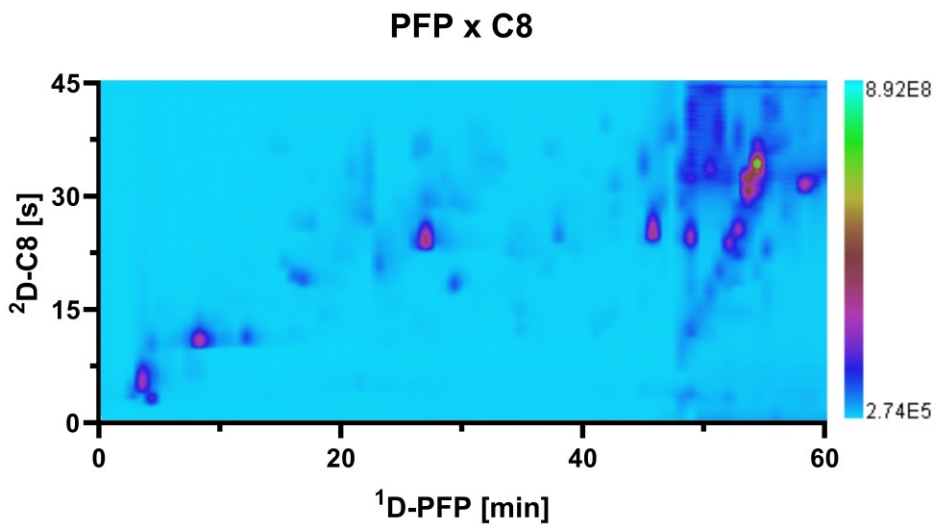




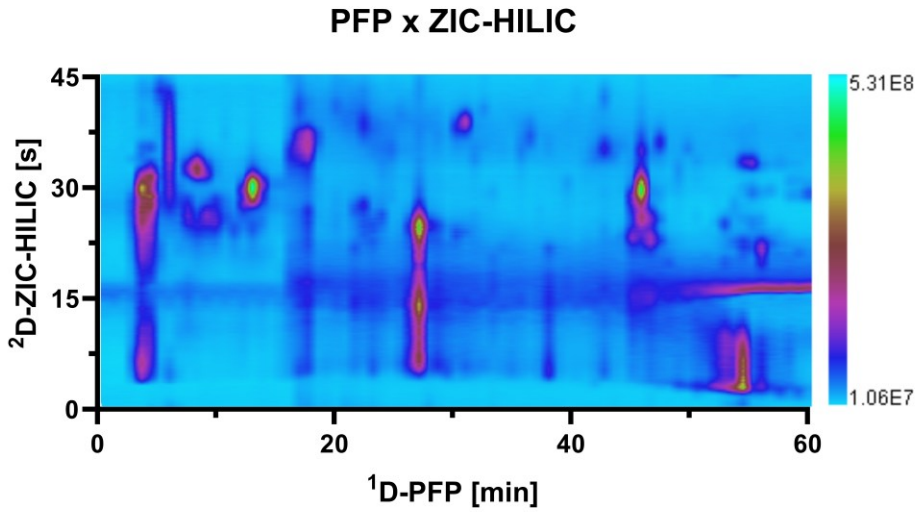


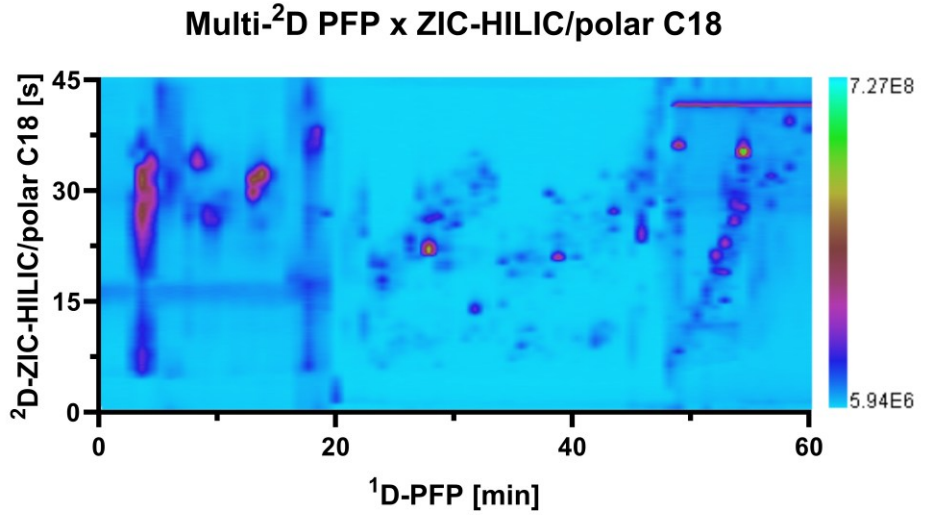
LC × LC and Multi-^{2}D LC × LC analysis of a *Agrimonia eupatoria*



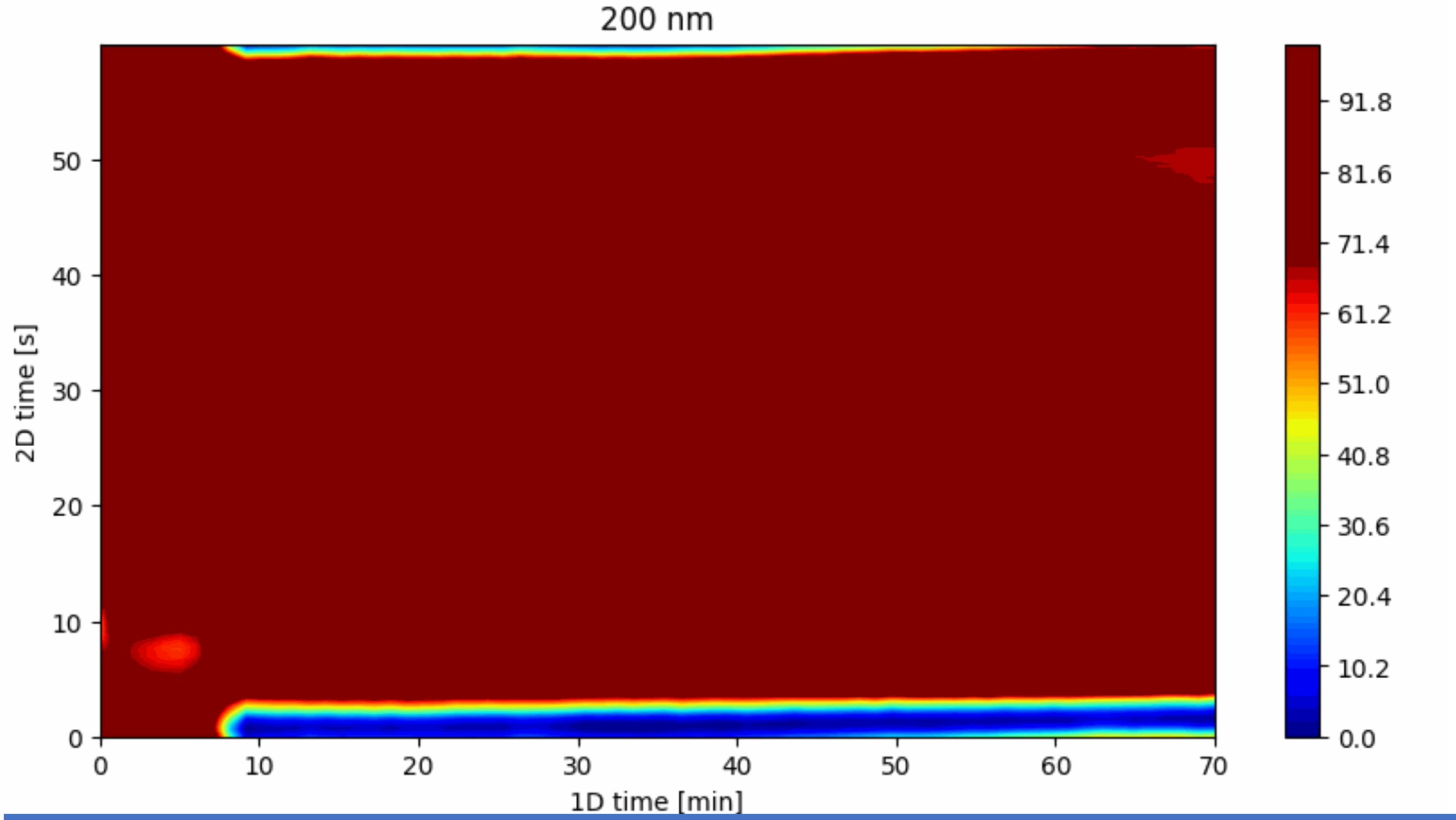


Katharina Wetzel





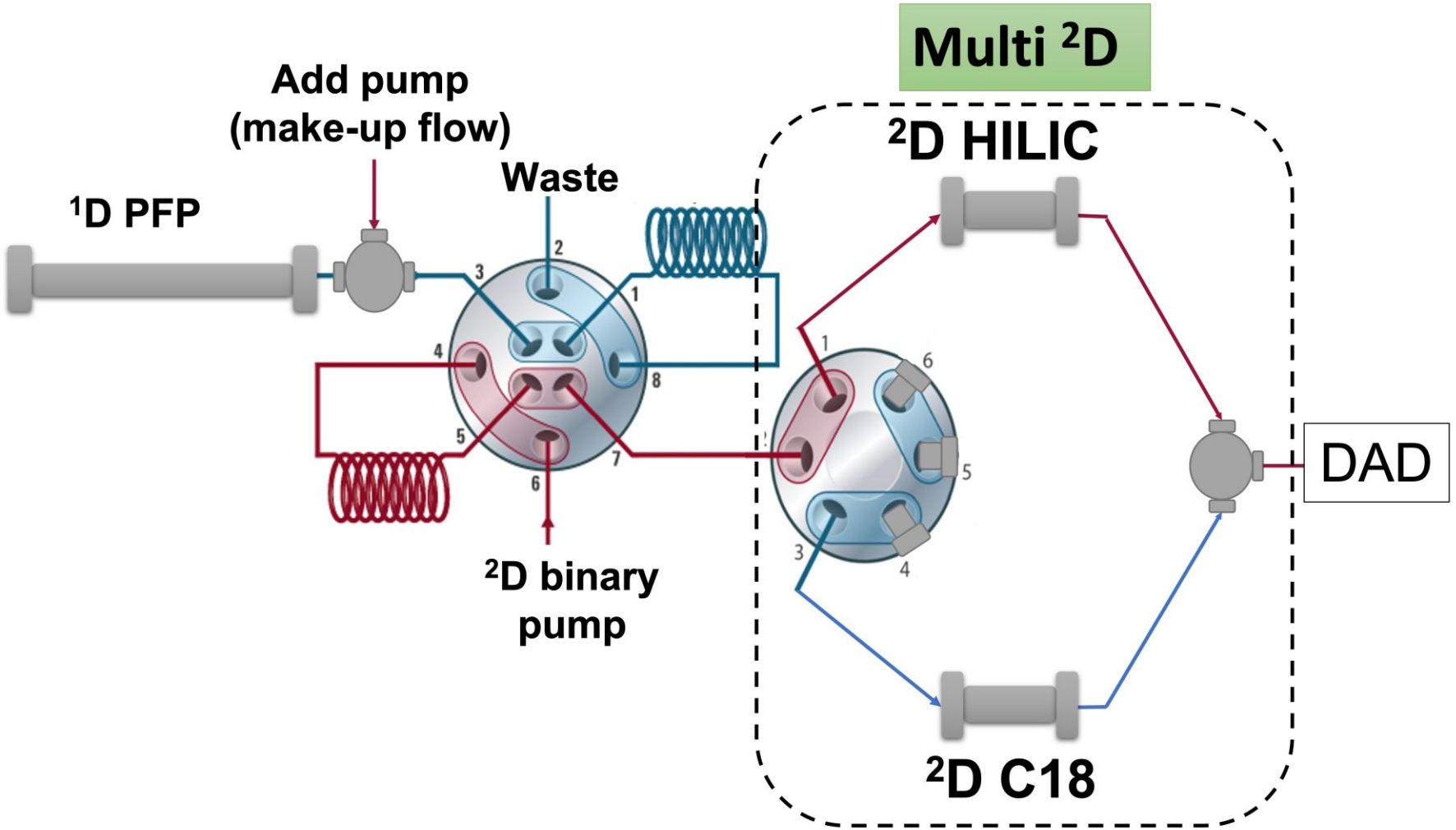
Initial progress in data analysis



Jaqueline Leddin

Y. Oulad El Majdoub, A. K. Mallik, L. Montero, J. Rösler, F. Stappert, J. Leddin, S. W. Meckelmann, O. J. Schmitz, *submitted to Analytical Chemistry*

Multi-^{2}D LC × LC-DAD

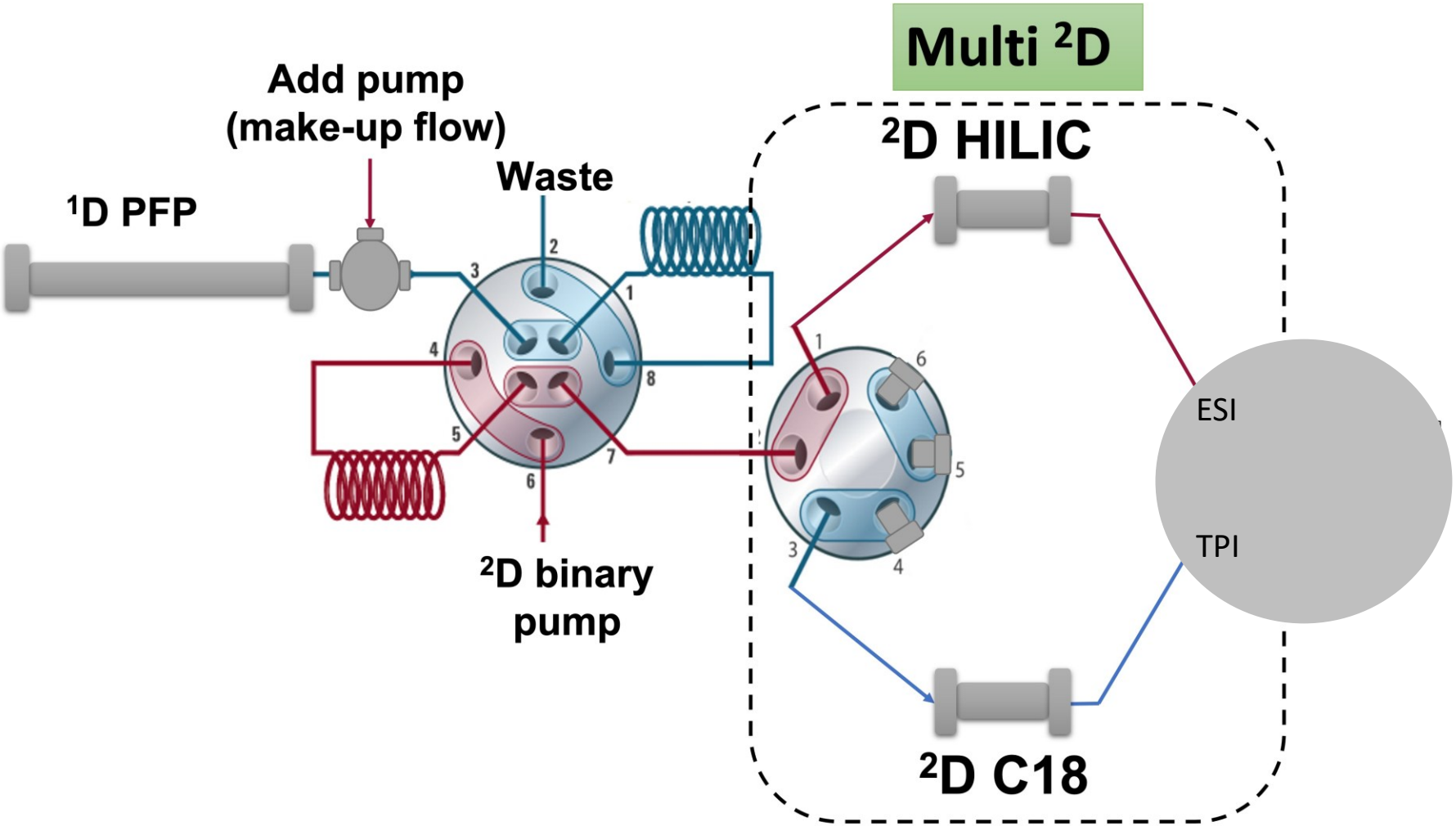


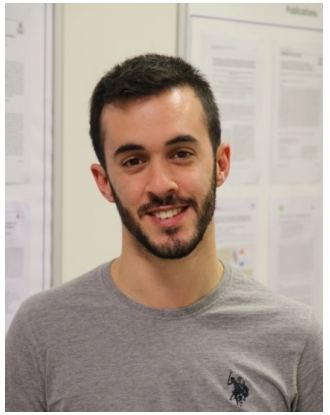
Separation looks good, but what is the situation in identification?

Of course, we need an MS!

But which ion source?

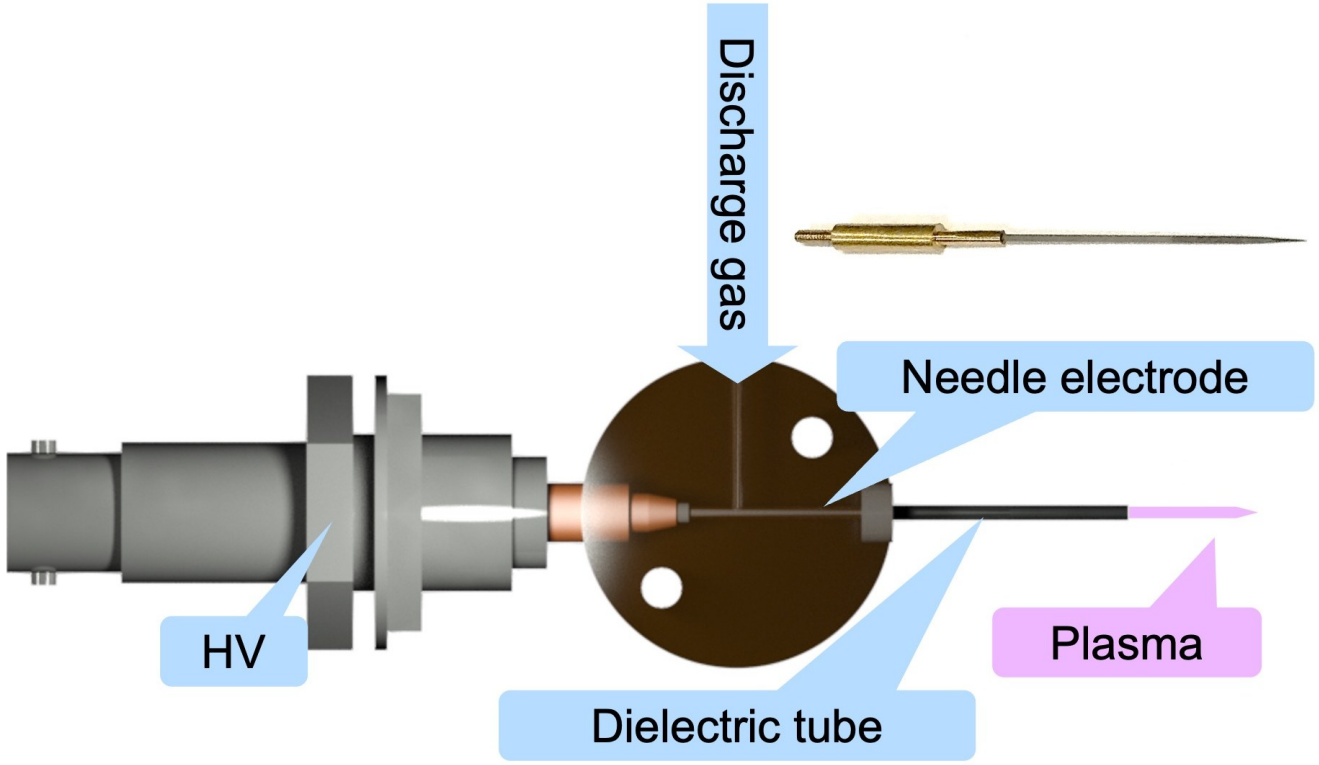
Multi-^{2}D LC × LC-dual source-qTOF-MS





Juan Ayala Cabrera

Dielectric barrier discharges (DBD) ion source



Counter electrode is the ion source housing

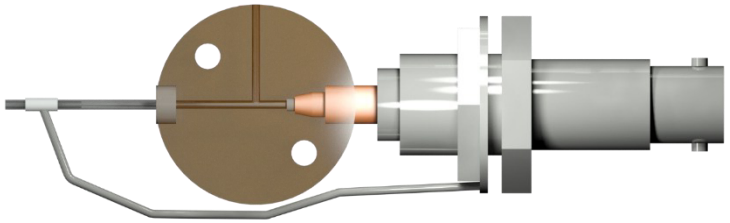
Alexandra Pape

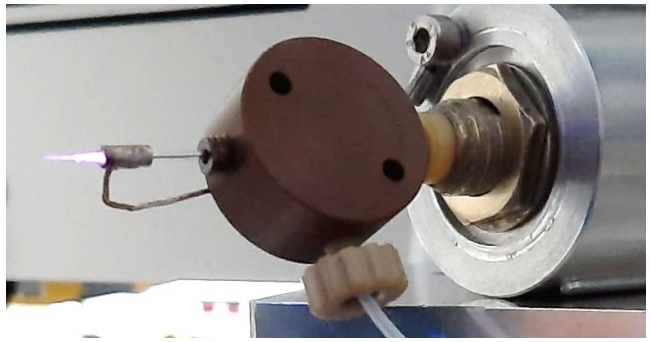
Sebastian Löbbecke

D. Brecht, F. Uteschil, O. J. Schmitz, **RCM** (2021) 34:e8845; A. Pape, O. J. Schmitz, **TrAC** (2024) 170:117420

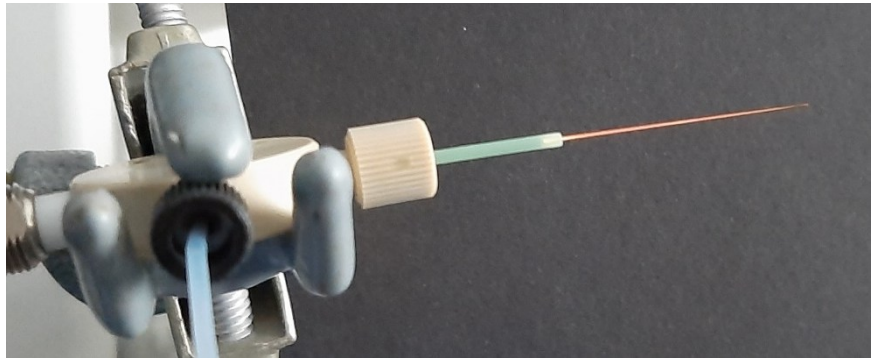
Dielectric barrier discharges (DBD) ion source

inverse low temperature plasma (iLTP)

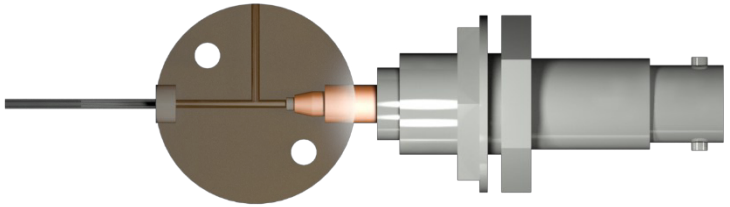


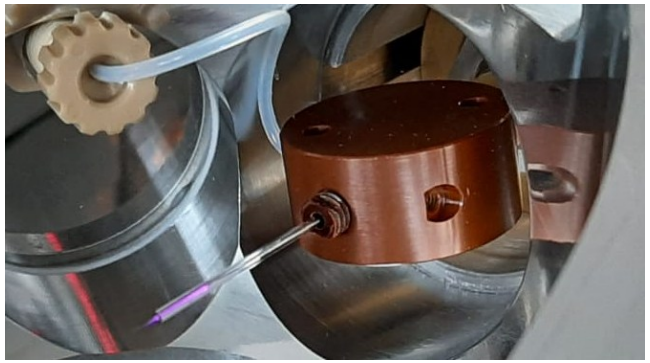


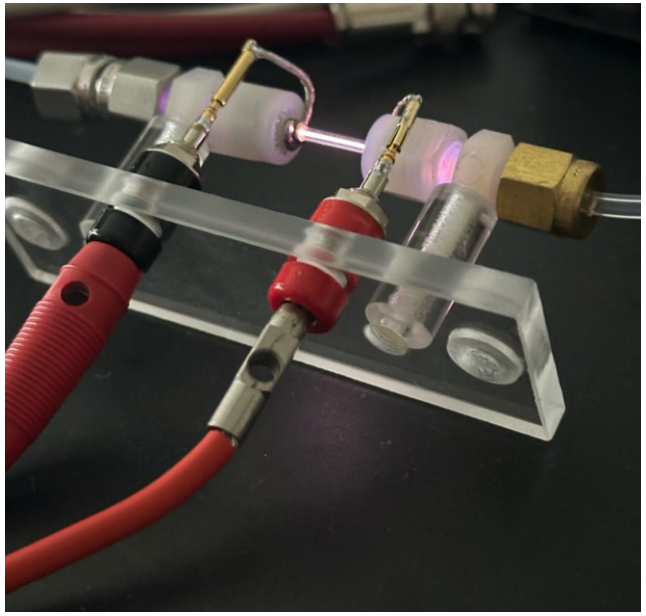
flexible microtube plasma (FμTP)



tube plasma ionization (TPI)

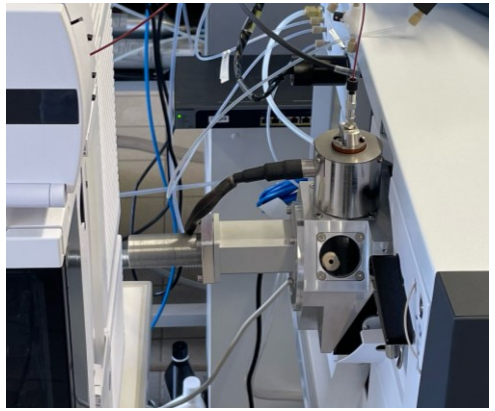






TPI source for LC-MS and GC-MS

Florian Uteschil



LC-TPI Configuration

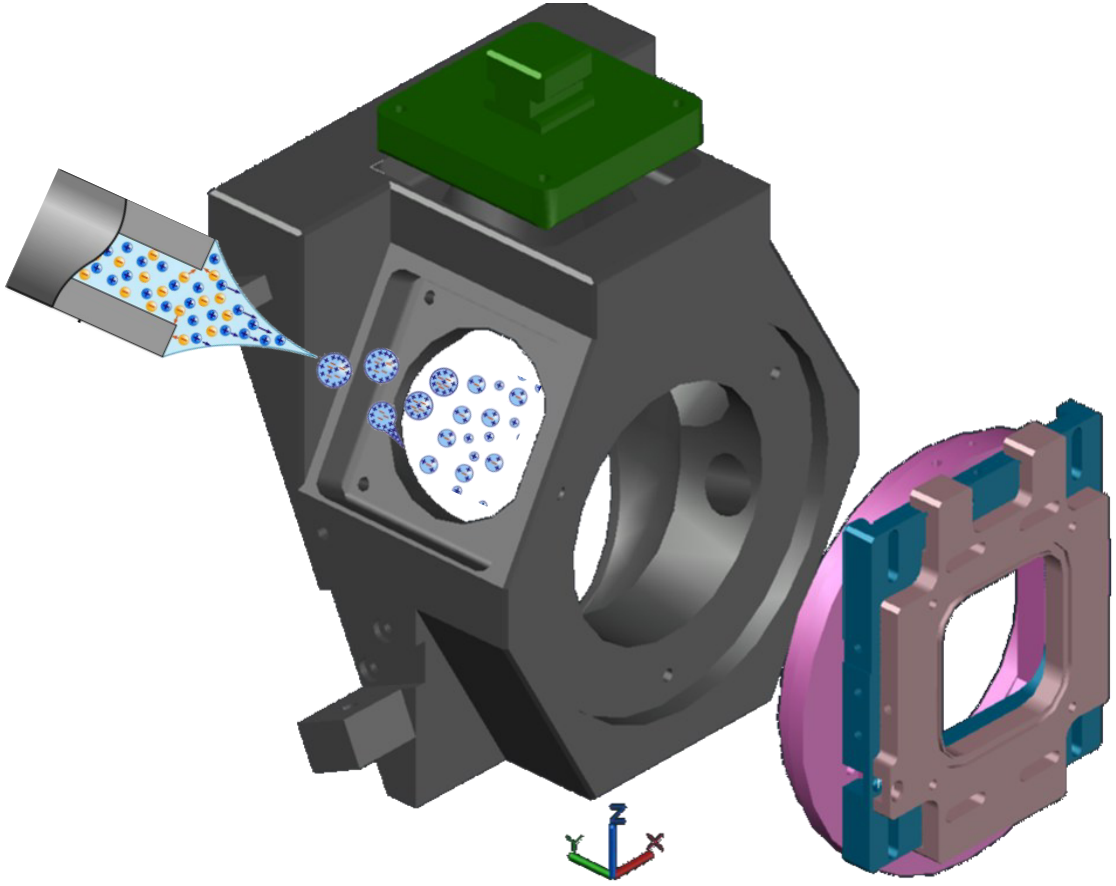
GC-TPI Configuration

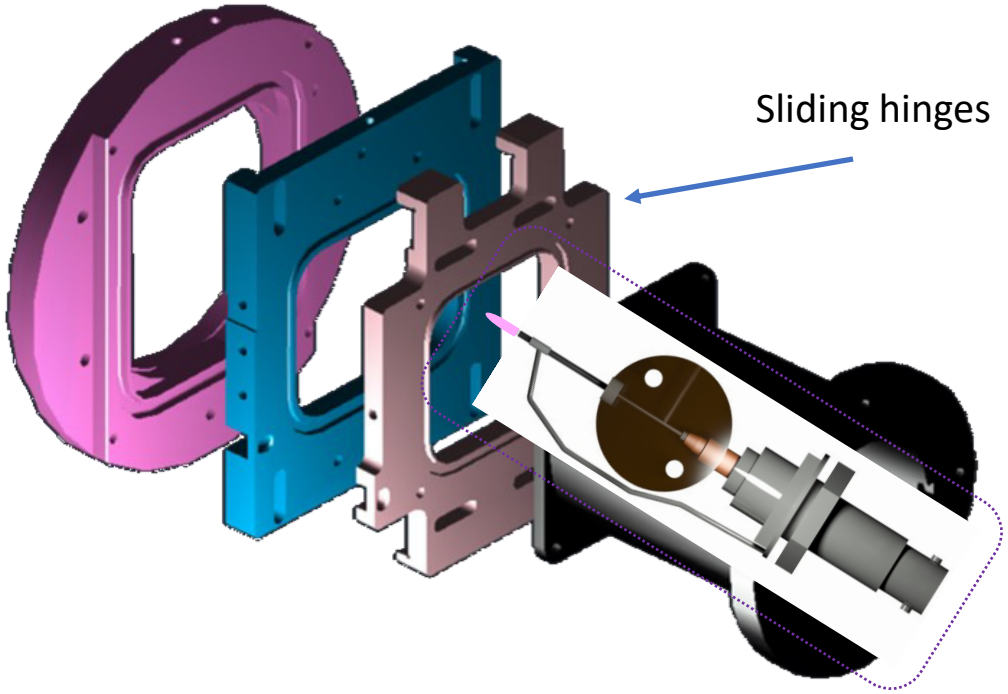
J. F. Ayala-Cabrera, J. Turkowski, F. Uteschil, O.J. Schmitz, **Anal Chem** (2022) 94:9595-9602; J. F. Ayala-Cabrera, L. Montero, T. Sahlabji, F. Uteschil, O.J. Schmitz, **Anal Bioanal Chem** (2023) 415(13):2561-2573;
S. Löbbecke, A. Pape, L. Montero, F. Uteschil, J. F. Ayala-Cabrera, O. J. Schmitz, **Talanta** (2025) 285:127388; *two further manuscripts about TPI are submitted*

Multi-^{2}D LCxLC-dual source-qTOF-MS

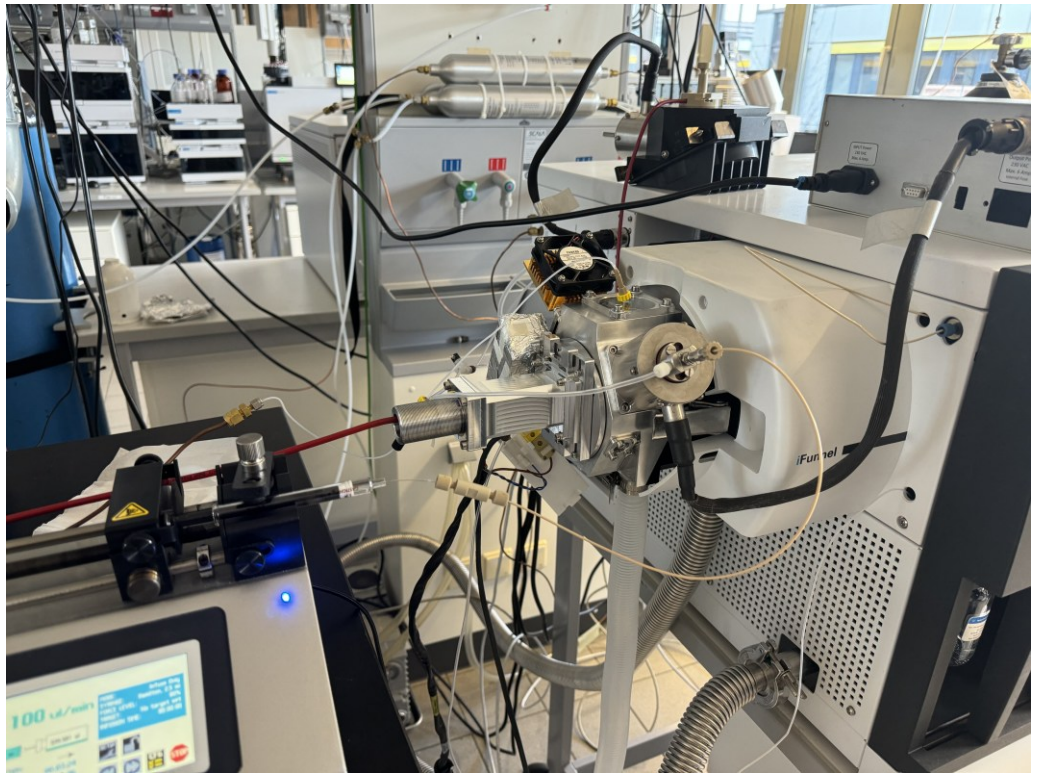
Combination of two-dimensional chromatography with a dual-ion source can increase the information of a complex sample.

Marvin Häßler





Multi-^{2}D LC × LC-dual source-qTOF-MS

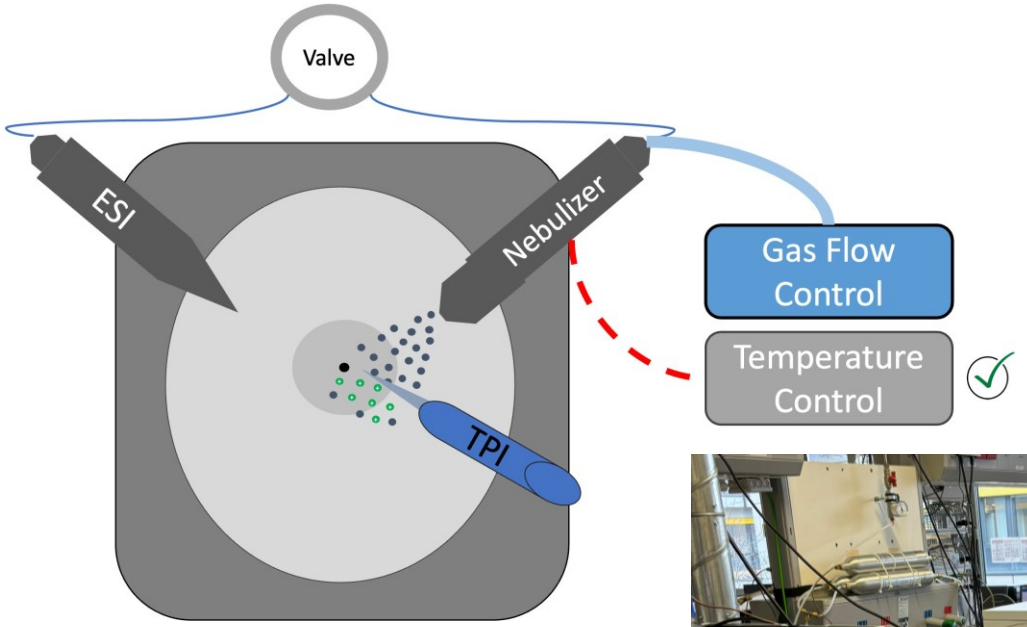


By using two probes in the same housing, it is possible to completely change the ion source during a run:

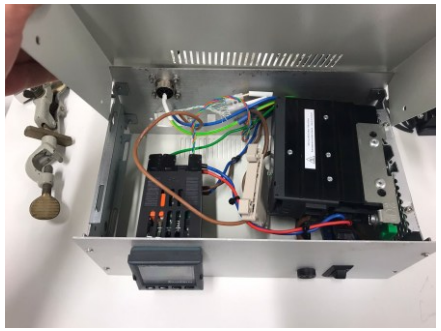
- Agilent Jet Stream ESI source
- APCI Nebulizer unit

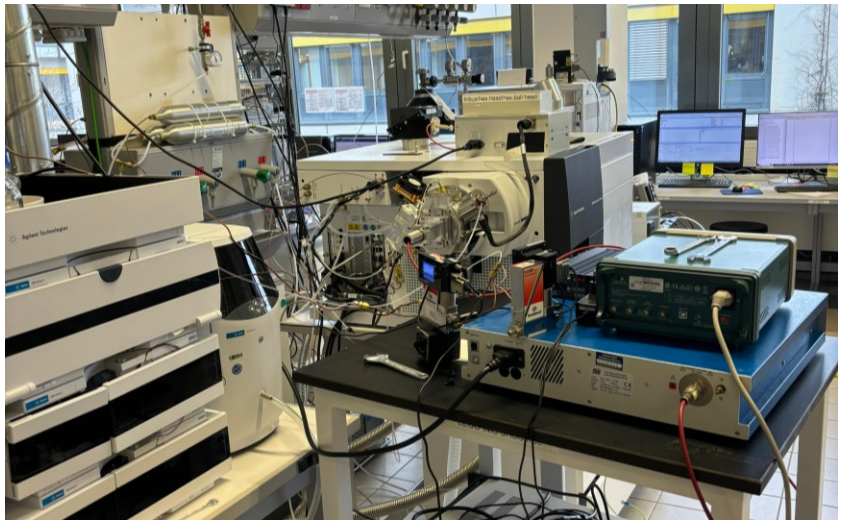
Dual ion source

	TPI mit Argon LOD µM	DualeAJS LOD µM	Orginale AJS LOD µM
2,4,6-trihydroxyacetophenon	0.179	0.107	0.134
3-Aminoquinoline	0.012	0.0013	0.0009
4-Hydroxybenzoicacidmethylester	9.541	0.034	0.155
4-Nitrophenol	34.914	3.486	0.210
6-(dimethylamino)purine	0.065	0.0070	0.0034
Adenosin	0.031	0.0034	0.0016
Apigenin	0.156	0.266	0.160
a-tocopherol	0.088	0.328	0.158
Bergapten	0.020	0.059	0.026
Caffeine	0.020	0.018	0.013
Capsaicin	0.0093	0.010	0.009
Catechin	0.434	0.203	0.080
Cinnamaldehyde	1.650	0.848	2.984
Colchicine	0.0012	0.003	0.001
Coumarin	0.072	0.070	0.050
Curcumin	0.0013	0.199	0.073
Cyanidin cloride	13.613	0.361	0.214
Delphinidin	1.718	8.757	3.883
Epicatechingallat	0.038	0.683	0.133
Hesperitin	0.00044	0.166	0.024
Hesperitin-7-rutinosid	0.014	0.270	0.073
Kaempferol	0.372	0.583	0.176
Luteolin	0.401	0.308	0.120
Mannit	2.527	0.019	0.004
Myricetin	32.016	23.478	6.575
naringenin	0.067	0.246	0.159
Naringin	0.009	0.030	0.016
Oleuropein	0.015	0.554	0.025
Polydatin	1.470	0.041	0.013
Pseudocapsaicin	0.013	0.011	0.013
Quercetin hydrate	12.144	7.584	0.378
Quercetin-3b-D-glucoside	1.738	0.431	0.130
Reserpine	0.0077	0.021	0.0066
Resveratrol	0.165	0.196	0.096
Riboflavin	0.00025	0.0056	0.0019
Rutin	0.008	0.145	0.030
Syringaldehyd	1.478	0.150	0.124
Taurine	1.746	0.045	0.060
Vanillin	0.307	0.096	0.124
Vitamin B12	0.021	0.366	0.230



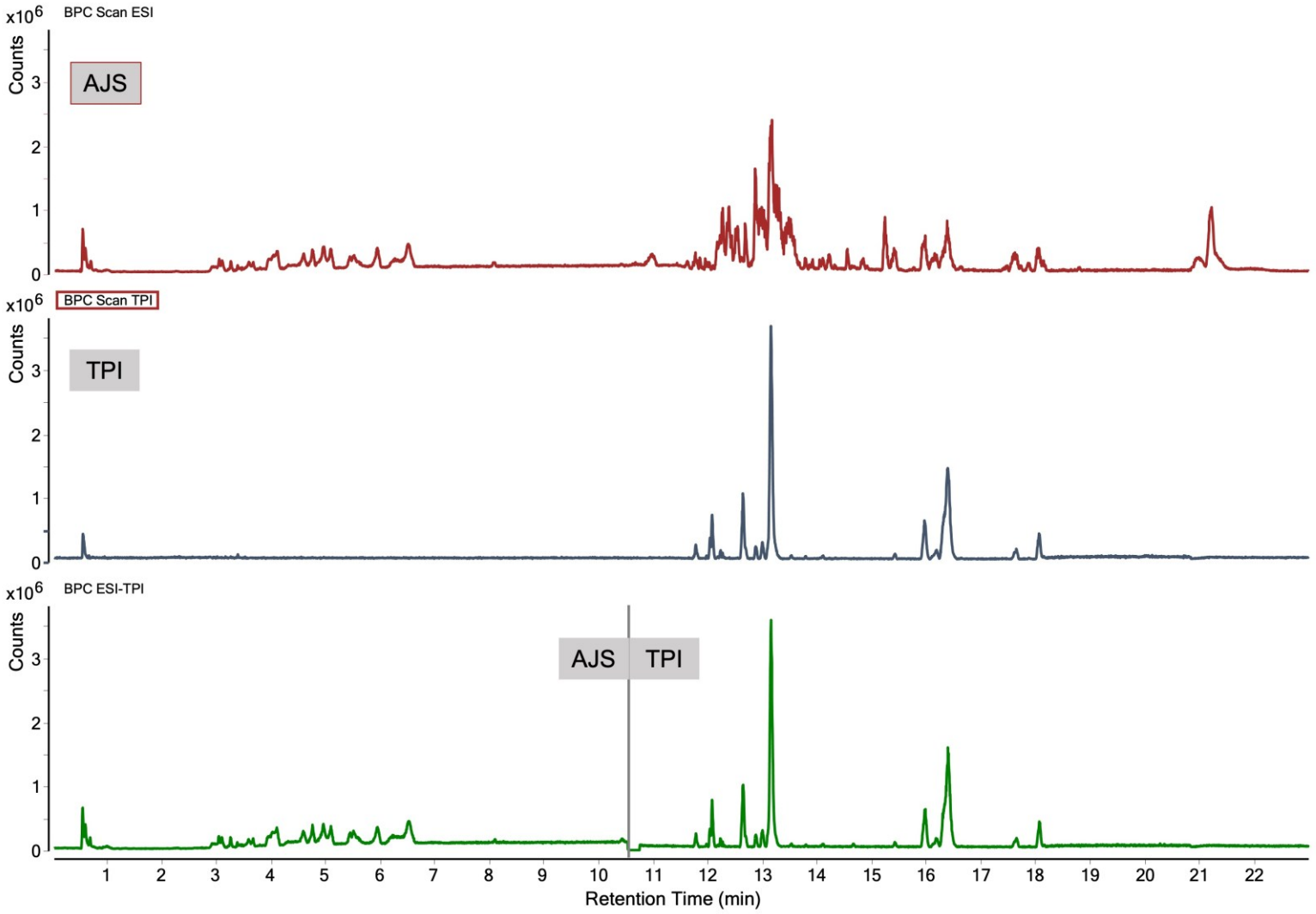
Marvin Häßler





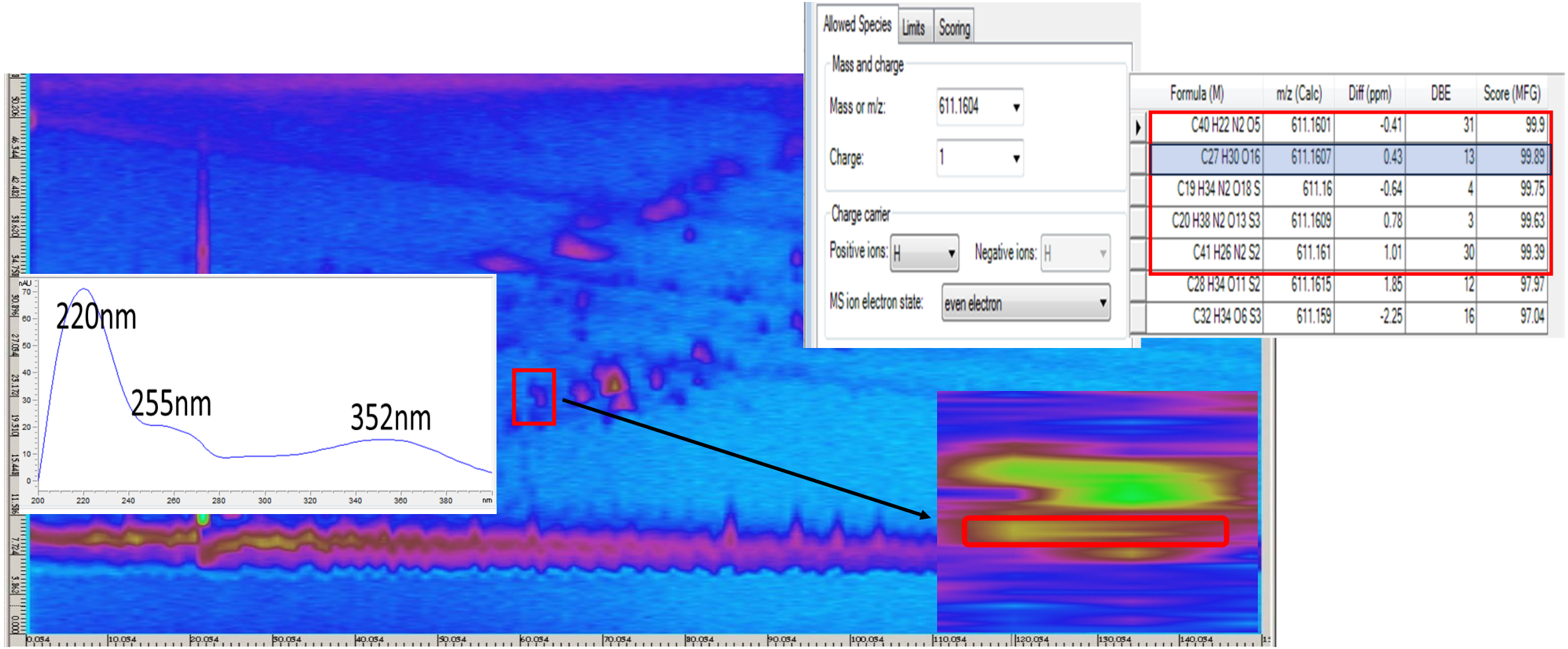
We used a Eurotherm EPC3016 and a Eurotherm ESwitch

First measurements of an *Agremonia Eupatoria* leaves‘ extract

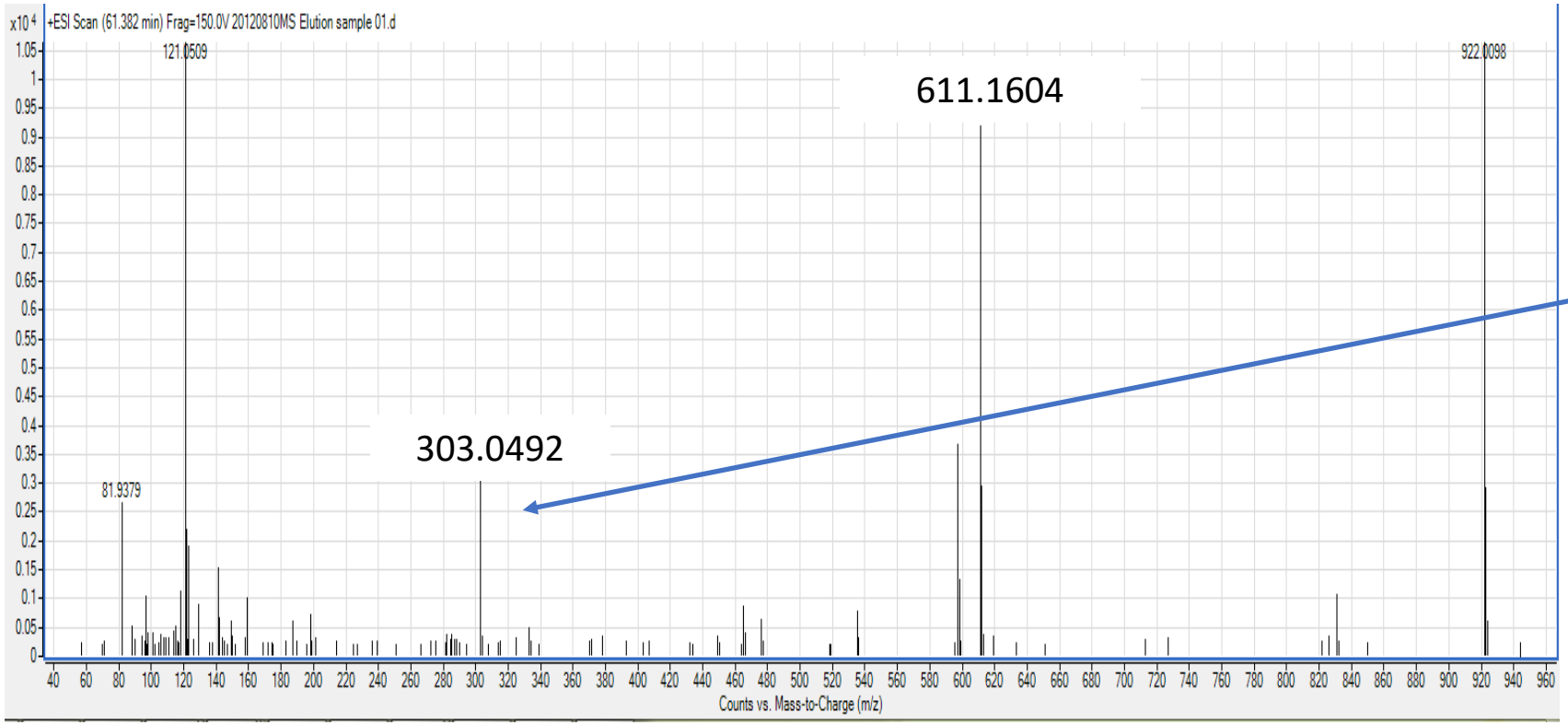


Agremonia Eupatoria

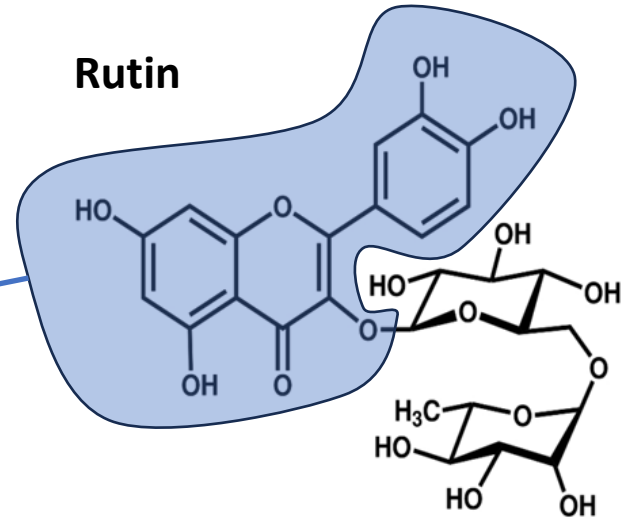
Separation and Detection vs Identification: *Hediotys diffusa*



Identification of the ingredients in *Hediotys diffusa*

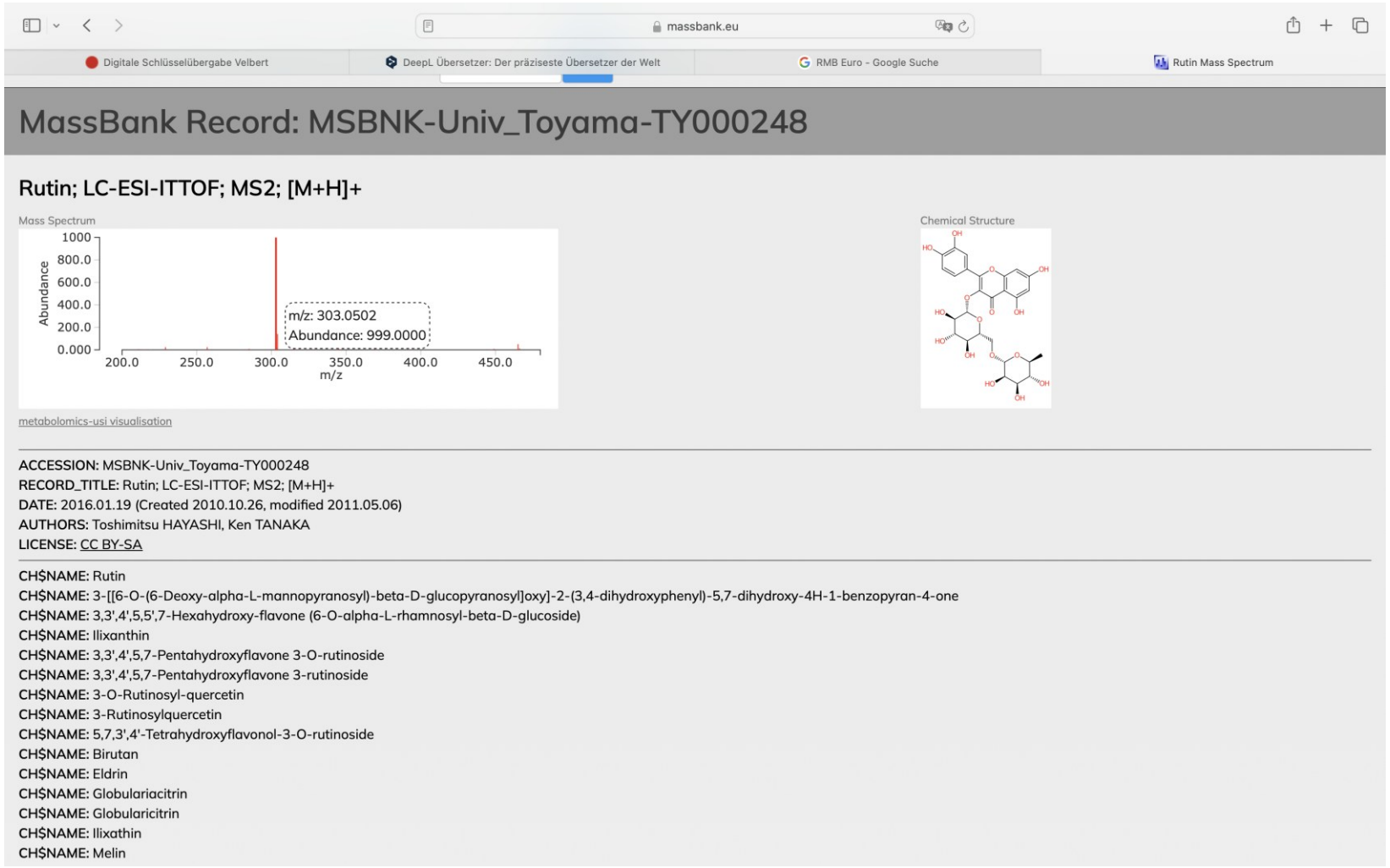


Rutin



Identification level 3

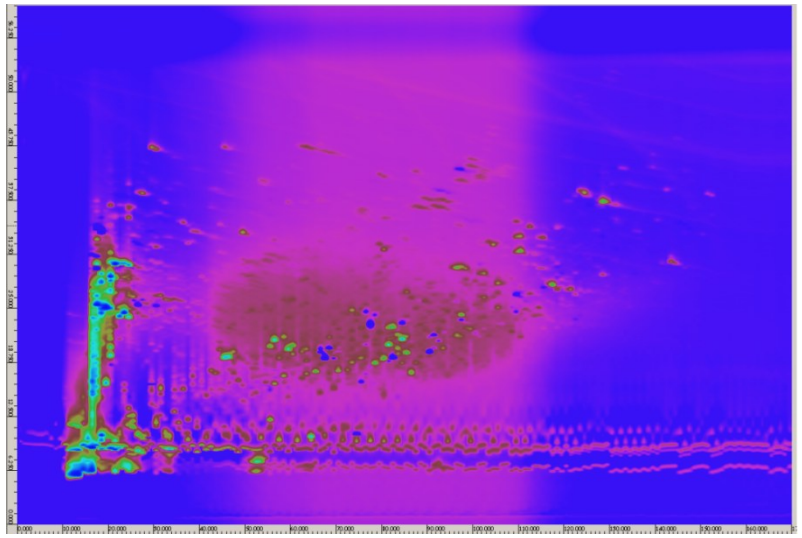
Identification of the ingredients in *Hediotys diffusa*

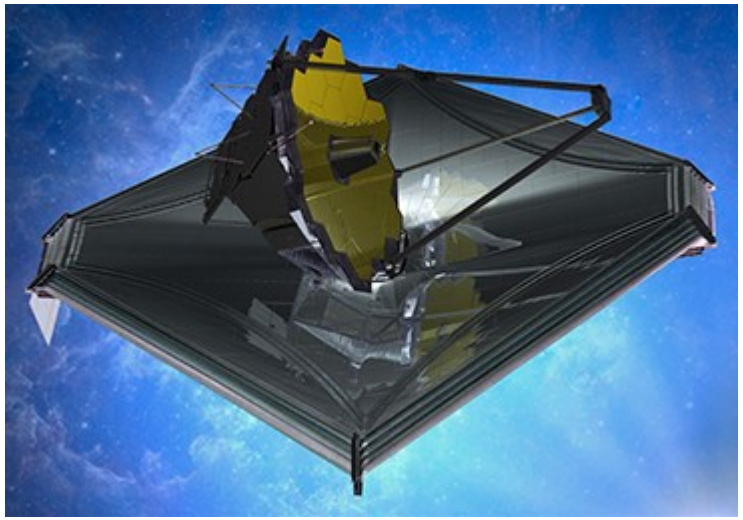


→ Identification level 2

https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-Univ_Toyama-TY000248

LCxLC versus Hubble

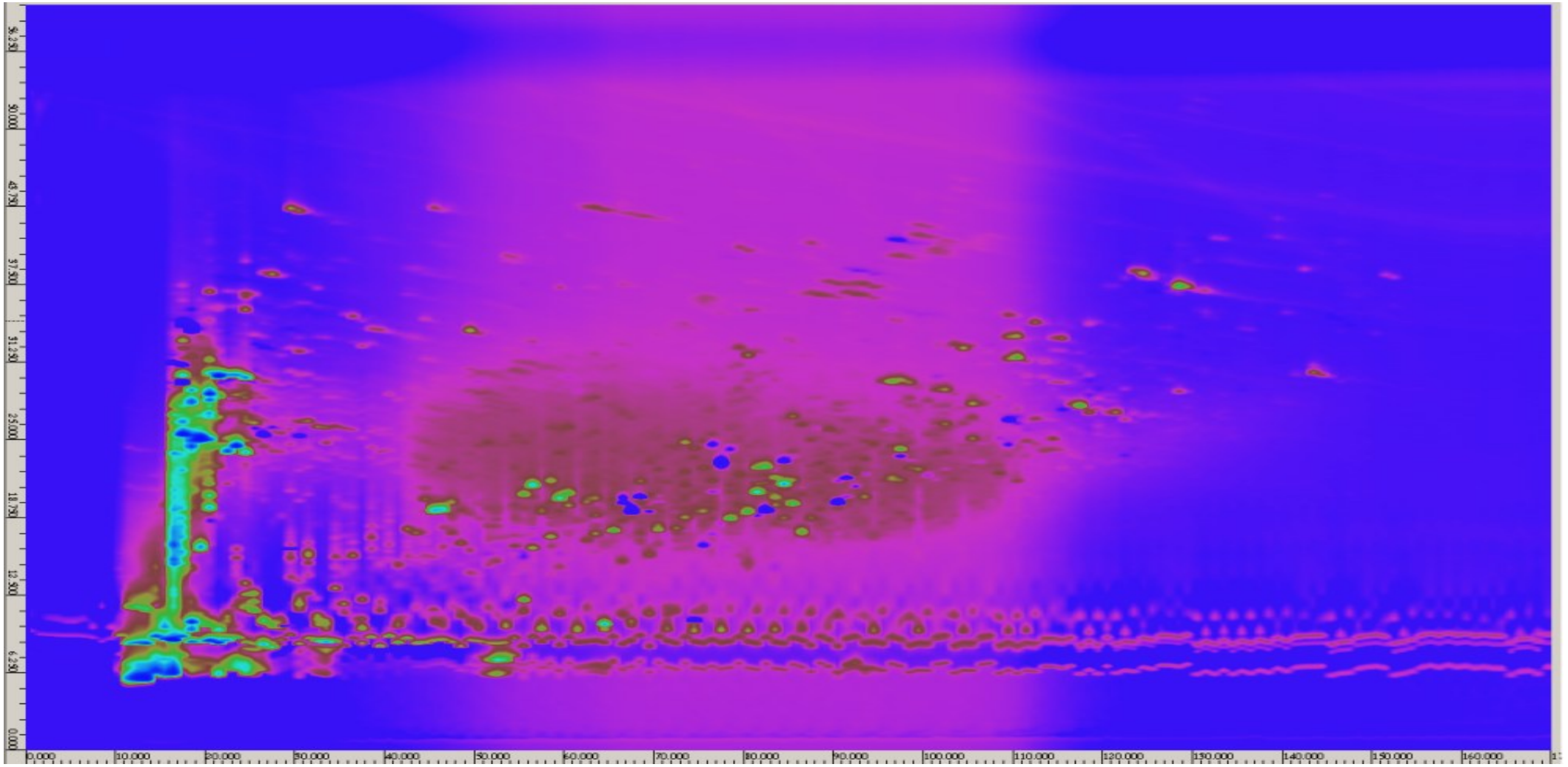




James Webb Space Telescope

Ion mobility mass spectrometry: DTIM-qTOF-MS

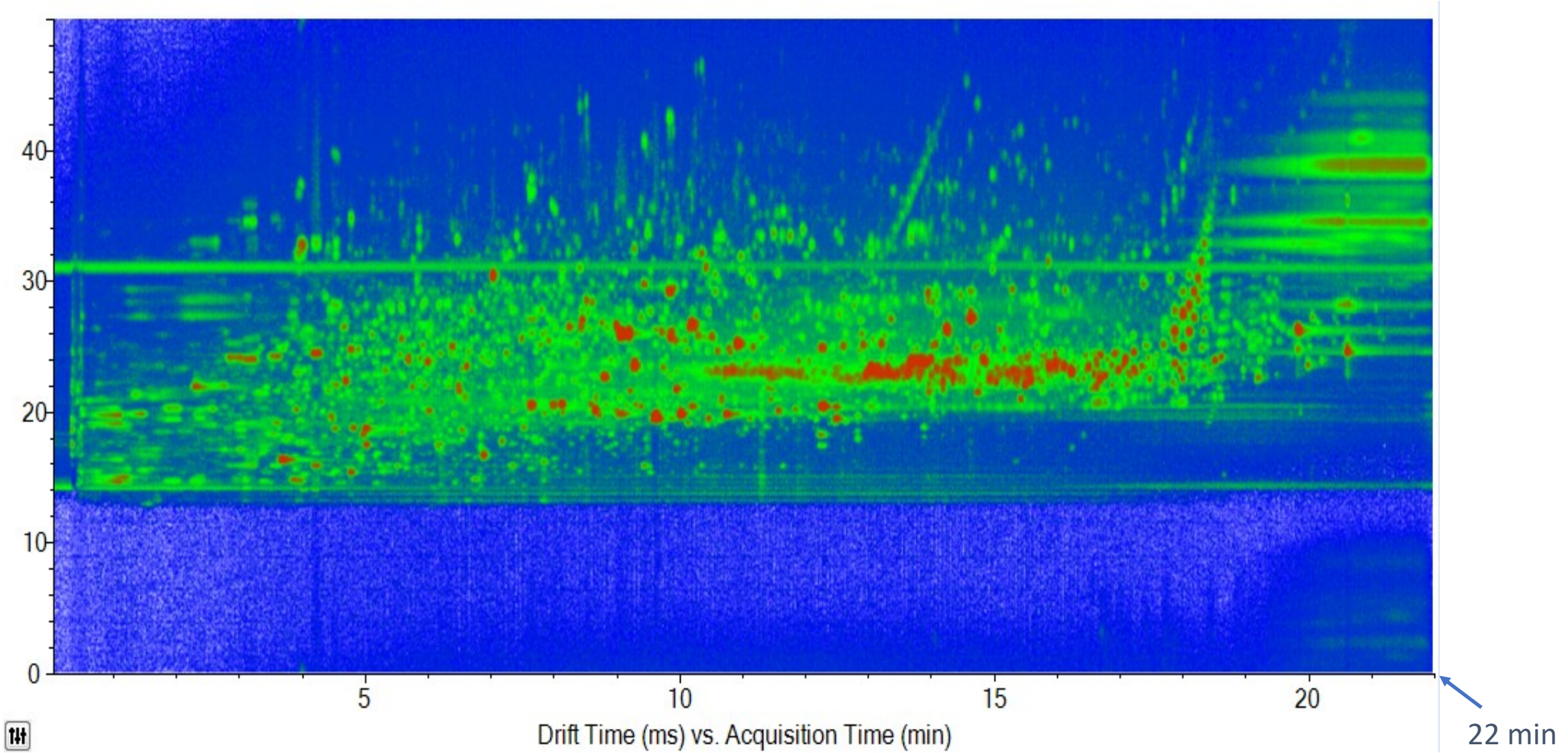
Analysis of *Scutellaria barbata* and *Hediotys diffusa* with LC × LC-DAD (270 nm)



180 min

D. Li, O. J. Schmitz, **Anal Bioanal Chem** 405 (2013) 6511-6517 and D. Li, C. Jakob, O. J. Schmitz, **Anal Bioanal Chem** 407 (2015) 153-167

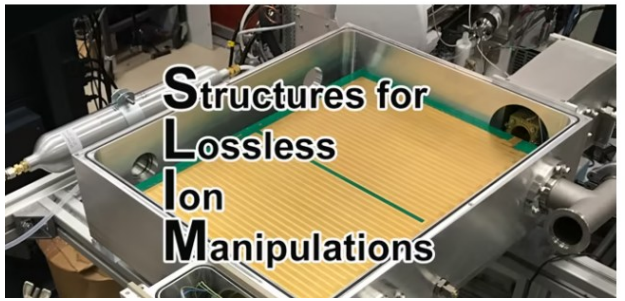
Analysis of *Scutellaria barbata* and *Hediotys diffusa* with LC-DTIM-qTOF-MS

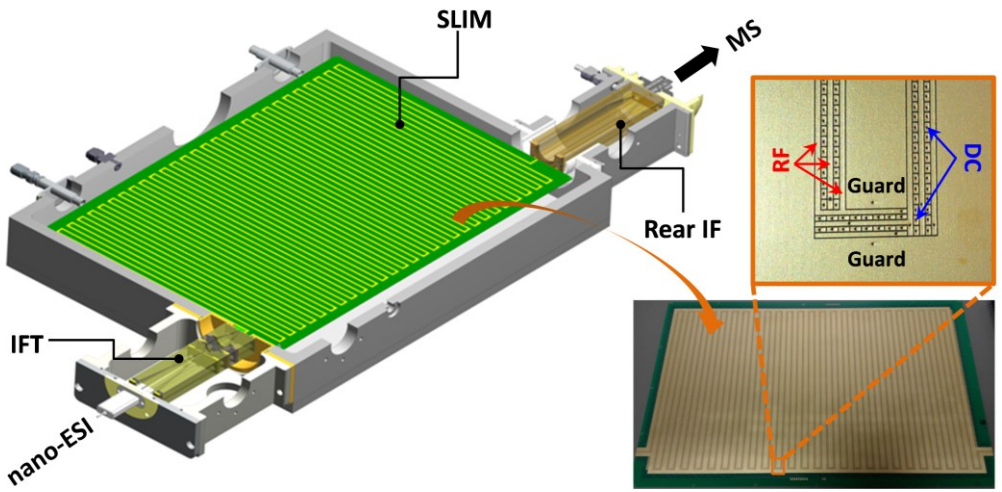


Ion mobility mass spectrometry of the next generation

Use of the next generation IM-MS (SLIM)

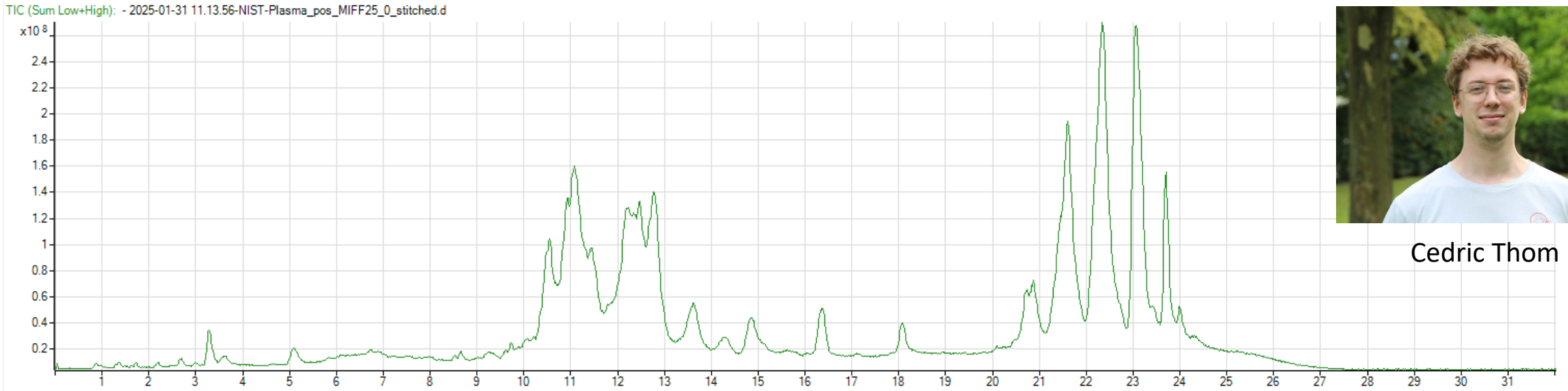
- Development of a platform for lossless ion manipulation and ion mobility (SLIM IM) connected to a commercial high-resolution mass spectrometer (MS).
- SLIM IM implements the travelling wave ion mobility technique (TWIMS) over a path length of ~13 m for high-resolution IM (HRIM) separations (with printed circuit boards).
- The resolving power (CCS/ΔCCS) of the SLIM IM stage is over 200 for a wide range of ion masses.



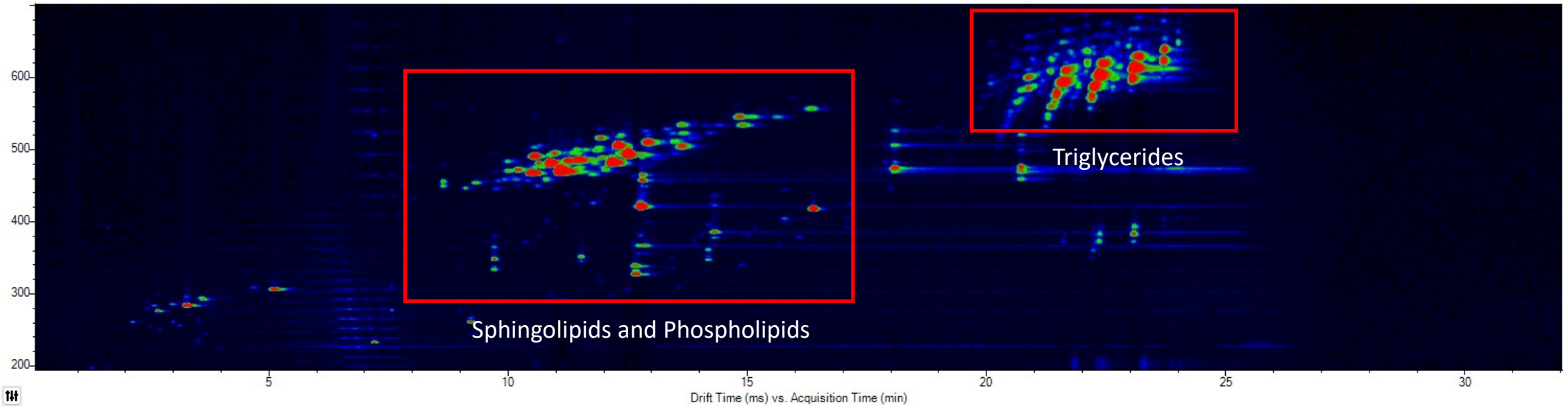


J. C. May, K. L. Leaptrot, B. S. Rose, K. L. Wormwood Moserm L. Deng, L. Maxon, D. DeBord, J. A. McLean, **J Am Soc Mass Spectrom** (2021) 32(4):1126-1137

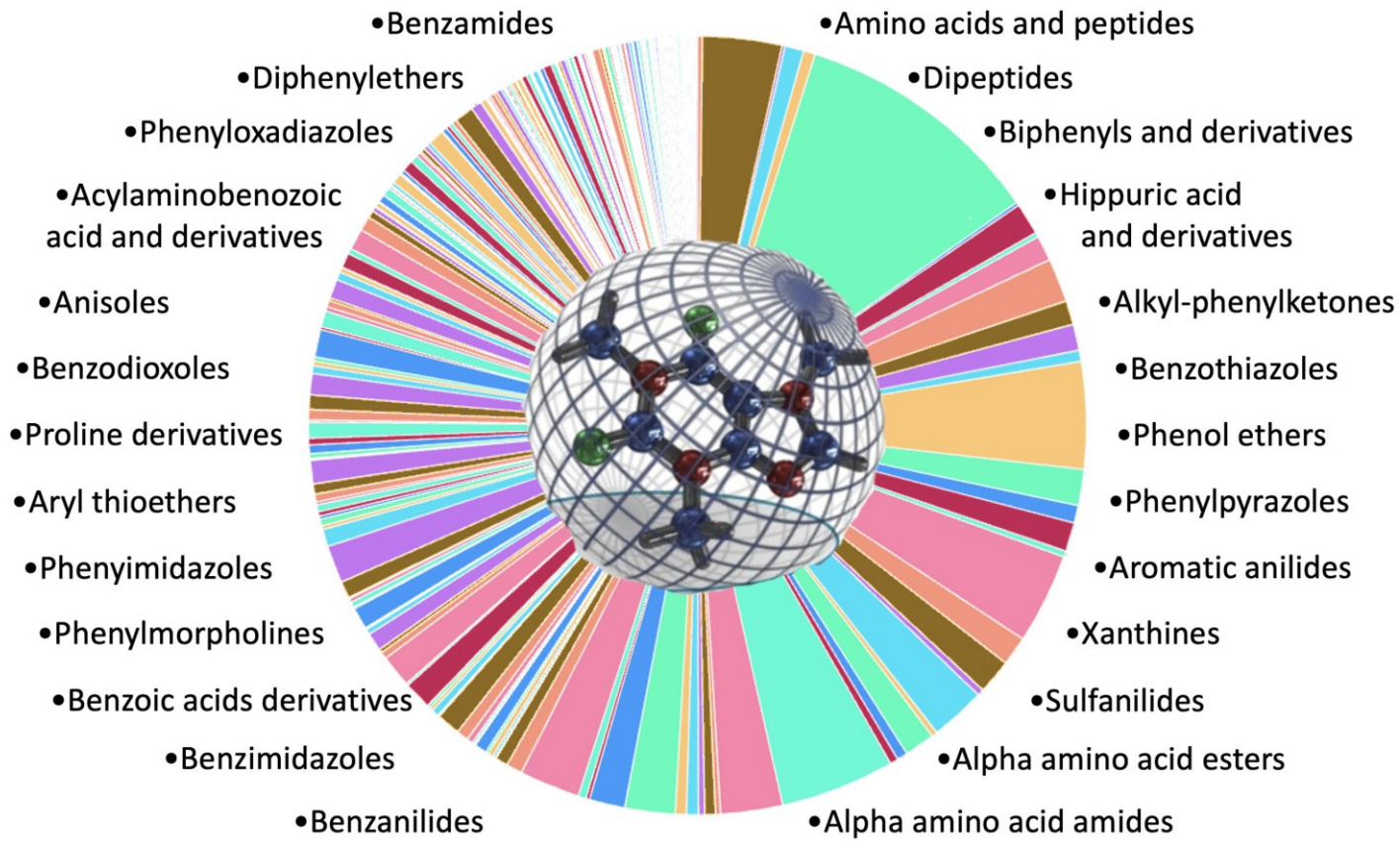
Human (NIST) plasma lipidome analysis



Cedric Thom



METLIN-CCS: An ion mobility spectrometry collision cross section database



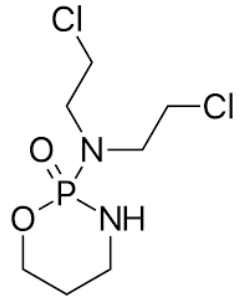
27,633 molecular standards across 79 chemical classes

E. S. Baker, C.Hoang, W. Uritboonthai, H. M. Heyman, B. Pratt, M. MacCoss, B. McLean, R. Plum, A. Aisporna, G. Siuzdak (2023) **Nature methods** 20:1836

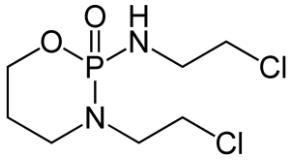
Ion suppression with 1D- and 2D-LC-ESI-qTOF-MS

$$F_{IS} = area_{sample}/area_{standard}$$

Reference	1D-LC			2D-LC		
	Plant	Waste water inflow	Bio coal	Plant	Waste water inflow	Bio coal
Cyclophosphamide	0.58	0.60	0.16	0.96	0.95	0.55
Ifosfamide	0.55	0.61	0.16	0.93	0.75	0.45



Cyclophosphamide

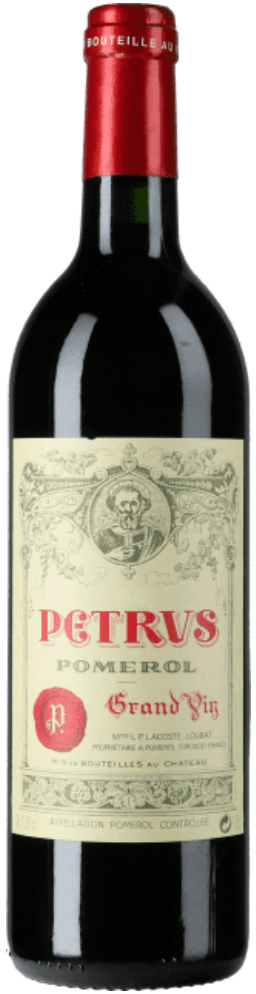


Ifosfamide

S. Stephan, J. Hippler, T. Köhler, D. Brecht, O. J. Schmitz, **J Anal Test** (2017)1:1-9

The best of two worlds: Coupling LC × LC with IM-MS

+



=

HEADACHE

The four dimensions of data processing are problematic:

- one retention time in the 1st dimension
- one retention time in the 2nd dimension
- one ion mobility drift time (CCS value)
- one m/z value

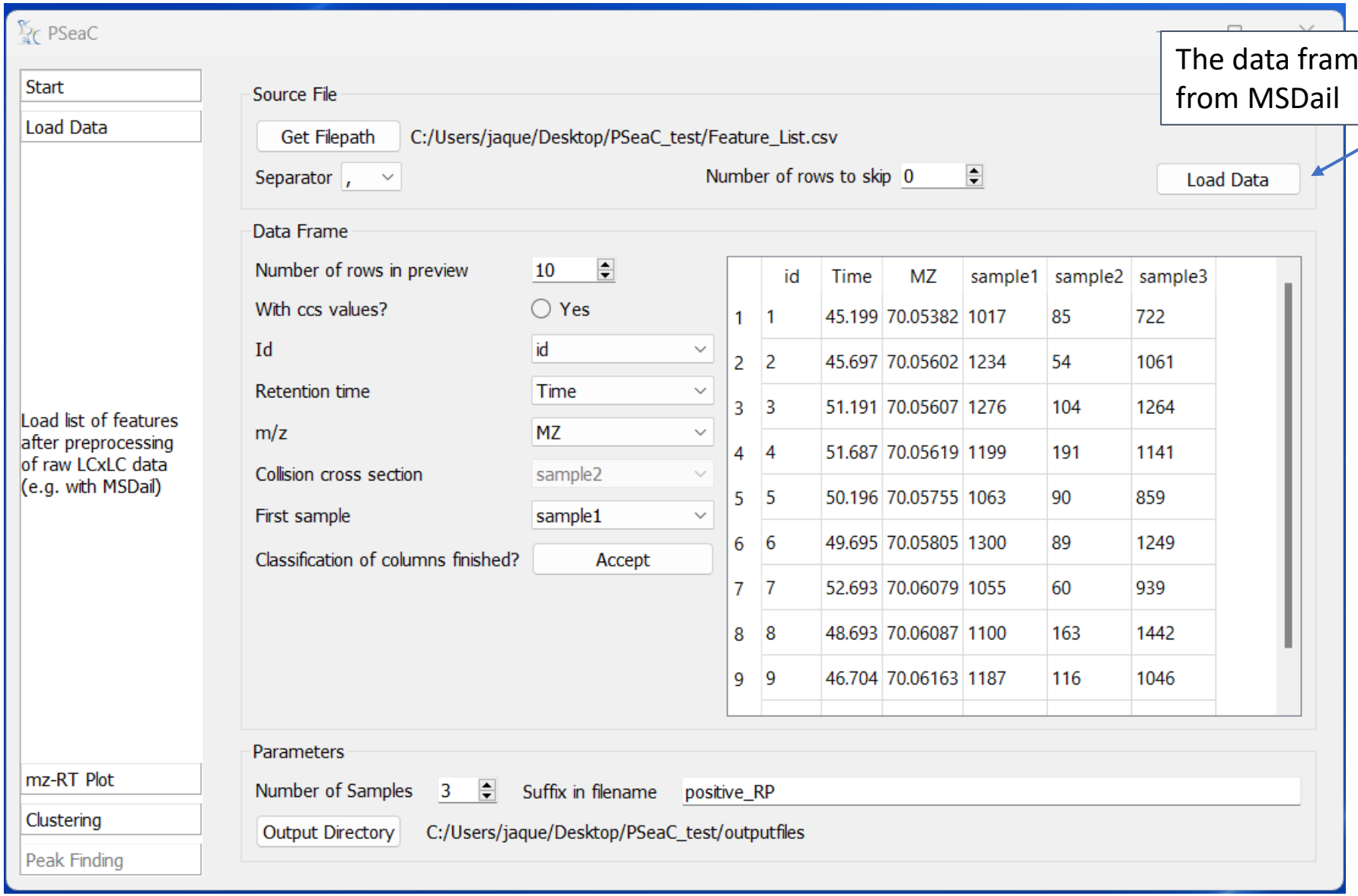
How should the data be displayed in four dimensions?

Stand-alone program for combining peaks in LC × LC experiments

Jaqueline Leddin

After measurements, MSDial is used for peak finding and integration. After this preprocessing a list of features with averaged m/z values, retention times and peak height or area for intensities is generated. The python script, named PSeaC, takes this list and clusters by m/z values and retention time to combine signals of one feature in different modulations. After clustering, the intensities are summed up, if they have peak shape.

How it works

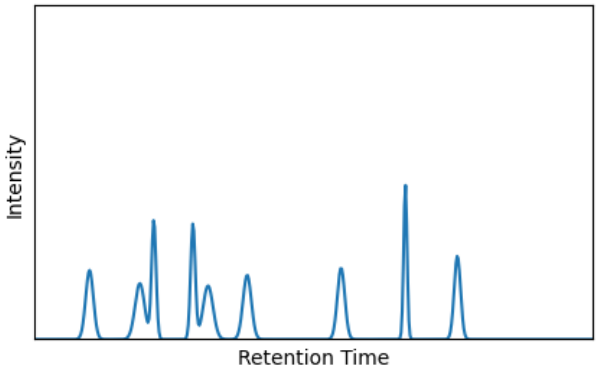


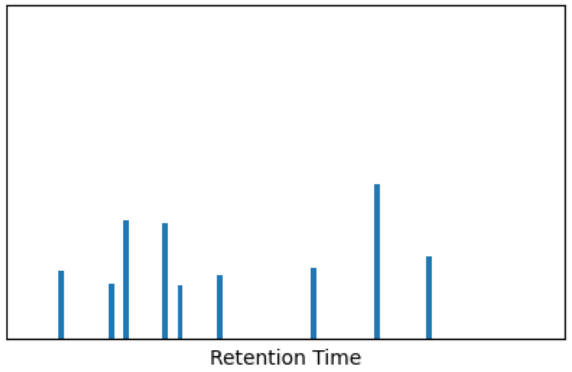
The data frame is exported from MSDail

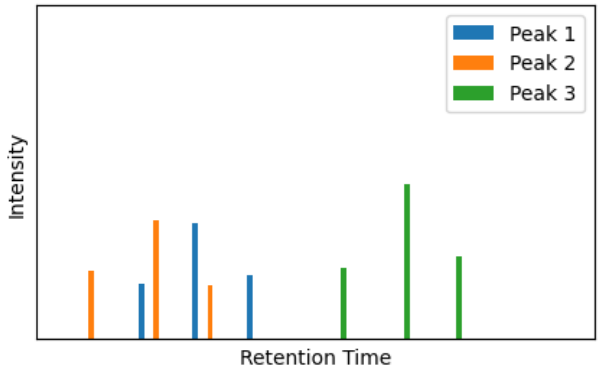
Combining features of LC × LC experiment

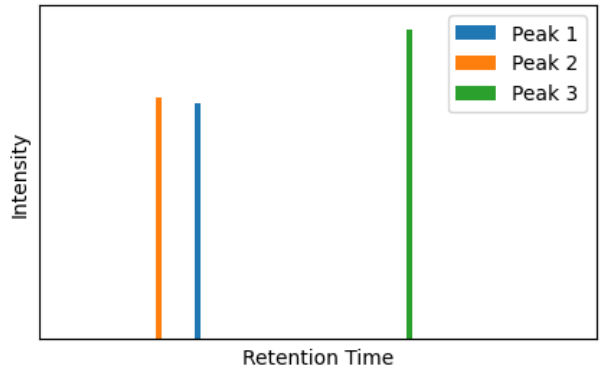
Peak **Se**lection **a**nd **C**ombining

LC x LC experiment → Feature finding → Combining of features









Time	MZ	Sample

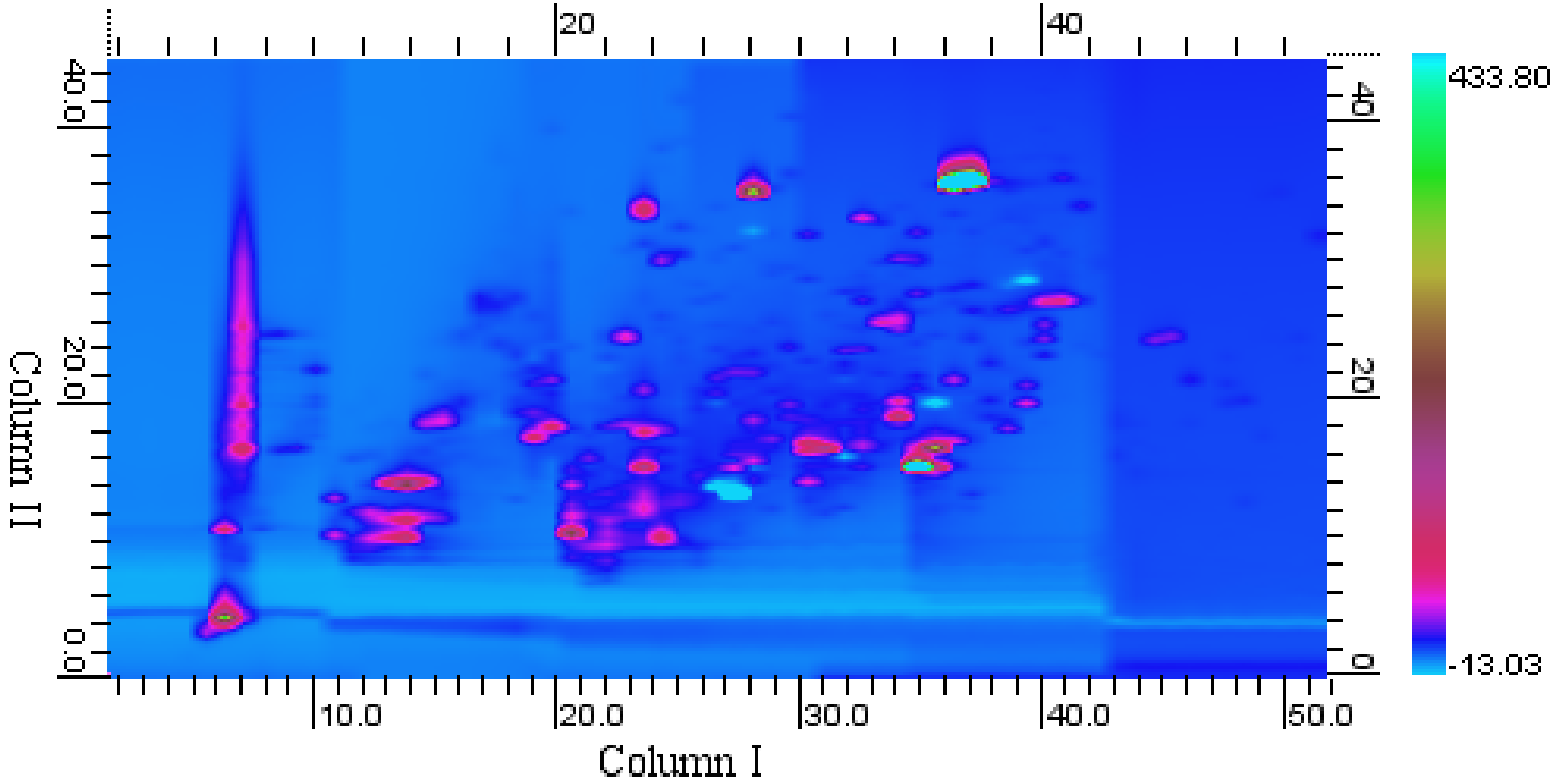
List of features

Time	MZ	Sample

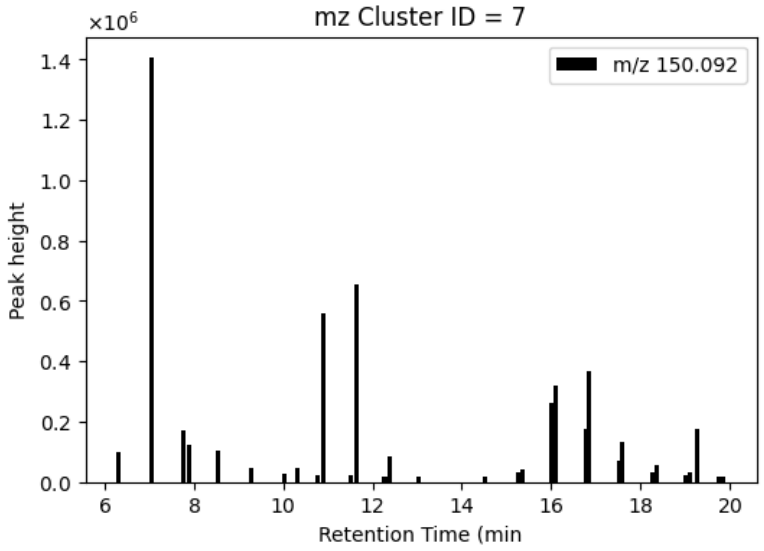
Time	MZ	Sample

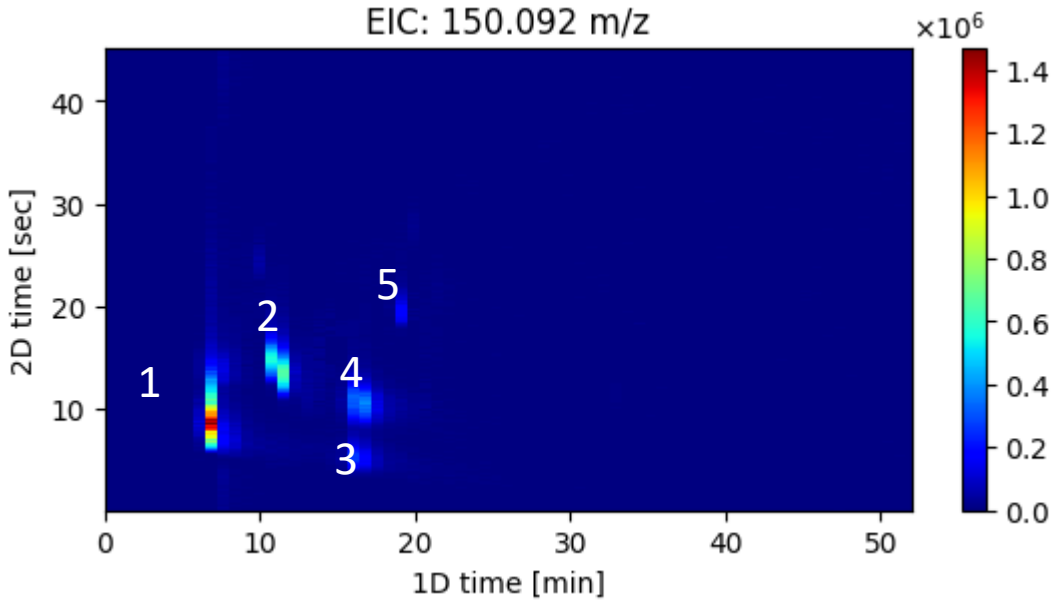
42

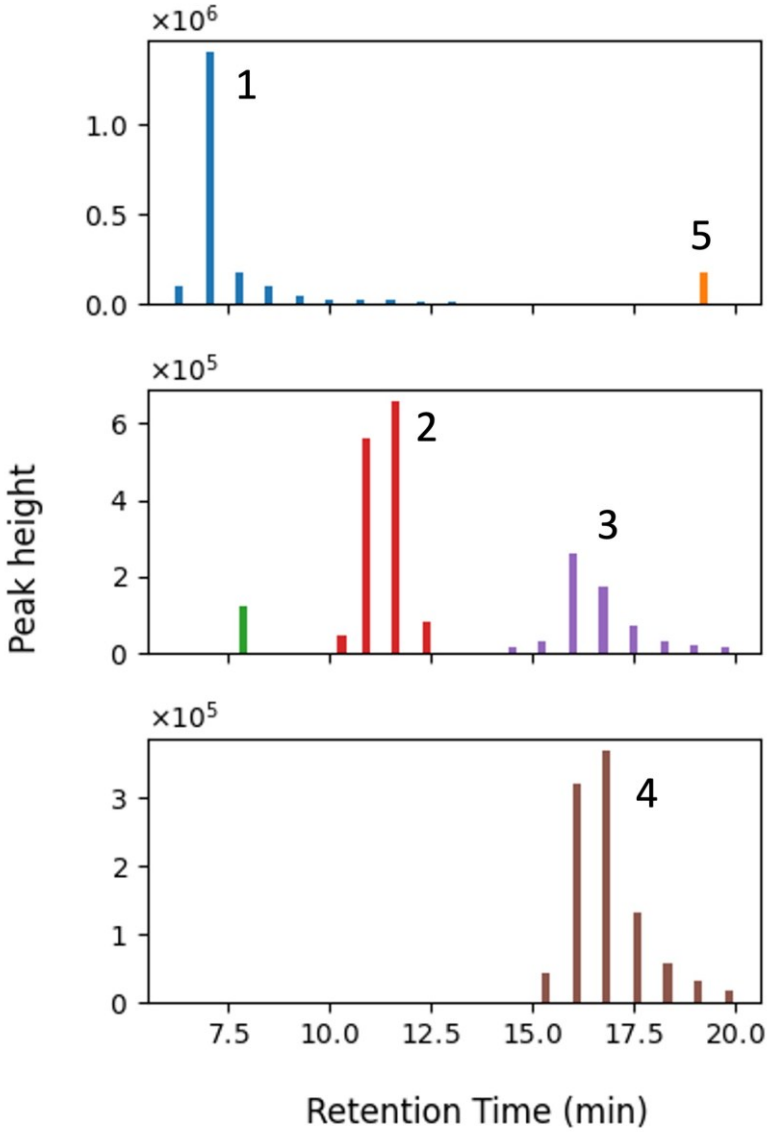
LC × LC-MS measurement with Underberg



m/z Cluster 150.092

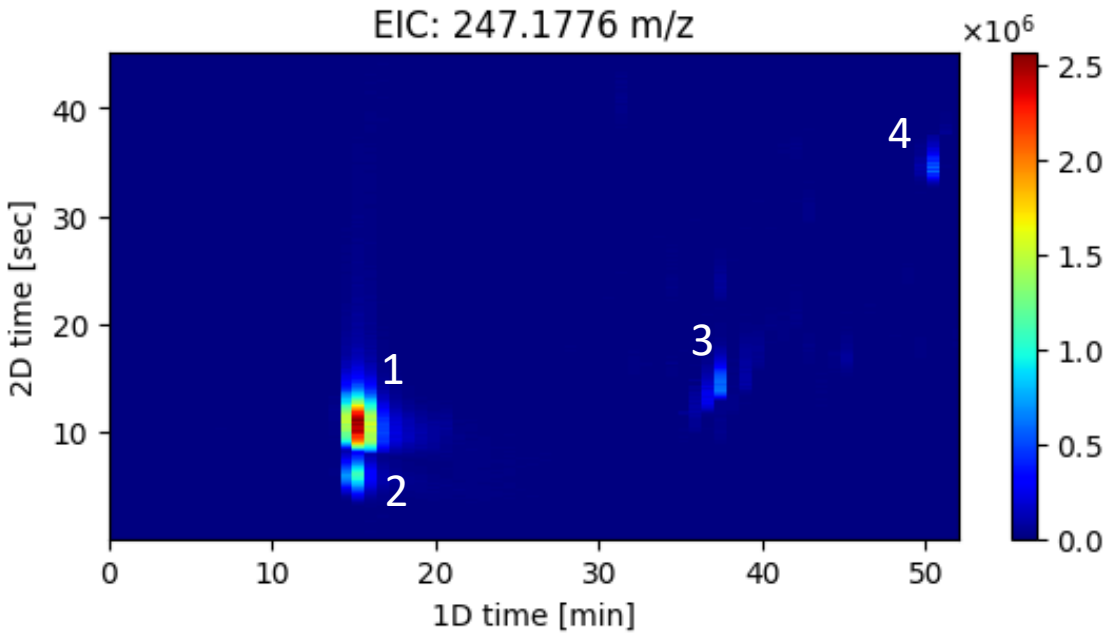


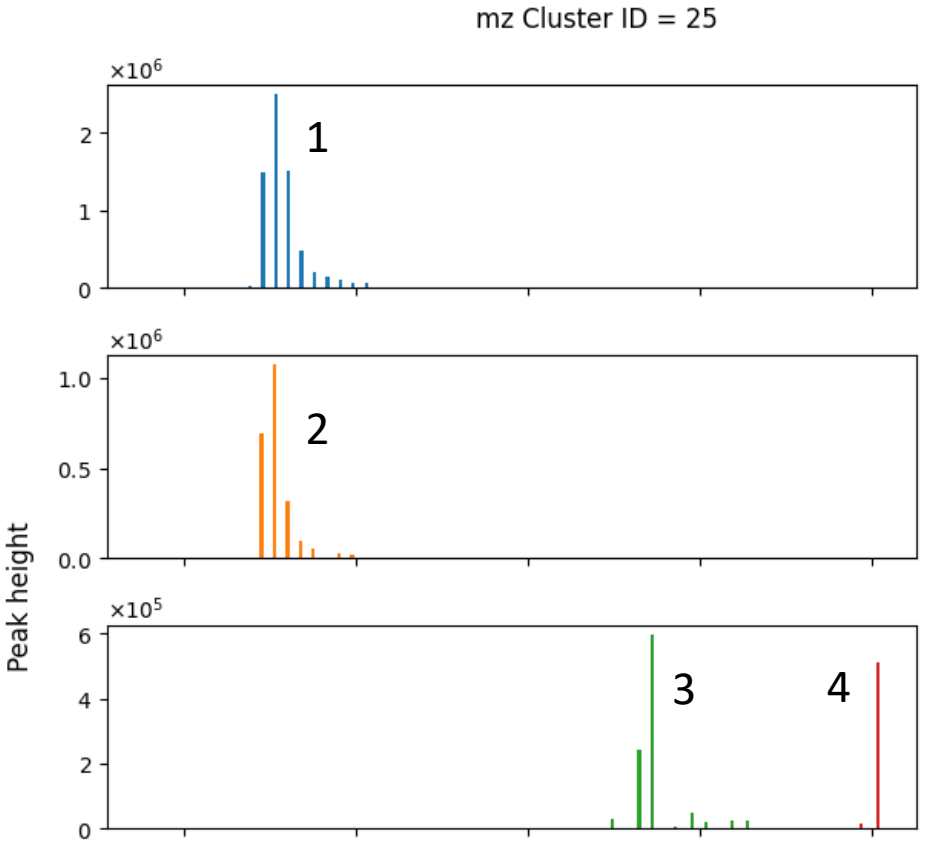




The algorithm can separate feature 3 and 4 by the modulation time.

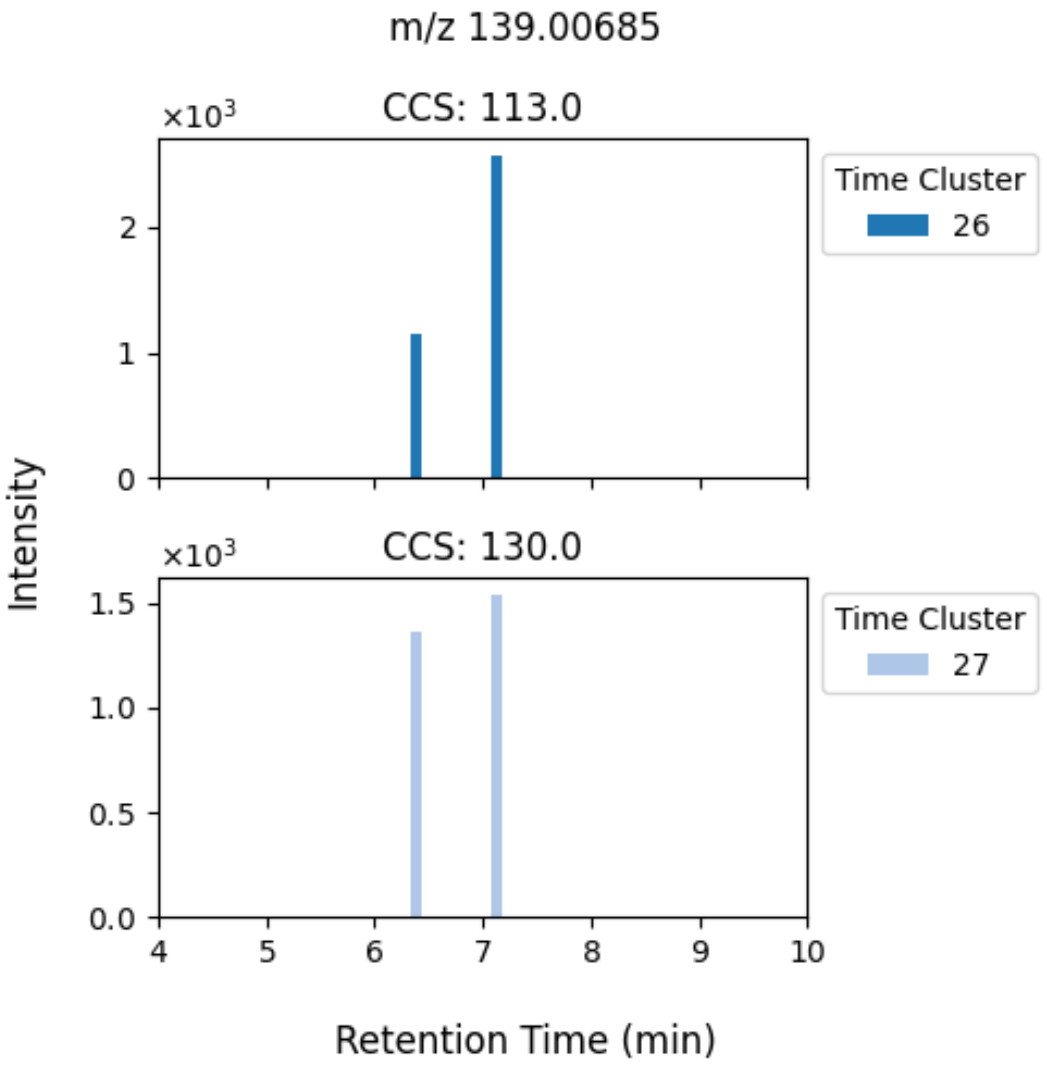
m/z Cluster 247.1776

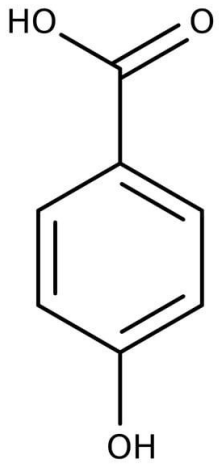


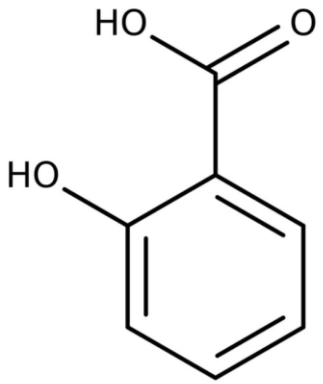


Peak 1 and 2 are separated by the time differences within a modulation.

LC × LC-DTIM-qTOF-MS analysis of 4-Hydroxybenzoic acid und Salicylic acid

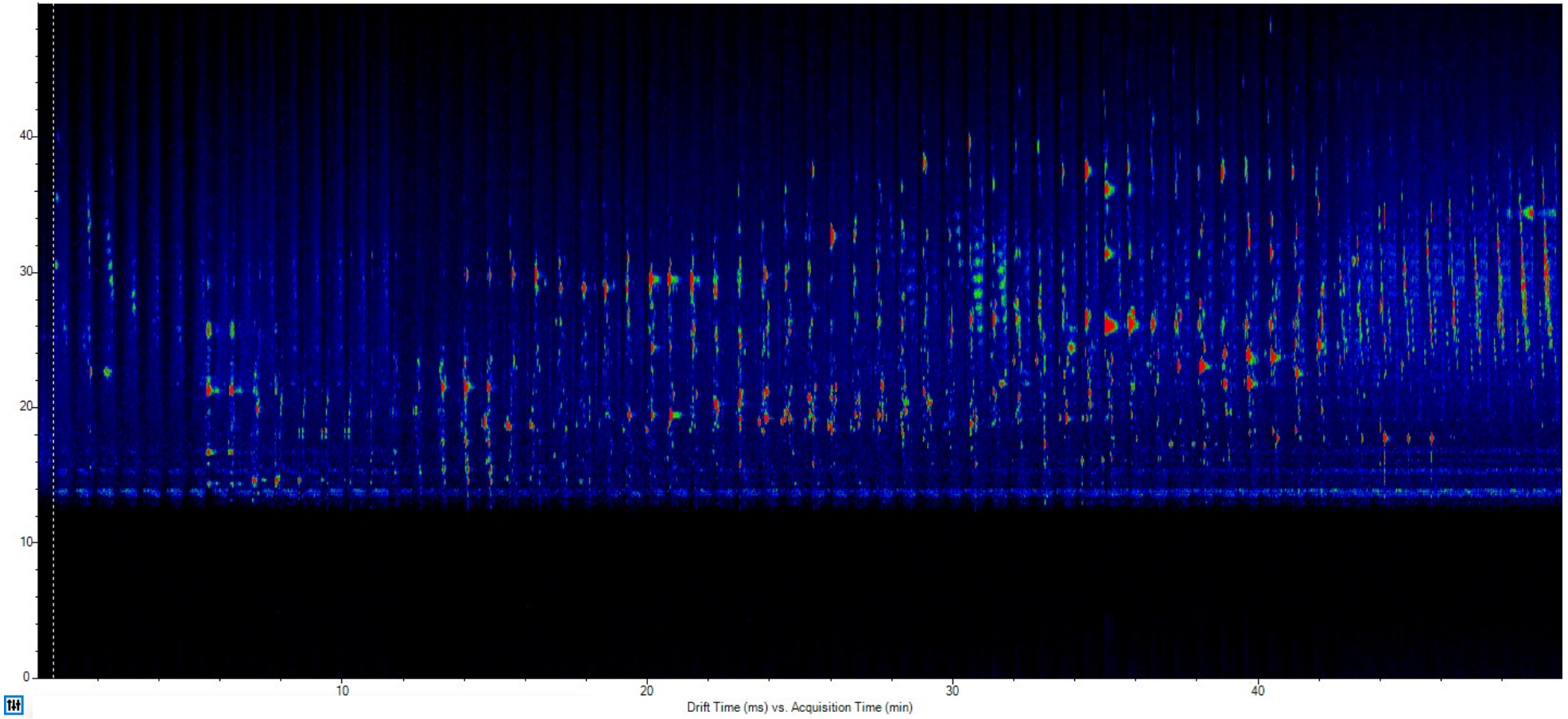




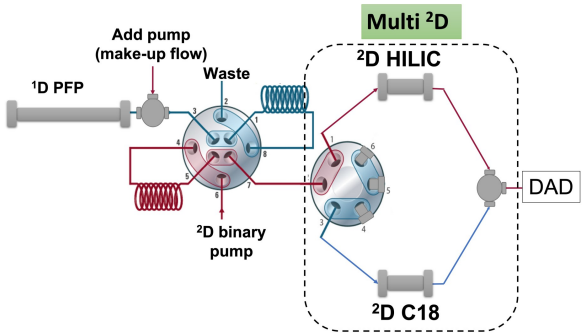


Both substances have the same retention time in two dimensions and the same m/z values, but different values for CCS

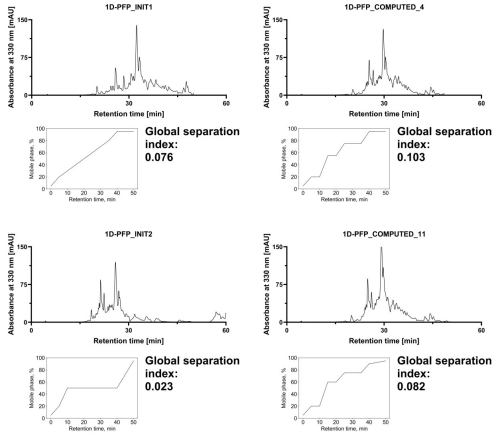
LC × LC-DTIM-MS results of Underberg



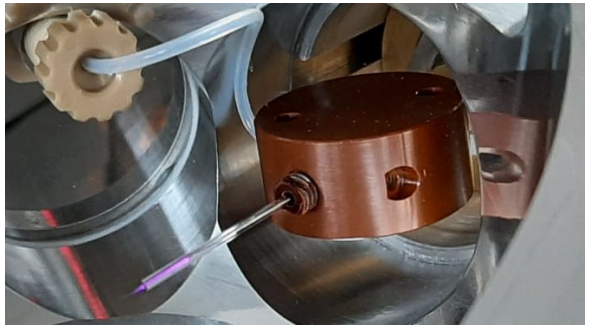
Summary



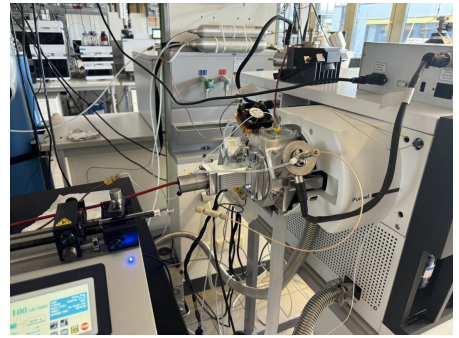
Multi-²D LC × LC-DAD



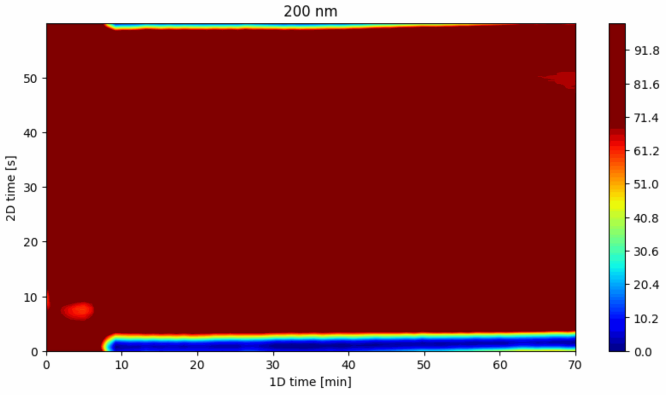
Bayesian optimization of separation gradients



Dielectric barrier discharges (DBD) ion source

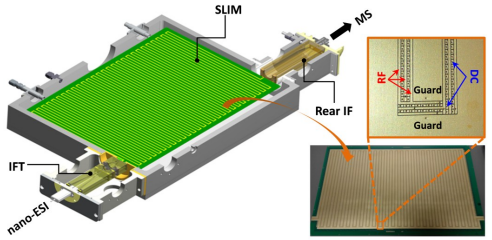


Dual ion source

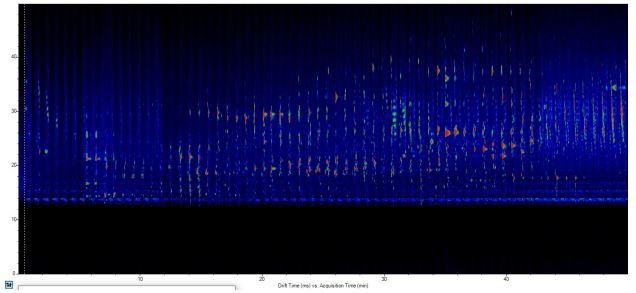


Useful tool for data analysis

Combining features of LC × LC experiment



LC-SLIM-MS for Lipidomics



LC × LC-DTIM-qTOF-MS

Special thanks go to my group

Agilent Technologies

Hitachi High-Tech

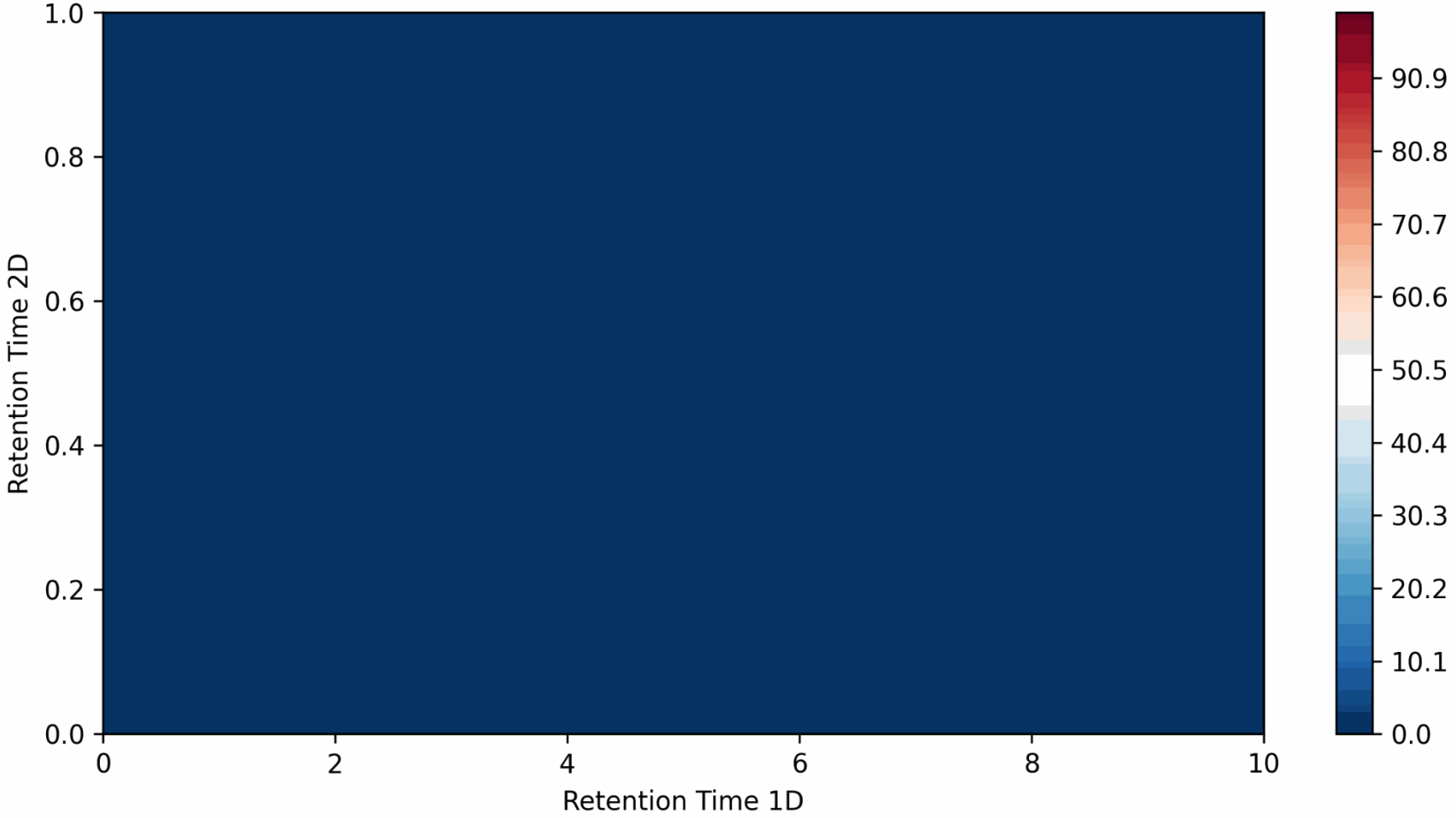
MOBILion SYSTEMS, INC.

DFG Deutsche Forschungsgemeinschaft

SPONSORED BY THE Federal Ministry of Education and Research

and former team members:

Lidia Montero
Juan F. Ayala-Cabrera



more information:

…for your Attention!