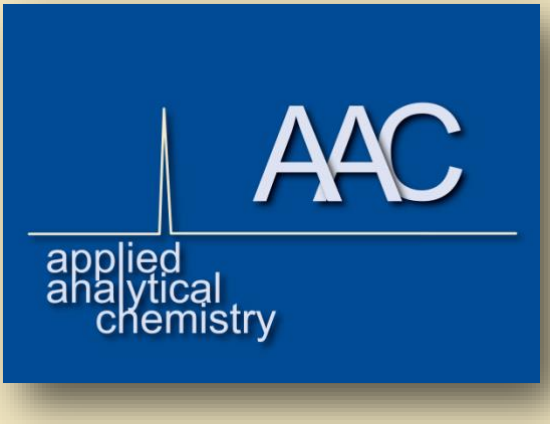
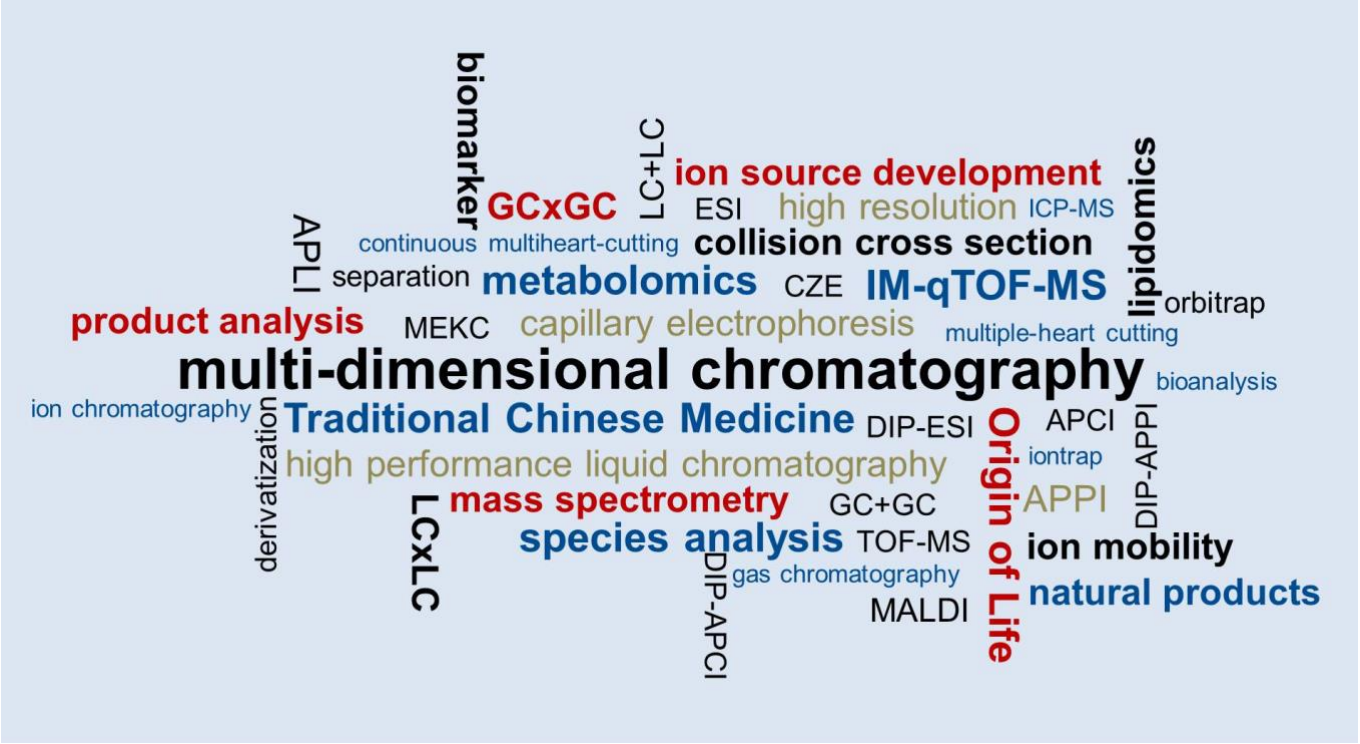
Applied Analytical Chemistry (AAC)

Annual Report 2024



Applied Analytical Chemistry

Annual Report 2024



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Faculty of Chemistry
Applied Analytical Chemistry
Universitaetsstr. 5
45141 Essen
Germany

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The Year 2024 at a Glance

As every year, shortly before Christmas, I am sending you the annual report of Applied Analytical Chemistry (AAC) at the University of Duisburg-Essen to give you a brief insight into the work carried out at the AAC this year.

The AAC is part of the Faculty of Chemistry at the University of Duisburg-Essen (UDE) and exists since September 2012 with the main focus on the development of novel ion-sources for mass spectrometry, the non-target analysis of complex samples by multi-dimensional separation techniques in combination with ion mobility and high-resolution mass spectrometry, metabolomics/lipidomics and investigation about single cell metabolome analysis.

2024 was the twelfth year of the Applied Analytical Chemistry research group at the University of Duisburg-Essen and the most successful to date. This year was a bit special as I took a sabbatical from July 15, 2024 to January 8, 2025 and spent that time in Vancouver, Canada.

The most important thing in 2024 was that we renewed old collaborations and started some important and forward-looking collaborations with different groups. I would like to highlight some cooperation partners here. First, we started a collaboration with Trent Northen from Lawrence Berkeley National Lab, David Chen and Huan Tao, both from the University of British Columbia, the company Thyssen-Krupp, Jean-Marie Demandja from the FDA and Ralf Schäfer and Alexander Probst, both from the University of Duisburg-Essen. The collaboration with Mobilion that began last year was successfully continued. A Slim 2.0 next-generation ion mobility mass spectrometer, the first in Germany, was installed in our laboratory in December and led to great joy among the team, as you can see in the photo.

In 2018, we entered into a partnership with Agilent Technologies. In the course of this cooperation, Agilent provides us with a variety of analytical systems. As in 2021, we also had the opportunity in 2024 to exchange these fantastic instruments for the latest versions. Without this outstanding equipment, much of the work in this report would not have been possible. Therefore, a big thank you to Agilent Technologies at this point.

Teaching and Research Center for Separation

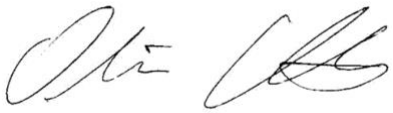
Agilent Technologies

In addition, we also want to mention our long-standing and extremely successful collaboration with Hitachi-High Tech (Japan), which unfortunately came to an end this year. Thank you very much for your trust in us.

In 2024 we managed to publish 13 scientific papers in peer-reviewed journals and seven further manuscripts are in the review process. Seven posters and 20 lectures at national and international conferences were given.

In addition, four PhD, five master's, and seven bachelor's theses were completed in 2024 in AAC and several projects, were started or continued, e.g. development and optimization of new ion sources and investigation of the metabolome/lipidome in cancer research.

We wish you all the best, good health, happiness, and success for the year 2025.



Vancouver, December 17, 2024

AAC Research Group

Regular Staff

Prof. Dr. Oliver J. Schmitz	Head
Dr. Sven Meckelmann	Senior Researcher
Dr. Florian Uteschil	Senior Researcher
Constanze Dietrich	Technician / Lab
Sandy Kerwien	Office Manager

Post-Docs

Dr. Işıl Gazioğlu
Dr. Jaqueline Leddin
Dr. Yassine Oulad El Majdoub
Dr. Florian Stappert
Dr. Tatyana Tishakova

Ph.D. Students

University Duisburg-Essen	External
Maha Alhasbani	Sarah Fasbender
Paul Görs	Anneke Niehuus
Marvin Häßler	Simon Schastok
Martin Meyer	
Alexandra Pape	
Jonas Rösler	
Cedric Thom	
Katharina Wetzel	
Pia Wittenhofer	
Ling Tang	

M.Sc. Students (Master's Theses Accomplished 2024)

Christopher Julian Jaeger, Sarah Klaus, Matthias Miertz, Laila Orell, Lennard Warnecke

B.Sc. Students (Bachelor's Theses Accomplished 2024)

Nadezhda Dimitrova, Friederike Jahr, Leonardo Nuredin, Nico Pernberg, Claudia Meike Rzepinski, Paula Schneyer, Anna Maria Wegenaer

Guest Scientists

Prof. Abdalla Ahmed Elbashir (King Faisal University, Saudi Arabia), Assoc. Prof. Dr. Abul Khayer Mallik (AvH-fellower), Dr. Taher Sahlabji (King Khalid University, Saudi Arabia), Federica Vento (University of Messina, Italy)

Apprentices

Leonie Nufer, Jan Müller

Major News 2024

New Cooperation

During a delegation trip by the Faculty of Chemistry at UDE to Lawrence Berkeley National Laboratory, Lawrence Livermore National Laboratory and NASA Ames in February, many cooperation talks took place and a cooperation between the AAC and the working group of Trent R. Northen from the Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory started shortly afterwards in the field of soil organic matter.

Mass Spectrometry Center for Omics

In 2024 we were able to continue our successful cooperation on metabolomics with the group of Prof. Alpaslan Tasdogan from the Clinic for Dermatology at University Hospital Essen (Germany), the group of Prof. Ömer H. Yilmaz from MIT (USA) and others.

To further expand our collaboration, Prof. Tasdogan and I share several PhD students and a new LC-qTOF has been funded by Prof. Tasdogan to increase our sample throughput in the future.

Some important publications from the cooperation with Prof. Alpaslan Tasdogan

Together with colleagues from Brazil, Germany, Italy, USA and Turkey, we have recently published an article about short-term post-fast refeeding in Nature:

S. Imada, H. Shin, S. Khawaled, S. W. Meckelmann, C. A Whittaker, R. Oliveira Corrêa, Y. Lu, G. Tie, D. Pradhan, G. Calibasi-Kocal, L. Martins Nascentes Melo, G. Allies, **J. Rösler, P. Wittenhofer**, J. Krystkiewicz, **O. J. Schmitz**, J. Roper, M. Aurelio Ramirez Vinolo, L. Ricciardiello, E. C. Lien, M. G. Vander Heiden, R. A. Shivdasani, C.-W. Cheng, A. Tasdogan, Ö. H Yilmaz, *Short-term post-fast refeeding enhances intestinal stemness via polyamines.* Nature (2024) https://doi.org/10.1038/s41586-024-07840-z. In this project we generated and analyzed the metabolomics data about the polyamines.

Another paper in the Nature family was published together with Prof. Tasdogan and colleagues from the USA in Nature Cancer about liver-metastatic organotropism:

M. Rogava, T. J. Aprati, W.-Y. Chi, J. C. Melms, C. Hug, S.H. Davis, E.M. Earlie, C. Chung, S. K. Deshmukh, S. Wu, G. Sledge, S. Tang, P. Ho, A. D. Amin, L. Caprio, C. Gurjao, S. Tagore, B. Ngo, M. J. Lee, G. Zanetti, Y. Wang, S. Chen, W. Ge, L. Martins Nascentes Melo, G. Akkies, **J. Rösler**, G. T. Gibney, **O. J. Schmitz**, M. Sykes, R. J. Creusot, T. Tüting, D. Schadendorf, M. Röcken, T. K. Eigentler, A. Molotkov, A. Mintz, S. F. Bakhoum, S. Beyaz, L. C. Cantley, P. K. Sorger, **S. W. Meckelmann**, A. Tasdogan, D. Liu, A. M. Laughney, B. Izar, *Loss of Pip4k2c confers liver-metastatic organotropism*, Nature Cancer (2024) doi.org/10.1038/s43018-023-00704-x

This work demonstrate a rare example of metastatic organotropism through co-option of physiological metabolic regulation, and proposes therapeutic avenues to abrogate these mechanisms. In this project we generated and analyzed metabolomics data.

A paper was recently published in Science Advances in which we could show that functional mtDNA is favored during melanoma growth and supports metastatic entry into the blood.

S. D. Shelton, S. House, L. Martins Nascentes Melo, V. Ramesh, Z. Chen, T. Wei, X. Wang, C. B. Llamas, S. Sai Krishna Venigalla, C. J. Menezes, G. Allies, J. Krystkiewicz, **J. Rösler**, **S. W. Meckelmann**, P. Zhao, F. Rambow, D. Schadendorf, Z. Zhao, J. G. Gill, R. J. DeBerardinis, S. J. Morrison, A. Tasdogan, P. Mishra, *Pathogenic mitochondrial DNA mutations inhibit melanoma metastasis*, Science Advances (2024) https://doi.org/10.1126/sciadv.adk8801

In this project we generated and analyzed metabolomics data.

Hero of the Year 2024

Sebastian Löbbecke

In 2024, Sebastian published one manuscript to Talanta and another one will be submitted very soon (both as the first author).

He also gave two oral presentations at an Agilent workshop and an international conference (ISC 2024 in Liverpool, UK).

And all of this before he had really started his Master's thesis.

List of Projects 2024

(Abstracts of these projects within the next pages)

Determination of collision cross sections of dendrimer samples by stepped field method for the use as a calibration standard in IM-MS applications
Cedric Thom, Florian Stappert

Challenges in protein analysis using HPLC-MS/MS
Matthias Miertz, Florian Uteschil

Design of a Dual Ion Source coupled to HRMS to enhance the chemical characterization of comprehensive 2D-LC of European Medicinal Plants
Marvin Häßler, Katharina Wetzel, Florian Uteschil, Juan Ayala Cabrera

Potential of tube plasma ionisation for the determination of growth promotors
Sebastian Löbbecke, Juan Ayala-Cabrera, Florian Stappert, Florian Uteschil

New Tools in Cancer Metabolomics – Ion Source Development for Single Cell Analysis
Jonas Rösler, Friederike Jahr, Florian Uteschil, Alpaslan Tasdogan

Evaluation of different electrode materials and dimensions for tube plasma ionisation for LC-MS of polyphenols and small organic molecules
Marvin Häßler, Sebastian Löbbecke, Florian Uteschil

Method development for aliphatic hydrocarbons in complex samples
Sebastian Löbbecke, Jaqueline Leddin, Florian Uteschil

Derivatization strategies for the determination of alkylamines (C2-C8) in yogurt by GC-APLI-(Iontrap)MS
Ling Tang, Florian Uteschil

Orthogonal separation of complex matrix in comprehensive two-dimensional liquid chromatography (LCxLC) employing Sil-Lys-2C18 as stationary phase in both dimensions
Abul K. Mallik, Yassine Oulad El Majdoub

Multi-^{2}D LC × LC-HRMS for the analysis of the *Sambucus nigra L.* leave extract to enhance the separation power of conventional LC × LC
Katharina Wetzel, Priscilla Nhan, Tatyana Tishakova

Effected-directed analysis for the identification of antioxidative compounds of European medicinal plants
Katharina Wetzel, Tatyana Tishakova, Marvin Häßler

Assessing the characterization and differentiation of *Sambucus Nigra* plants from different European geographical areas by LC-HRMS
Marvin Häßler, Katharina Wetzel, Lennard Warnecke, Lidia Montero, Juan Ayala Cabrera

Characterisation and identification of antioxidant compounds from *A. Eupatorium, A. Archangelica, S. Nigra* and *S. Ebulus* by effector-oriented analysis of their fractions by LC-MS and elemental distribution by ICP-OES
Marvin Häßler, Katharina Wetzel, Nadezhda Dimitrova, Juan Ayala Cabrera

Identification of double bond positions in fatty acids by in-source fragmentation
Paul E. Görs, Sven W. Meckelmann

Green lipid extraction
Pia Wittenhofer, Sven W. Meckelmann

Heart-cut liquid chromatography and mass spectrometrie to separate structural isomers of the cholesterol biosynthesis
Pia Wittenhofer, Sven W. Meckelmann

Unveiling the metabolome and lipidome of *Haloarcula sp.*
Marvin Häßler, Sven W. Meckelmann

Application for visualizing LC × LC – DAD data
Jaqueline Leddin, Katharina Wetzel

PSeaC – Application for feature combining in LC × LC data
Jaqueline Leddin, Sven W. Meckelmann

Targeted metabolite identification and quantification for Enzyme activity profiling
Constantin P. Krempe, Jonas Rösler, Sven W. Meckelmann

Fast and robust metabolome screening in cancer research
Jonas Rösler, Constantin P. Krempe, Jaqueline Leddin, Sven W. Meckelmann, Alpaslan Tasdogan

Quantitative analysis of polyamine synthesis in intestinal stem cells
Jonas Rösler, Pia Wittenhofer, Sven. W. Meckelmann

Implementation of automated sample preparation and script assisted data analysis in the metabolome analysis
Jonas Rösler, Jost Guinand, Jaqueline Leddin, Sven W. Meckelmann, Alpaslan Tasdogan

Changes within the lipidomic profile induced by different medication strategies in cancer therapy
Jonas Rösler, Luiza Martins, Nascentes Melo, Sven W. Meckelmann, Alpaslan Tasdogan

Metabolic effect in treatment strategies for heart disease
Jonas Rösler, Feyza Cansiz, Luiza Martins Nascentes Melo, Gabriele Allies, Sven W. Meckelmann, Alpaslan Tasdogan

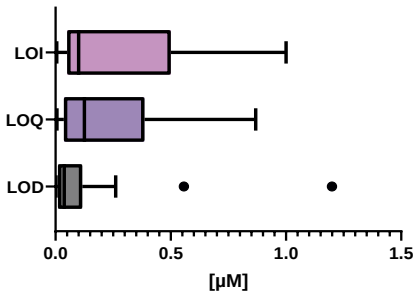
Effects on the metabolome in the central carbon metabolism for genetic deficient liver cells by HILIC-Orbitrap-MS
Jonas Rösler, Feyza Cansiz, Luiza Martins Nascentes Melo, Gabriele Allies, Sven W. Meckelmann, Alpaslan Tasdogan

Dose dependent impact of polyamine inhibition on the global metabolome
Jonas Rösler, Feyza Cansiz, Sven W. Meckelmann, Alpaslan Tasdogan

Metabolic differences of efficient and inefficient metastasizers in cancer disease
Jonas Rösler, Feyza Cansiz, Sven W. Meckelmann, Alpaslan Tasdogan

Determination of collision cross sections of dendrimer samples by stepped field method for the use as a calibration standard in IM-MS applications

Cedric Thom, Florian Stappert



Ion mobility mass spectrometry (IM-MS) is rising in popularity over the last years due to its application fields and improving instruments. Its ability to separate ions based on their collision-cross section (CCS) provides another compound specific parameter which can be used for identification. Since the CCS value of an analyte is dependent on external parameters calibration of m/z and CCS is mandatory, for repeatable and accurate results in IM-MS measurements. Dendrimers, highly branched polymers used for calibrating MALDI-MS, have been proposed as calibration standards for IM-MS applications due to their broad mass range and monodisperse synthesis. Up to this date the IM-MS community has not agreed on an universal calibration standard.

In a previous study, we measured four dendrimer mix standards provided by a collaboration partner to determine their CCS values and compare them across different IM-MS platforms. Therefore the stepped-field method was used to enable the CCS determination without calibration standards. The results of this study were published in J. Am. Soc. Mass Spectrom. 2024, 35, 1101-1109.

In this study the CCS of three different dendrimer mix standards, containing ten dendrimers each, were determined by the stepped-field method using an Agilent 6560 DTIMS-QTOF.

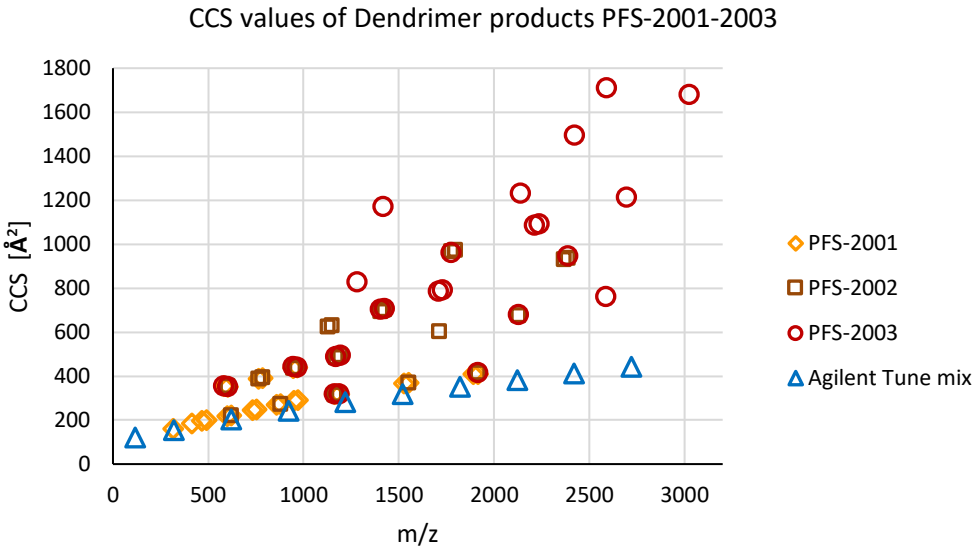


Fig. 1: Comparison of collision-cross section (CCS) values of dendrimer samples PFS-2001, PFS-2002, and PFS-2003 and the Agilent ESI Low concentration Tune mix.

The here shown mix standards consisted of more analytes which resulted in a higher coverage. Further the CCS calibration using the dendrimers will be compared to the conventional calibration standard to determine whether a larger coverage in CCS values is beneficial. Therefore, protein standards will be measured and the resulting CCS values will be compared with the literature to determine the effects of different calibration standards on their CCS values.

Collaborative Project – Project Partner: Jens Sommertune (Polymer Factory Sweden AB, Sweden, Stockholm)

Challenges in protein analysis using HPLC-MS/MS

Matthias Miertz, Florian Uteschil

Several challenges in protein analysis using HPLC-MS/MS are addressed, identifying key issues and implementing effective optimization strategies to improve performance and reliability. A significant problem was protein adsorption to the surfaces of sample containers and the LC-system's interior walls, which caused inconsistent results and carry-over effects. This was mitigated by using low-binding protein tubes and TORAST-H-Bio vials, which reduced protein waste and enabled overnight measurements. Additionally, replacing steel tubing with PEEK tubing in the LC system minimized adsorption-related carry-over effects. The transition to the more selective MRM mode was achieved by increasing the collision gas flow rate, enabling the detection of unique and reproducible fragment ions with sufficient intensity. Autotuning procedures further enhanced the system's sensitivity by improving ion transmission, although auto-calibration's impact on resolution was minimal. Optimization of the mobile phase composition significantly improved the sensitivity and resolved peak tailing issues. Adjusting from 0.1% FA to a mix of 0.2% FA and 0.1% DFA led to better peak shapes and more consistent results. Furthermore, the established workflow proved adaptable to different analytes, demonstrated by the inclusion of β-Amyloid 40 in the analysis. However, carry-over for this protein highlighted the need for further refinements in mobile phase composition. While the separation aspect was deprioritized to focus on faster analysis times, the system successfully operated within the prescribed flow rate of 50 µL/min. Despite not achieving the requested low pM range limits of detection (LoDs) for PTH and insulin, the LoDs for β-Amyloid 40 and IGF-1 in the low nM range are now attainable. This represents significant progress, especially given that intact protein analysis using HPLC-MS/MS is a relatively undeveloped research area, with comparable advancements only recently reported in 2023. The improvements in sensitivity and peak quality achieved during this thesis underscore the potential of the optimized workflow. These advancements provide a strong foundation for further exploration and refinement in the field of protein analysis. A comparison of insulin analysis results highlights the clear benefits of these innovations, showcasing the system's evolution toward more reliable and efficient performance.

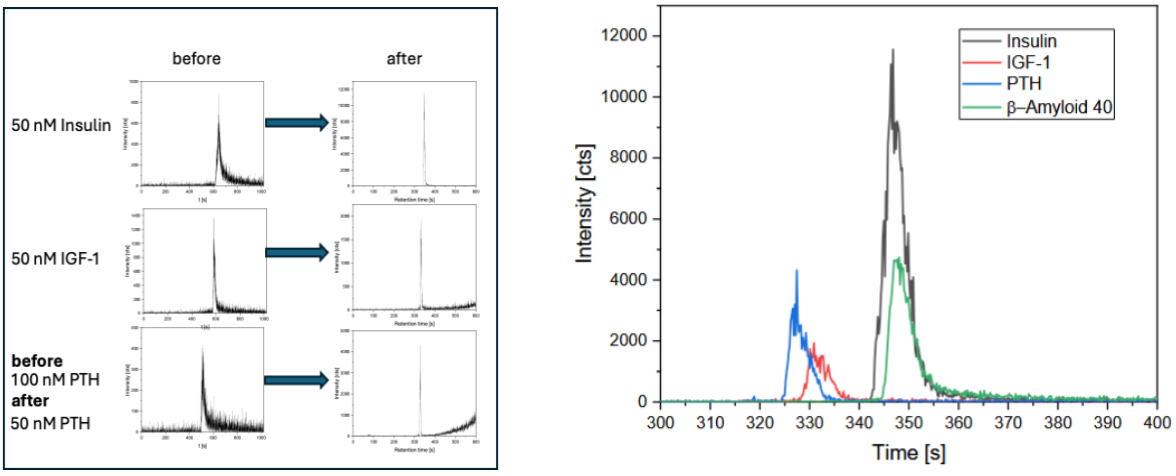


Fig. 1: Optimization of targeted intact proteins analysis and HPLC-MS/MS run of targeted low molecular weight proteins

Collaborative Project – Project Partner: Hitachi High-Tech Corporation, Tokio, Japan
Funded by: Hitachi High-Tech Corporation, Tokio, Japan

Design of a dual ion source coupled to HRMS to enhance the chemical characterization of comprehensive 2D-LC of European medicinal plants

Marvin Häßler, Katharina Wetzel, Florian Uteschil, Juan Ayala Cabrera

Traditional European plant medicine is among the most popular and strongest growing forms of alternative medicine and based on ancient healing practices it still plays a significant role in the treatment of many health conditions. Its impact significantly influenced the creation of therapeutic products by using medical herbs and the market is growing. Because of the complexity of metabolic profiles, the potential of medicinal plants has not been explored yet since the chemical characterization remains a challenge. Comprehensive two-dimensional liquid chromatography (LC x LC) has previously shown the capability of separating very complex matrices [1]. A dual-ion source combining various API techniques has been developed for more universal metabolite detection [2]. Switching ionization sources based on analyte polarity eluting from the LC improves detection. This project aims combining ESI for polar compounds with TPI (Figure 2) for less polar ones in a single measurement. Figure 2 highlights the first proof of concept differences between ion sources. ESI ionizes more compounds, while TPI generates fewer signals but usually avoids ion suppression.

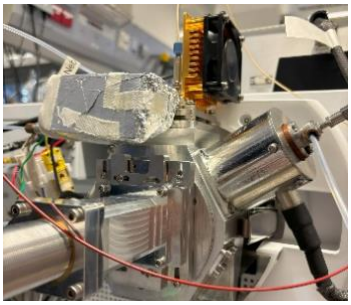


Fig. 1: Dual Ion source. In this configuration, the dual ion source is mounted onto the Agilent Q-TOF mass spectrometer for analytical measurements. The two nebulizers get used separately by switching a valve during the run.

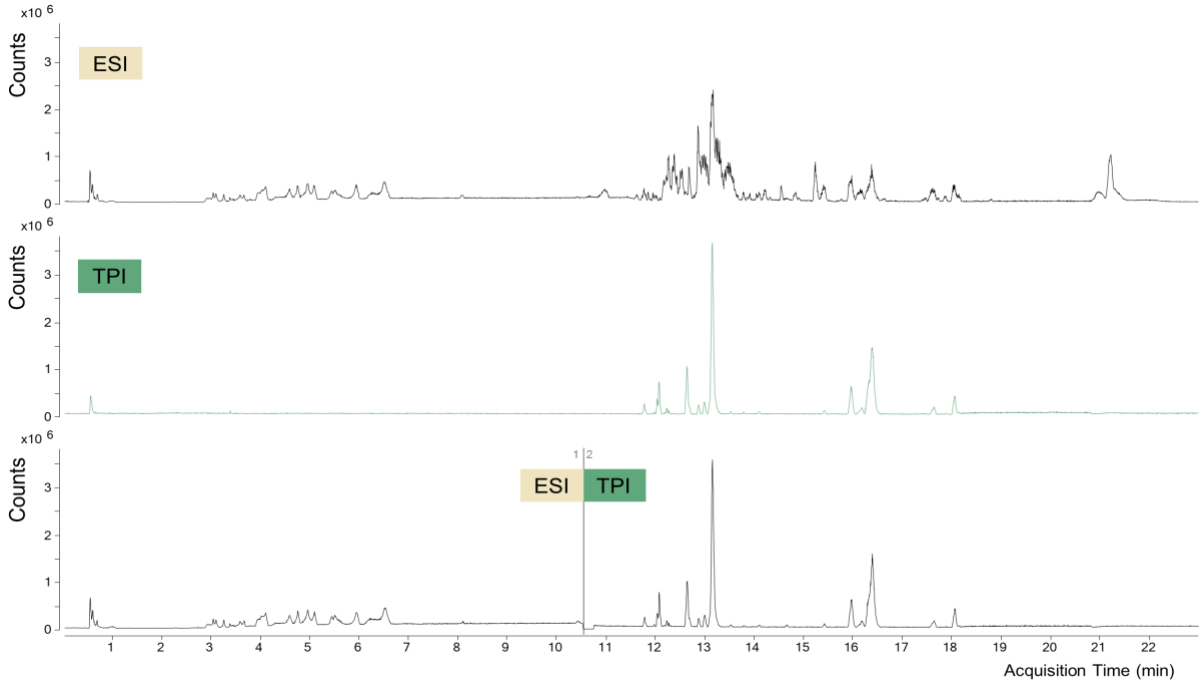


Fig. 2: First proof of concept of ion source switching during a run of *Agrimonia eupatoria* leaves extract. The ion source switching (10.5 min) takes about 10 seconds, because of the plasma equilibration time and the ignition.

Collaborative Project – Project Partner: Agilent Technologies, Santa Clara, USA
Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143

Potential of tube plasma ionisation for the determination of growth promotors

Sebastian Löbbecke, Juan Ayala-Cabrera, Florian Stappert, Florian Uteschil

Certain steroid compounds, including estrogens, androgens, progestogens and stilbestrols, can be used to promote growth in livestock. In the European Union Regilation 96/22/EC banns the use of those compounds in animals due to adverse effects in human and animal health. The analytical methodologies are usually based on either liquid chromatography (LC) or gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS). When using LC-MS electrospray ionisation (ESI) is most widely applied. However, it comes with drawbacks due to ion supression and lower overall performance. Alternative GC-MS usually uses electron ionisation (EI) which leads to high in-source fragmentation and therefore a loss in selectivity. Tube plasma ionisation (TPI) was introduced to overcome the presented analytical challenges and offer soft ionisation of growth promoters. After a careful optimisation of plasma, source and acquisition parameters comparison between different analytical platforms were made. In LC-MS determination TPI was compared to ESI and atmospheric pressure chemical ionisation (APCI), whilst in GC-MS determinations a comparison between TPI and EI can be made. TPI offered instrumental detection limits (iLOD) similar to APCI and significantly lower than those achieved by ESI in a 0.007 – 5.085 µg L^{-1} range. Linearity was comparable with all ion sources whilst APCI offered better sensitivity than TPI. For precision and trueness, TPI and APCI achieved low relative standard deviations and relative errors and showed increased performance compared to ESI. Furthermore, the influence of matrix (bovine urine and meat) on the measurements of TPI and ESI was studied. These experiments showed a decreased matrix effect (40 % lower) in TPI compared to ESI. For the GC-MS approach derivatiation with N-methyl-N-(trimethylsilyl)-trifluoracetamid (MSTFA) is necessary. The GC-MS approach whilst having the downside of necessary derivatisation offers shorter analysis time than LC-MS methods and lower detection limits (0.009 – 3.460 µg L^{-1}). GC-TPI-MS/MS offered soft ionisation of the $[M+2TMS+H]^+$ species with litte in-source fragmentation, increasing selectivity over the GC-EI-MS/MS approach. An interplatform comparison with 10 samples from EURL Growth Promoters (located at WFSR) revealed similar results across the different platforms and proved the good performance of TPI and APCI compared to the routine GC-EI-MS/MS methodology. A high precision (RSD < 15%) was found for EI, TPI and APCI.

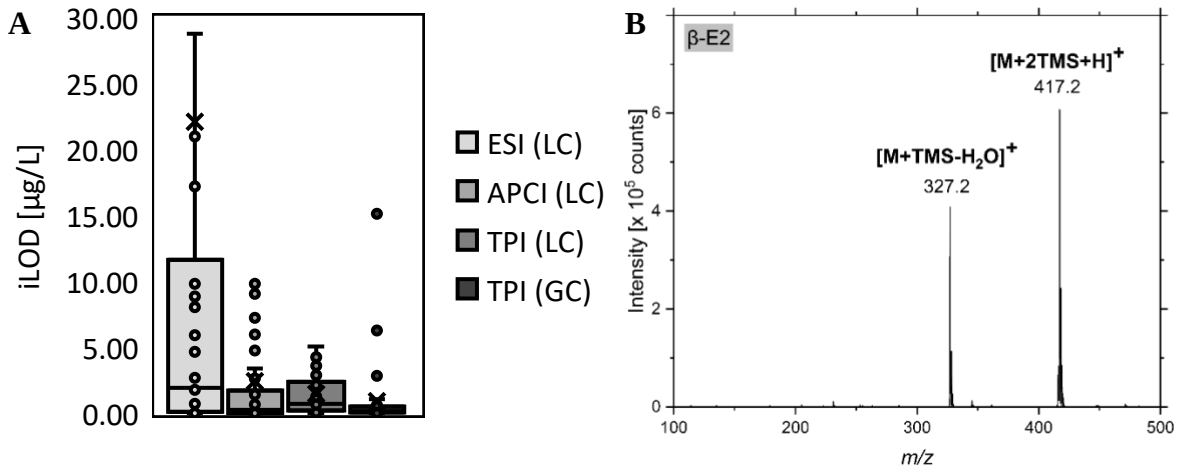


Fig. 1: Detection limits with different ion sources and mass spectro of β-Estradiol.

A: Instrumental detection limits (iLOD) with different ion sources in GC-MS and LC-MS showcasing the good performance of TPI and APCI. B: Mass spectrum of β-Estradiol (β-E2) in GC-MS showing the $[M+2TMS+H]^+$ ion and the $[M+TMS-H_2O]^+$ fragment with very low in source fragmentation.

Collaborative Project – Project Partner: Ane Arrizabalaga-Larrañaga and Marco H. Blokland, Wageningen Food Safety Research (WFSR), Wageningen University and Reseach, The Netherlands

Funded by: Agilent Technologies

New tools in cancer metabolomics – Ion source development for single cell analysis

Jonas Rösler, Friederike Jahr, Florian Uteschil, Alpaslan Tasdogan

Metastasising of cancer cells is responsible for about 90 % of cancer related deaths and therefore key target in cancer research. A major challenge in this field is to unravel the mechanisms of metabolic reprogramming of cancer cells during metastasis, which allows for efficient adaptations to the changing chemical microenvironment. For now, cancer metabolomics still relies on bulk analysis technologies, which are not capable to picture the heterogeneity of the disease and by this lose a big part of the desired information. The emerging field of single cell metabolomics is aiming to cover this gap, but challenges in sensitivity and keeping the cells metabolome unaffected are limiting the current used designs.

This project is aiming to develop a new ion source for single cell mass spectrometry, using ultrasonic nebulization for instant cell lysis during sample vaporization and low temperature plasma ionization for efficient ionization prior to detection by high resolution mass spectrometry.

Building up on previous constructions, already indicating promising results in cells, an improved prototype could be established as shown in figure 1, enabling the exact control of vaporizing and injection conditions. This setup overall lead to a better handling and a vast improvement of repeatability. The operation parameters have been subsequently optimized and the ion source has been tested in different modes of plasma ionization, somehow also indicating possiblities for the more sensitive measurement of polar metabolites.

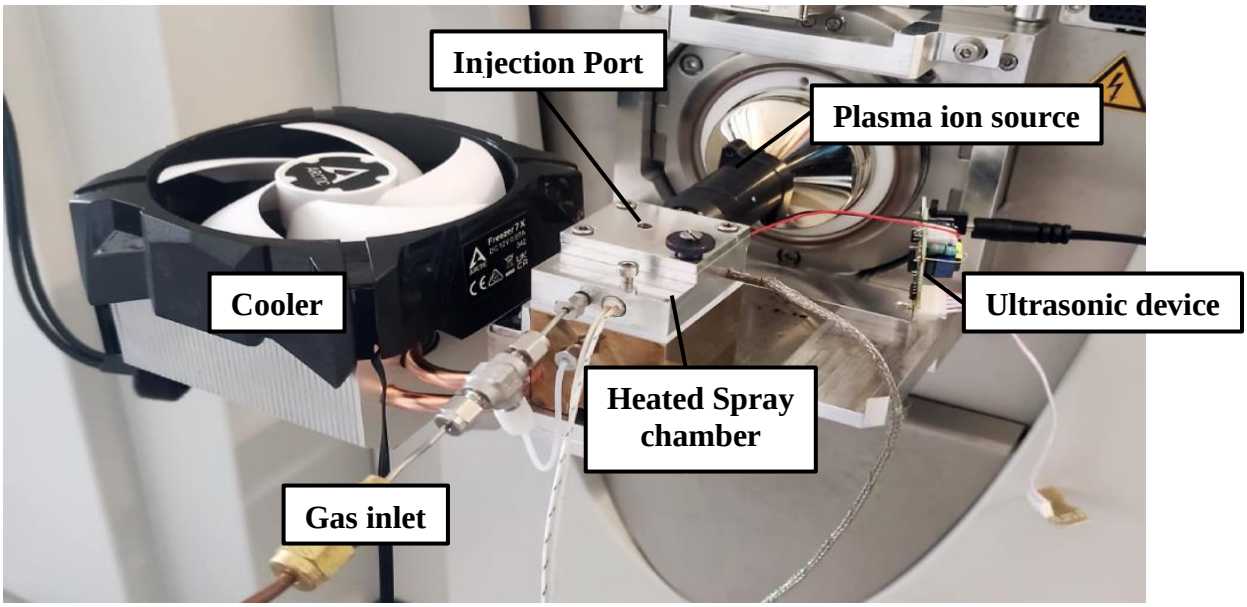


Fig. 1: Setup of the developed ion source. Depicted is the ultrasonic device, which is inside of the heated spray chamber and below the injection port. A distinct gas flow is carrying the formed vapor towards the plasma ion source and subsequently the MS. The ultrasonic device is constantly cooled to maintain a stable performance.

The novel prototype is currently under investigation for further applications in different ionization modes and for coupling to ion mobility spectrometry, further enhancing the scope of this technology.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm)

Evaluation of different electrode materials and dimensions for tube plasma ionisation for LC-MS of polyphenols and small organic molecules

Marvin Häßler, Sebastian Löbbecke, Florian Uteschil

Tube plasma ionisation (TPI), based on a dielectric barrier discharge with a needle electrode, can be used as an alternative ion source to conventional electrospray ionisation (ESI) in LC-MS determination. TPI is, in comparison to ESI, with its APCI-like mechanism especially well suited for smaller and non-polar compounds. To study and possibly enhance the performance of the TPI source further, different electrode materials and diameters were optimised with a design-of-experiment approach and evaluated for thier performance and quality parameters. The materials considered were stainless steel, tungsten, gold, copper, titanium and chromium. Depending on the electrode material and diameter different optimal setpoints for the plasma parameters were found, generally with either short high frequency pulses (2 µs / 18kHz) or longer low frequency (20 µs / 5 kHz) pulses. The high voltage required was usually lower for smaller electrodes and more conductive materials like Gold. Additionally, the discharge gas flow required to be increased with lower electrode diameter, probably due to the increased gas volume in the dielectric tube. With regards to performance no significant differences were found between the electrode materials allowing for the use of cost-effective materials as needle electrodes. Additionally, the diemeter of the electrode was not found to have an influence on the analytical performance in LC-MS. A comparison to electrospray ionisation revealed lower detection limits in ESI, but higher accuracy with TPI. Generally, the performance of the two sources is similar and comparably to the commercially available AJS-ESI source from Agilent. It is to be noted that the analytes tested so far are rather polar and therefore are better ionised by ESI. Further studies in this project incluede less polar analytes as well as an evaluation of the different electrodes for GC-MS.

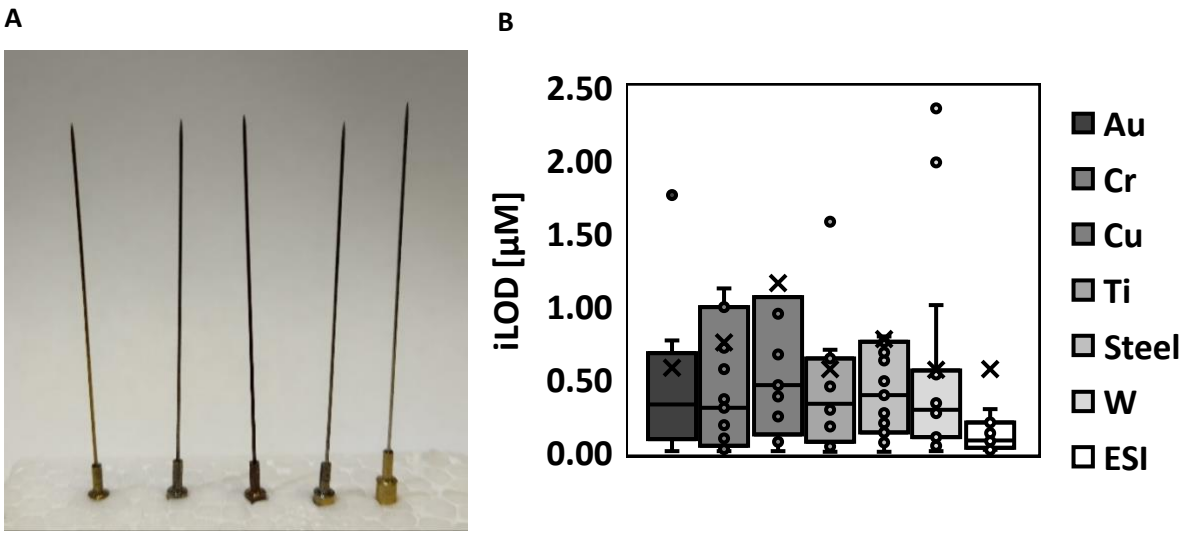


Fig. 1: Different 0.35 mm electrode materials and respective instrumental detection limits (iLOD).

A: showcases the five different electrodes from left to right: Gold, Chromium, Copper, Titanium and Stainless Steel. All shown electrodes are of 0.35 mm diameter. B: shows the respective iLOD reached by the 0.35 mm electrodes as well as the iLOD with a Tungsten electrode (0.52 mm) and the comparison to ESI.

Funded by: Agilent Technologies

Method development for aliphatic hydrocarbons in complex samples

Sebastian Löbbecke, Jaqueline Leddin, Florian Uteschil

The aviation sector was responsible for 3.8 to 4% of all greenhouse gas emissions in the European Union in 2022. With the aim to reduce the carbon footprint in this sector sustainable aviation fuels (SAF) are in the current focus of academic and industrial research. The products, like fossil fuels, are a complex mixture consisting of a large number of hydrocarbons posing an analytical challenge due to their number and structural similarity. Conventional analytical methods include gas chromatography (GC) and two-dimensional gas chromatography (GCxGC) for separation and, amongst others, mass spectrometric (MS) detection. For GC-MS electron ionisation (EI) at 70 eV is most widely applied for ionisation resulting in high fragmentation and loss of the molecular ion. With soft ionisation selectivity and sensitivity may be increased. Therefore, tube plasma ionisation (TPI) and atmospheric pressure photoionization (APPI) are deployed alongside conventional GC-EI-MS. In the first steps of the method development, TPI and APPI methods are optimised for the detection of Olefins. In TPI Olefins are observed as $[M+NO]^+$ adduct ions with very little fragmentation to the carbon chain, whilst in APPI both $[M-nH]^+$ (n = 1 or 2) and $[2M]^+$ dimers were observed. After a carful optimisation of plasma, source and acquisition parameters, detection limits were found to be in the high µg L^{-1} range for TPI and APPI. The next project aims include a comparison to GC-EI-MS as well as the extension of the analyte mix to allow detailed hydrocarbon analysis with paraffins, isoparaffins, olefins, naphthenes and aromatic compounds (PIONA). Further, the application of the method for the analysis of fuel samples is planned.

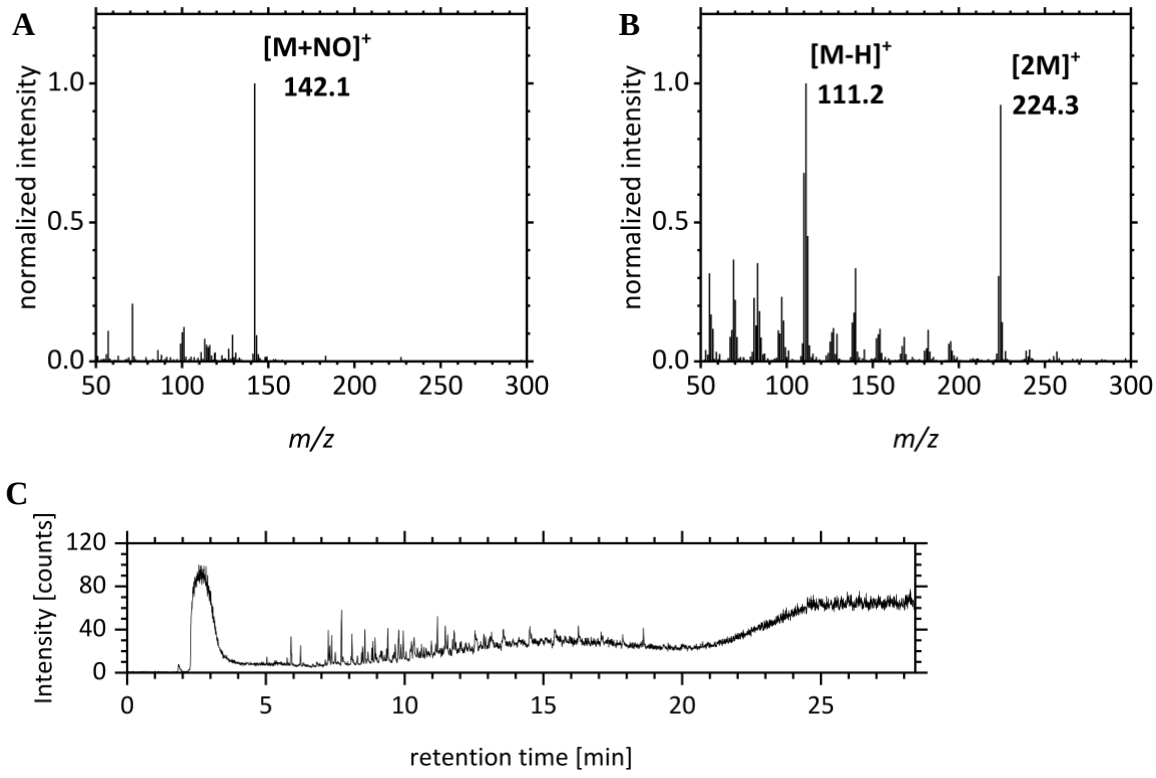


Fig. 1: Mass spectra of 1-octene with TPI and APPI.

A: Mass spectrum of 1-octene with TPI, showing the $[M+NO]^+$ adduct. B: Mass spectrum of 1-octene with APPI showing $[M-H]^+$ and $[2M]^+$ ions. C: GC-TPI-MS chromatogram of diesel on a DB-5ms column.

Funded by: Agilent Technologies

Derivatization strategies for the determination of alkylamines (C2-C8) in yogurt by GC-APLI-(Iontrap)MS

Ling Tang, Florian Uteschil

Atmospheric pressure laser ionization (APLI) is a powerful complement to existing atmospheric pressure (AP) ionization techniques such as atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI) and atmospheric pressure photoionization (APPI). APLI is based on two photon ionization (REMPI) at atmospheric pressure. It is a soft ionization method with no tendency for fragmentation of the analytes and enables high selectivity and high sensitivity detection of aromatic compounds. APLI has been used in many applications such as environmental, petroleomics, and clinical analysis. Up to now, there is no report on the coupling of APLI and ion traps. In here, the ion trap mass spectrometry and GC-APLI was coupled at the first time and the schematic and photograph of this construction are shown in Fig. 1.

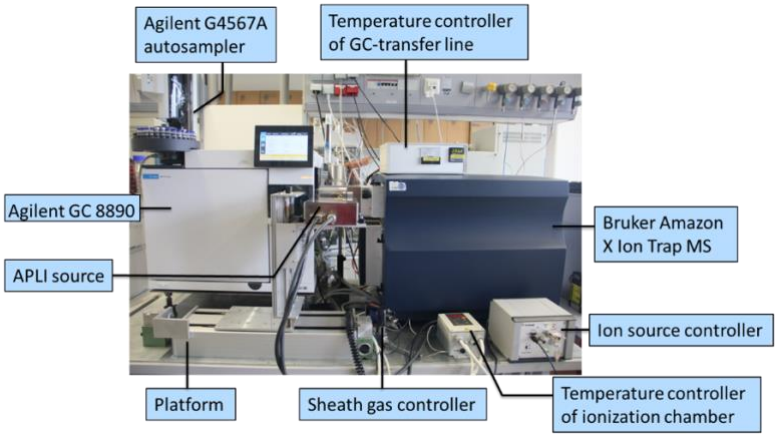


Fig. 1: Photograph of GC–APLI–(Ion trap) MS used in this work.

To detect non-aromatic compounds, derivatization strategies were used to address this problem. Alkylamines have been widely used in the production of surfactants, cosmetic products and food packaging materials. They were frequently found in water, wine, food, and human serum due to the migration and the biological accumulation effect, which may pose a threat to human health. In this report, the aim of the study was to achieve the quantification of alkylamines using yogurt as an example by GC-APLI-(Iontrap)MS (Fig. 2).

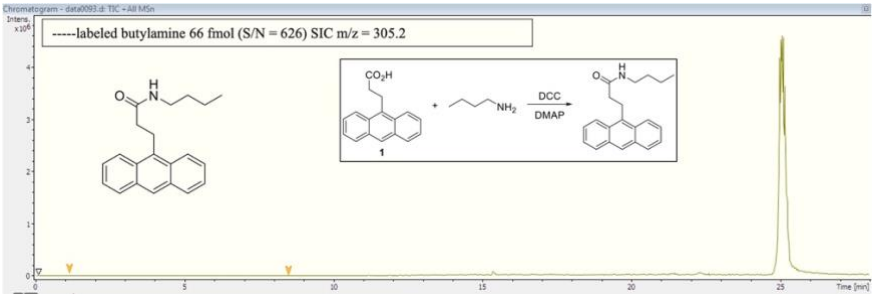


Fig. 2: GC-APLI-(Iontrap)MS analysis of butylamine after derivatization with an ionization marker

Orthogonal separation of complex matrix in comprehensive two-dimensional liquid chromatography (LCxLC) employing Sil-Lys-2C18 as stationary phase in both dimensions

Abul K. Mallik, Yassine Oulad El Majdoub

Comprehensive two-dimensional liquid chromatography (LCxLC) allows the separation of complex matrices in two orthogonal dimensions which is typically achieved by employing different stationary phases and separation mechanisms to achieve high peak capacity and improved resolution.

Employing the same stationary phase in both dimensions can simplify method development, reduce costs, and abridge instrument configuration. However, this setup can hinder achieving high orthogonality due to similar interaction mechanisms that may lead to correlated stationary phase interaction across dimensions. To overcome this limitation, L-lysine-derived two urea and one amide functional group including 2C18 phase (Sil-Lys-2C18) which contains different active sites was synthesized. The same stationary phase was packed into different dimensions 150 × 2.1 mm and 30 × 3 mm for ^{1}D and ^{2}D, respectively. Since the Sil-Lys-2C18 has proved its separation efficiency towards polar compounds, a similar HILICxC18 mobile phase was applied for the separation of complex Underberg compounds.

Sil-Lys-2C18 stationary phase employed in both dimensions of LCxLC, has shown a better separation with higher orthogonality compared to commercialized columns HILICxC18 under similar analytical conditions.

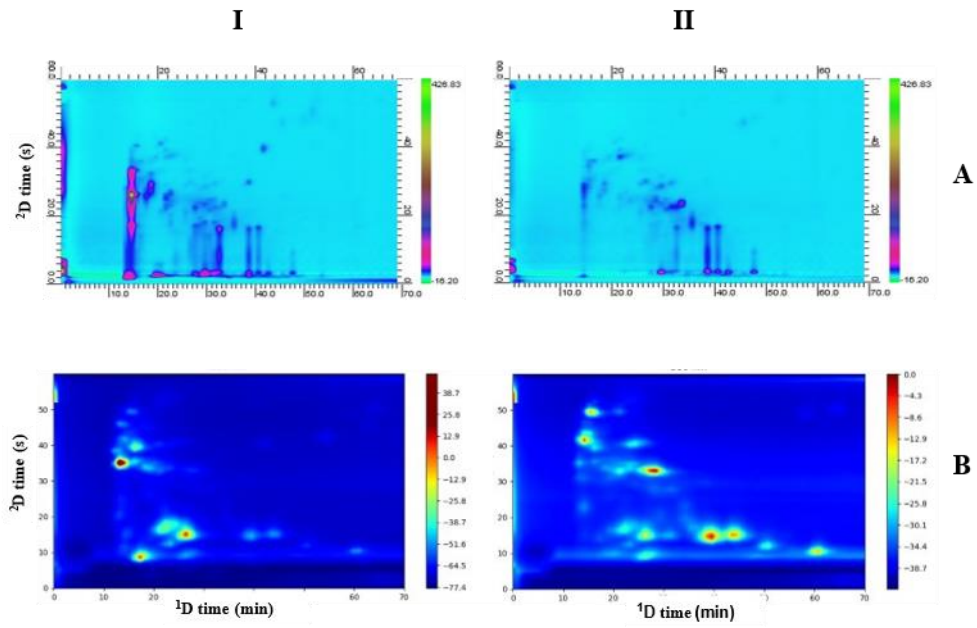


Fig. 1: A comparison between the commercialized and the crafted columns A. Luna-HILICxC18 and B. Sil-Lys-2C18 × Sil-Lys-2C18 through the separation of the components of Underberg by LCxLC coupled to DAD under the following extracted wavelengths I. 280 nm and II. 330 nm.

Funded by: Alexander von Humboldt Foundation through a fellowship to Dr. Abul K. Mallik.

Multi-^{2}D LC × LC-HRMS for the analysis of the *Sambucus nigra L.* leave extract to enhance the separation power of conventional LC × LC

Katharina Wetzel, Priscilla Nhan, Tatyana Tishakova

Comprehensive liquid chromatography (LC × LC) is the gold standard nowadays for the analysis of complex samples originated from various fields such as environment, food or omics. On the basis of one dimensional HPLC it was established to overcome coeluting compounds but what if this coelution still occurs in LC × LC maybe due to the wide range of polarities or other properties of a complex sample? Comprehensive multi-^{2}D liquid chromatography (multi-^{2}D LC × LC) is a novel multidimensional technique that uses not only two orthogonal columns in the first (^{1}D) and second dimension (^{2}D), but also two complementary ^{2}D columns which are switched according to the polarity of the analytes. By coupling a PFP (Kinetex, 150 x 2.1 mm, 1.7 µm) column in the ^{1}D to polar C18, C8 (Kinetex, 50 x 4.6 mm, 2.6 µm) or ZIC-HILIC (50 x 4.6 mm, 5 µm) in the ^{2}D, orthogonality values between 67% and 85% determined by the bin counting method were reached (Fig. 2). The multi-^{2}D LC × LC method using both ZIC-HILIC and polar C18 in the ^{2}D resulted in an outstanding orthogonality of 92% and yielded higher peak capacities than the respective LC × LC methods. The multi-^{2}D LC × LC method was coupled to high resolution mass spectrometry (HRMS/MS) for the chemical characterization of *Agrimonia eupatoria*, *Angelica archangelica*, *Angelica sylvestris*, *Sambucus nigra*, and *Sambucus ebulus* based on a non-target approach. Compared to the individual LC x LC separations, multi-^{2}D LC x LC provided a maximized separation in just one run and was successfully applied to unveil the metabolic profiles of herbal remedies.

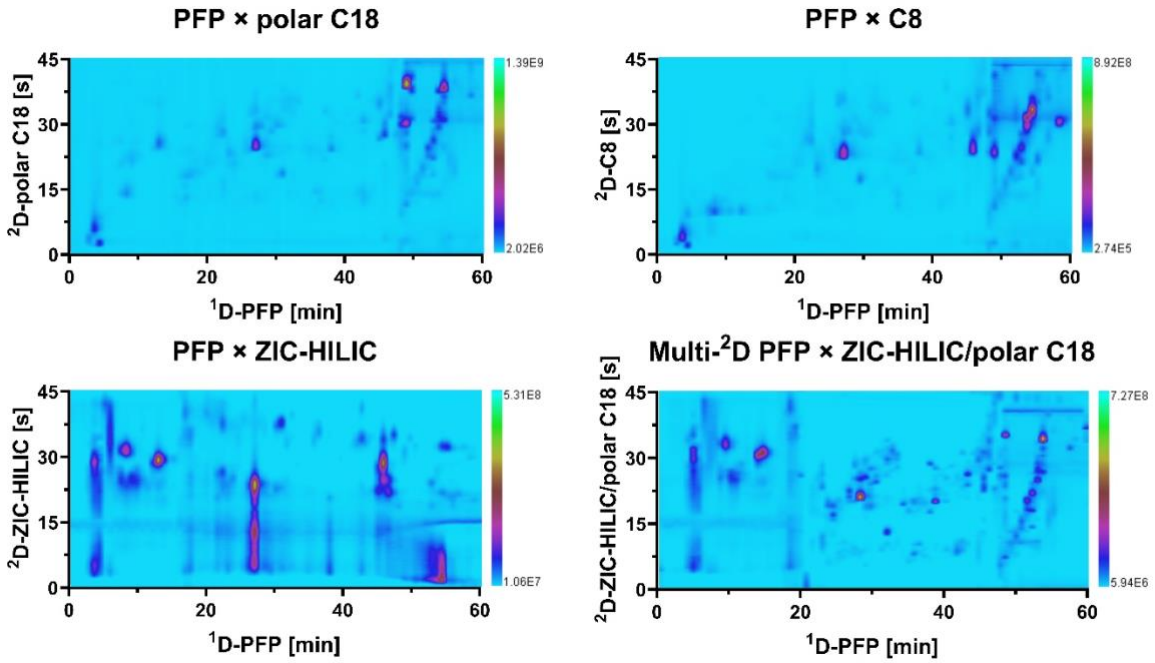


Fig. 2: HRMS-contour plots of *S. nigra L.* leave extract measured by PFP × polar C18, PFP × C8 and PFP × ZIC-HILIC compared to multi-^{2}D PFP × ZIC-HILIC/polar C18.

Collaborative Project – Project Partner: T. Niedenthal, Forschergruppe Klostermedizin GmbH, Würzburg, Germany
Funded by: Deutsche Forschungsgemeinschaft (DFG) - Projectnumber 504370143 and Agilent Technologies

Effected-directed analysis for the identification of antioxidative compounds of European medicinal plants

Katharina Wetzel, Tatyana Tishakova, Marvin Häßler

Despite the expertise about the therapeutic effects of traditional plants, there is a lack of understanding about how these effects are achieved and which compounds are responsible for such beneficial effects. Consequently, further information about their chemical composition, the identification of the compounds as well as the bioactivity is needed. Five European medicinal plants (*Agrimonia eupatoria*, *Angelica archangelica*, *Angelica sylvestris*, *Sambucus nigra*, and *Sambucus ebulus*) known for their hepatoprotective activity were chosen and extracted using an optimized green microwave-assisted extraction (MAE) method. For further insights about the chemical profile and their effects, the extracts were fractionated by preparative LC and the individual fractions were tested for their antioxidant activity (Fig. 1). Fractions that yielded above-average values will be further fractionated and evaluated for their antioxidant activity in order to correlate the respective effect to specific compounds. The first fractionation step reduced the chemical background of these complex samples for an identification by HPLC-MS/MS of the chemical profile of the plants. By combining all these different techniques for an effected-directed analysis, it was aimed to correlate antioxidant activity of these plant extracts to identified compounds in order to understand their beneficial effects.

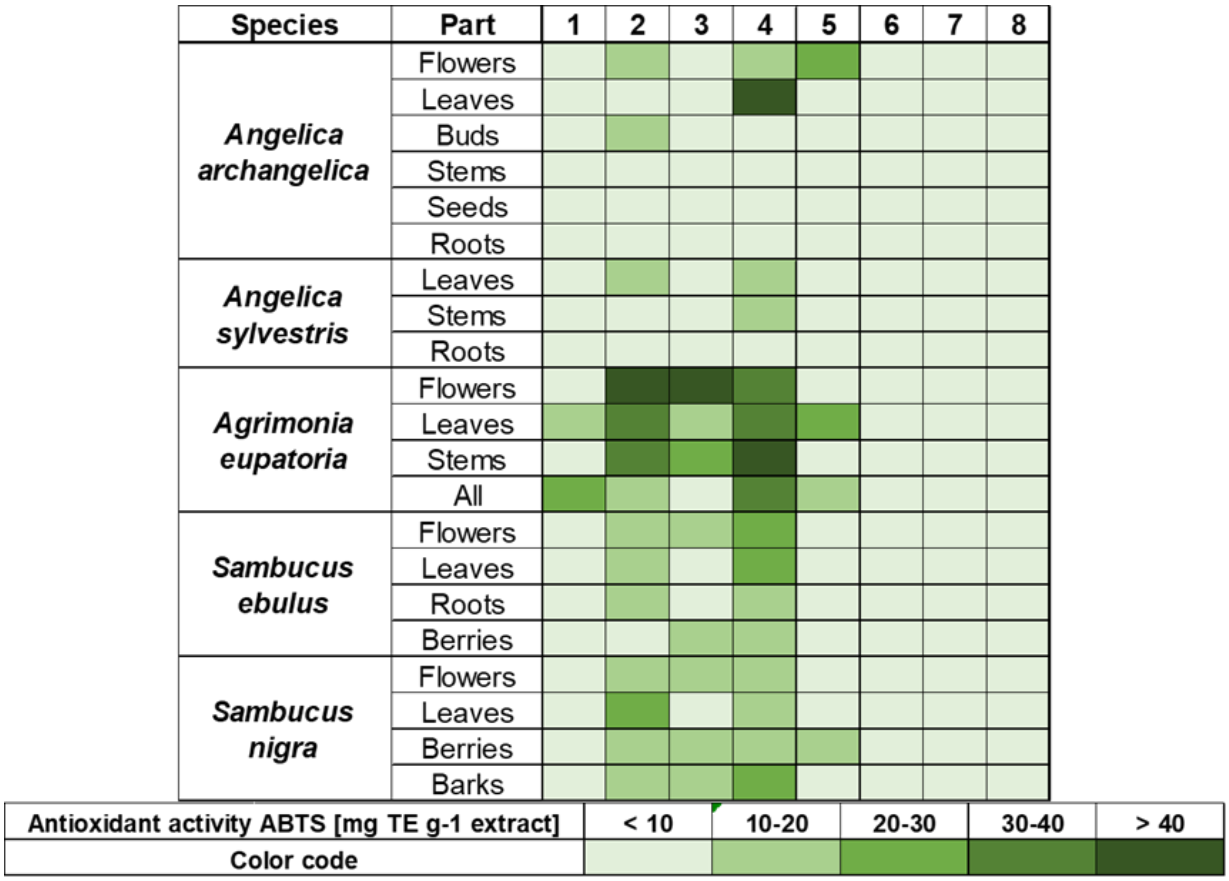


Fig. 1: Quantification of the antioxidant activity measured by a miniaturized assay of ABTS of the fractions collected by preparative LC from plant extracts.

Collaborative Project – Project Partner: T. Niedenthal, Forschergruppe Klostermedizin GmbH, Würzburg, Germany
Funded by: Deutsche Forschungsgemeinschaft (DFG) - Projectnumber 504370143 and Agilent Technologies

Assessing the characterization and differentiation of *Sambucus nigra* plants from different European geographical areas by LC-HRMS

Marvin Häßler, Katharina Wetzel, Lennard Warnecke, Lidia Montero, Juan Ayala Cabrera

Traditional European plant medicine is among the most popular and strongest growing forms of alternative medicine, based on ancient healing practices it still plays a significant role in the treatment of many health conditions. Its impact significantly influenced the creation of therapeutic products by using medical herbs and the market is growing. From 2020 to 2022 just the local German market grew by 158 € million in sales. Because of this indication more research on European medicinal plants is needed. In this study a reliable method using ICP-OES for elemental analysis and liquid chromatography in combination with high-resolution mass spectrometry for the qualitative analysis of bioactive compounds from European medicinal plants is developed. The extraction was carried out using ethanol of varying concentrations by means of microwave-assisted extraction (MAE). The evaluation of the data was primarily based on various plant components of elderberry (*Sambucus nigra*). 193 features were detected during the study being most features identified as polyphenols. Among the level 1 identifications, these accounted for over 90%. Of these, 29% can be assigned to the flavonoid group. Of the level 2 substances 40% are identified as polyphenols, of which 50% are flavonoids. Those substances vary within the plant a lot but stay the same within the same plant part (Figure 2) even though they are harvested in different years and countries.

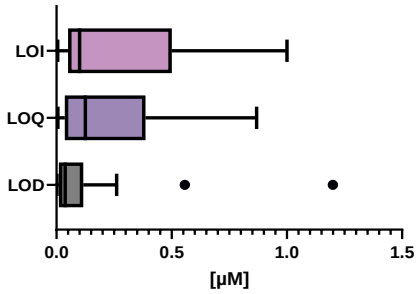


Fig. 1: Analytical quality figures of merit box and whiskers plot for the Identification

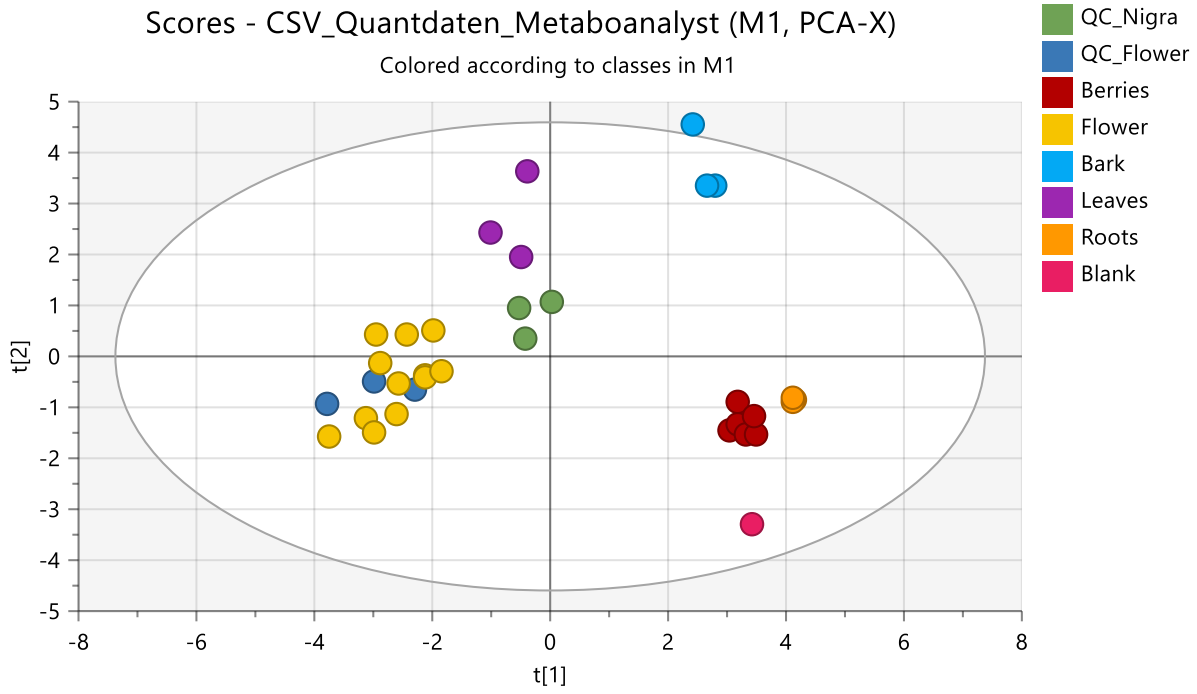


Fig. 2: PCA of the area from the level 1 identified compounds.

Collaborative Project – Project Partner: T. Niedenthal, Forschergruppe Klostermedizin GmbH, Würzburg, Germany
Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143 and Agilent Technologies

Characterisation and identification of antioxidant compounds from *A. eupatorium, A. archangelica, S. nigra,* and *S. ebulus* by effector-oriented analysis of their fractions by LC-MS and elemental distribution by ICP-OES

Marvin Häßler, Katharina Wetzel, Nadezhda Dimitrova, Juan Ayala Cabrera

Growing interest in traditional medicine, fueled by side effects and patient-specific reactions to current drugs, is advancing knowledge of herbal remedies. Medicinal plants show promising hepatoprotective properties due to bioactive compounds, but their complex metabolic profiles challenge analysis, leaving much untapped. This study systematically examines the metabolomic diversity of four European medicinal plants: *A. eupatoria*, *A. archangelica*, *S. nigra*, and *S. ebulus*.

The aim of this work is to present an effect-based analysis and to determine the bioactive potential of the analysed plant parts. Numerous potentially pharmacologically valuable compounds were found in all plants analysed. Therapeutic effects have already been documented for certain compounds such as quercetin and caffeic acid. The high content of flavonoids in the plants confirms this, as this class of substances is generally considered to be beneficial to health, because of its antioxidant capabilities. Coumarins, similar to flavonoids, are regarded as highly bioactive secondary metabolites. They are particularly valued for their antioxidant and hepatoprotective properties. Certain coumarin derivatives, such as esculetin and osthol, can mitigate oxidative stress and tissue damage caused by free radicals through their antioxidant activity.

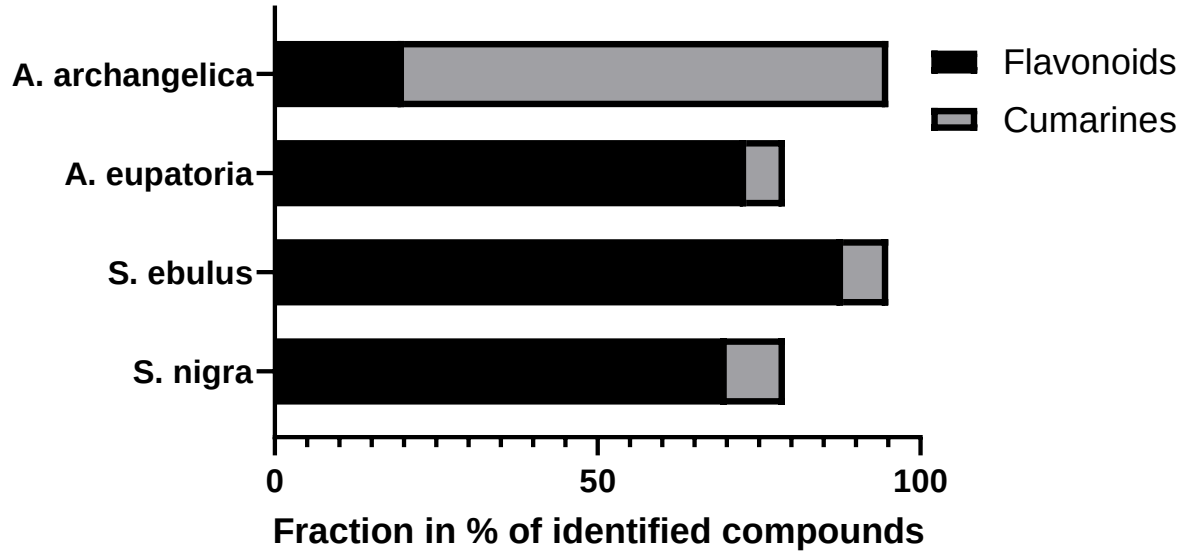


Fig. 1: Polyphenolic compounds identified in *A. eupatoria*, *A. archangelica*, *S. nigra*, and *S. ebulus*.

Collaborative Project – Project Partner: T. Niedenthal, Forschergruppe Klostermedizin GmbH, Würzburg, Germany
Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143 and Agilent Technologies

Identification of double bond positions in fatty acids by in-source fragmentation

Paul E. Görs, Sven W. Meckelmann

Fatty acids (FAs) play essential roles in energy storage, as precursors for complex lipids, and in relation to inflammatory diseases. Among these, unsaturated FAs—with one or more double bonds—are particularly important, as their effects depend on the number and position of these bonds. Detecting FA regioisomers, which may indicate diseases like multiple sclerosis or cancer, poses challenges due to their structural similarity. Techniques like ozonolysis and the Paternò-Buchi (PB) reaction have been used to identify double bond positions.

This study explored atmospheric pressure chemical ionization (APCI) with GC-HRMS to improve FA analysis. By introducing benzaldehyde as a reagent, specific fragment patterns related to double bond positions were generated, enhancing the method's sensitivity. This optimized approach was applied successfully to analyze FAs in fish oil samples. [1]

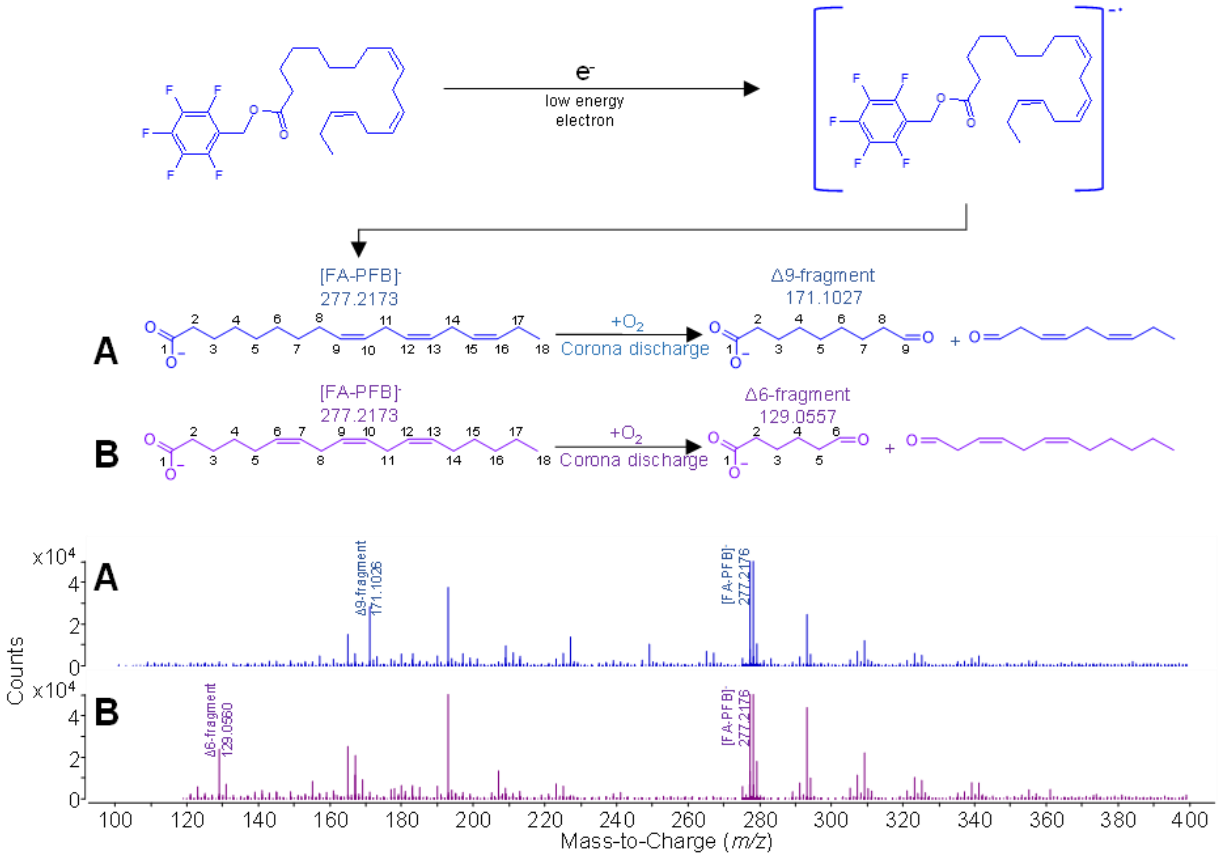


Fig. 1: Structures and MS spectra of FA 18:3 in alpha (ω3/Δ9) and gamma (ω6/Δ6) configurations. The regiospecific in-source fragment ions, m/z 171.1027 for the alpha configuration (A) and m/z 129.0557 for the gamma configuration (B), shown on the right, enable determination of the first double bond position. Simultaneous quantification is achieved using the [FA-PFB]⁻ ion.

[1] P.E. Görs, et al., J. Am. Soc. Mass Spectrom. 2023, 34, 11, 2538-2546

Collaborative Project – Project Partner: Prof. Dr. Bettina Siebers, Dr. Christopher Bräsens (University Duisburg Essen, Essen, Germany)

Funded by: VolkswagenStiftung as part of the project LipidDivide: "Resolving the 'lipid divide' by unravelling the evolution and role of fatty acid metabolic pathways in Archaea."

Green lipid extraction

Pia Wittenhofer, Sven W. Meckelmann

Lipidomics has demonstrated considerable promise in clinical, biological, and food sciences by enabling the comprehensive identification and quantification of lipids. Shifts in lipid profiles are often linked to disease states and serve as valuable biomarkers.

However, lipid analysis is complex due to the diversity of lipid structures. Advanced chromatographic techniques like HILIC, SFC, and RP chromatography, coupled with mass spectrometry (MS), are key in identifying and quantifying specific lipid compounds. A crucial first step in lipidomics workflows is extracting lipids from various biological samples. Traditionally, solvents like MTBE, hexane, or chlorinated solvents such as dichloromethane and chloroform have been used. However, green solvents like ethanol and ethyl acetate are preferred for their lower environmental and health impacts. Additionally, automation in lipid extraction enhances throughput, consistency, and minimizes solvent exposure.

This study presents an automated lipid extraction method using ethanol and ethyl acetate, achieving high recovery rates for key lipid classes in human plasma, serum, and HepG2 cells, offering a viable solution for lipidomics studies. [1]

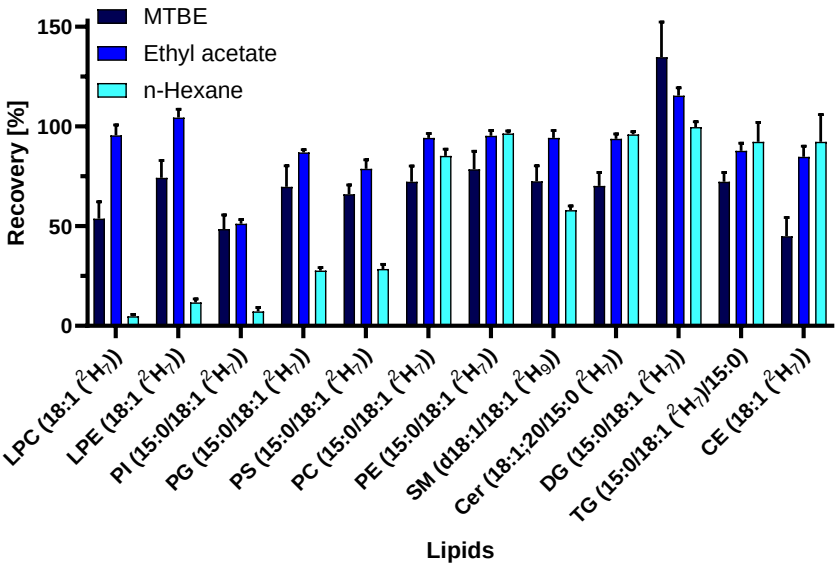


Fig. 1: Recovery rates of various deuterated lipid standards in human plasma following extraction with different solvents (MTBE, ethyl acetate, or n-hexane). Analysis was conducted using LC-ESI-QqQ-MS, with bars indicating the mean and standard deviation for n = 4 samples. Recovery rates were calculated by spiking each compound before and after extraction and comparing peak areas. Each lipid concentration was 1 µg/mL.

[1] Pia Wittenhofer, et al., Green Anal. Chem. 2024, 10, 100128

Collaborative Project – Project Partner: Prof. Dr. Bettina Siebers, Dr. Christopher Bräsens (University Duisburg Essen, Essen, Germany)

Funded by: Deutsche Forschungsgemeinschaft as part of the project "A novel target approach to characterize the Biosynthesis of Cholesterol in Cancer Cells" (ME678328 and SCHM 678329) and Agilent Technologies

Heart-cut liquid chromatography and mass spectrometrie to separate structural isomers of the cholesterol biosynthesis

Pia Wittenhofer, Sven W. Meckelmann

Cholesterol biosynthesis and homeostasis are fundamental to various biological processes. Altered cholesterol metabolism is implicated in hereditary diseases and also linked to cancer, where feedback control mechanisms like HMG-CoA reductase inhibition are often bypassed, resulting in unrestricted synthesis. The biosynthesis involves a complex pathway starting with the mevalonate pathway and divides in two parallel routes: the Bloch and Kandutsch-Russell pathways and differ by a double bond at C24. Analyzing these intermediates poses challenges due to structural similarities and vast concentration differences between cholesterol (mmol/L) and its precursors (nmol/L).

To overcome these challenges, we developed a multidimensional heart-cut LC method coupled with triple quadrupole MS. This approach combines PFP and C18 columns for selective and sensitive detection of all sterol intermediates in a single run, enabling quantification across large concentration ranges. Validated by ICH guidelines, this method effectively detects biosynthesis intermediates and was applied to assess statin effects on lung carcinoma cells, showcasing its utility for biological investigations [1].

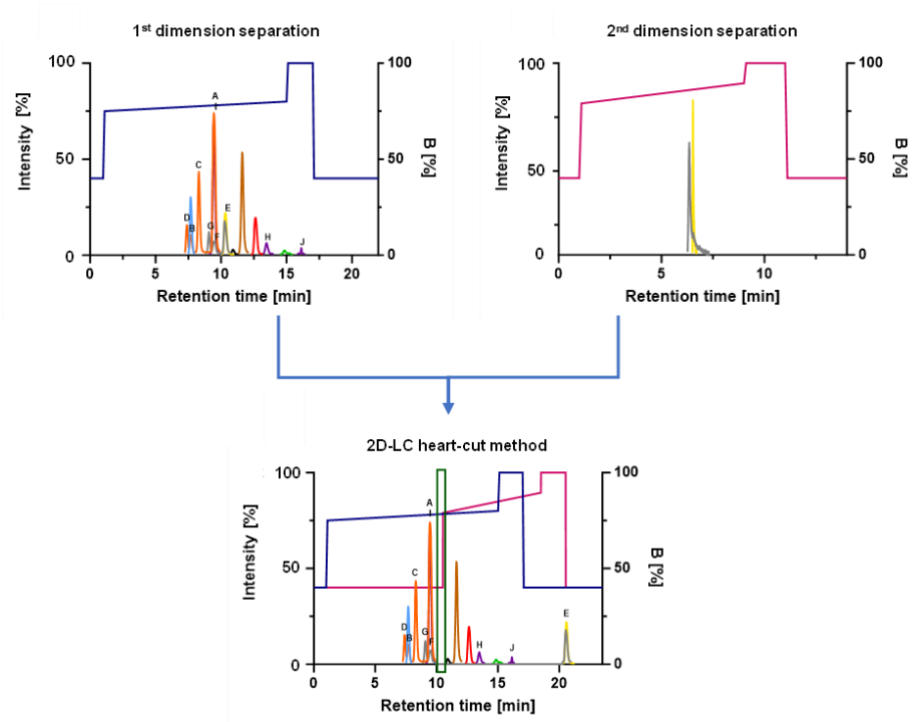


Fig. 1: Left: Chromatographic separation of sterols on the first dimension using a Kinetex PFP column (1.7 µm; 2.1 x 30 mm). **Right:** second dimension chromatogram of cholesterol and T-MAS using an InfinityLab Poroshell 120 EC-C18 column (1.7 µm, 2.1 x 100 mm). **Bottom:** chromatogram of the 2D heart-cut analysis (zymosterol (orange D), dehydrodesmoterol (blue), dehydrolathosterol (orange B), desmosterol (orange C), zymostenol (grey G), FF-MAS (purple I), 7-dehydrocholesterol (orange A), lathosterol (grey F), cholesterol (grey E), T-MAS (yellow), lanosterol (black), 2,3-oxidosqualene (brown), dihydro FF-MAS (red), dihydrolanosterol (purple (H)), dihydro T-MAS (green), squalene (purple J)). The amount of organic modifier for each gradient is provided on the right axis in each chromatogram.

[1] P. Wittenhofer, et al., J. Chrom. A 1738 (2024): 465475

Funded by: Deutsche Forschungsgemeinschaft "A novel target approach to characterize the Biosynthesis of Cholesterol in Cancer Cells" (ME 678328 and SCHM 678329) and Agilent Technologies

Unveiling the metabolome and lipidome of *Haloarcula sp.*

Marvin Häßler, Sven W. Meckelmann

Haloarcula sp. are extreme halophilic archaea that thrive in hypersaline environments, such as salt lakes, with NaCl concentrations reaching up to 4.5 mol/L. In collaboration with the Aerospace Microbiology Research Group at the DLR, we aim to characterize extremophiles and their environments, with a focus on *Haloarcula sp.* Using established metabolomics and lipidomics workflows, we are investigating its unique metabolic profile and biochemical pathways to gain deeper insights into archaeal biology. Our study includes analyses of *Haloarcula sp.* at various cultivation stages. Figure 1 illustrates cells at two growth intervals: after 2 days and 10 days. Initially, no vacuoles are visible, but after 10 days, nearly all cells develop prominent vacuoles. This time-dependent vacuole formation suggests a potential role in long-term stress adaptation or in the storage of specific compounds, likely linked to the organism's metabolic processes (Figure 2).

These observations underscore the dynamic nature of *Haloarcula sp.* cellular structures and their metabolic significance. By exploring these changes, this research offers an exciting opportunity to uncover novel aspects of archaeal adaptations to extreme environments.

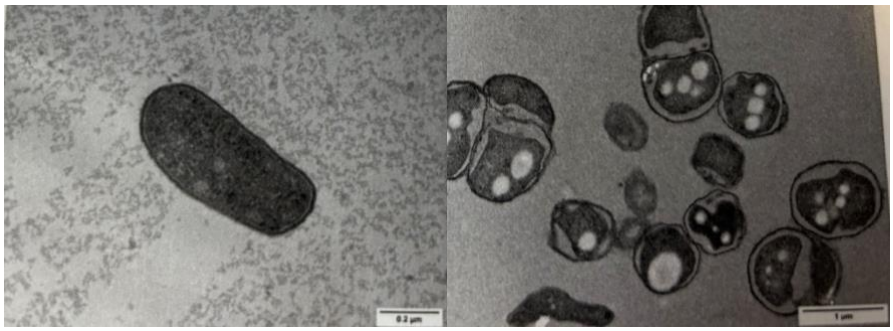


Fig. 1: Two growing periods of *Haloarcula sp.* after two (left) and ten (right) days of cultivation.

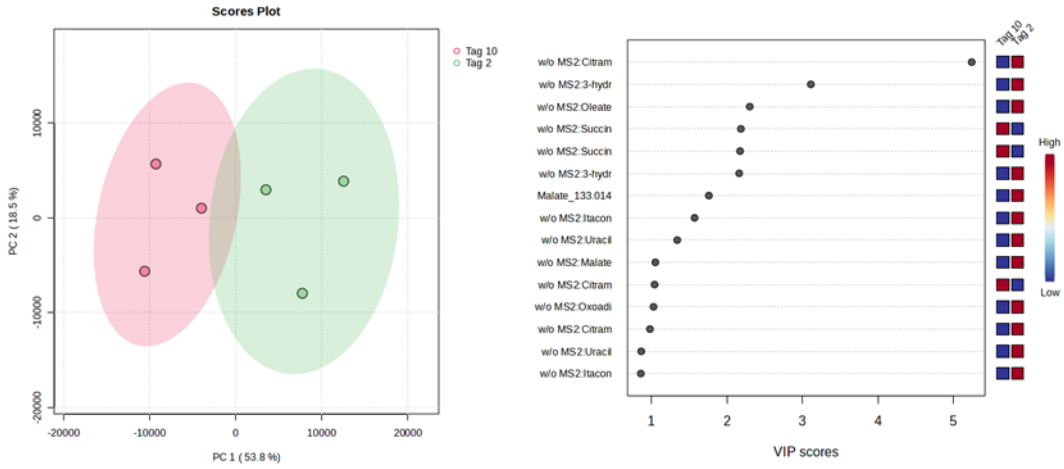


Fig. 2: Comparison of the metabolic profiles of Haloarcular sp. grown for 2 days and 10 days. Left: score plot of a PLS-Da analysis showing a clear distinction between both investigated groups. Right: VIP-plot showing the impact of individual metabolites indicating strong changes in the energy metabolism after 10 days.

Collaborative Project – Project Partner: Stefan Leuko and Katharina Runzheimer (DLR, Cologne, Germany)
Funded by: Agilent Technologies

Application for visualizing LC × LC – DAD data

Jaqueline Leddin, Katharina Wetzel

In LC × LC experiments, diode array detectors (DAD) are often used to measure the absorption of the mobile phase in the UV-VIS range. The diode array detector from Agilent records a UV-VIS spectrum depending on the retention time and saves the intensities in a binary file format. A programm was written in python, which can read out simultaneously all measured wavelengths and plot the two-dimensional chromatogram for each by selecting the wavelength in the list of the programm. The plots can be saved and also combinded to a video in gif format to get an overview of the data. To enable processing with other programmes, the retention time and intensity can also be exported as a text file. Fig. 1 shows some results of two-dimensional chromatographic separation of the herbal liqueur Underberg. A 1290 Infinity II LC × LC-DAD system was used with a PFP (150 x 2.1 mm, 1.7 µm) column in the first dimension and a C18 (50 x 4.6 mm, 2.6 µm) column in the second dimension. The absorbance of the DAD was recorded in the range of 200 - 500 nm with a resolution of 4 nm. The plots of an undiluted 10 µL sample show different signals. The programme thus offers the possibility of viewing the entire range of the measured spectrum, which is particularly helpful for non-target analysis.

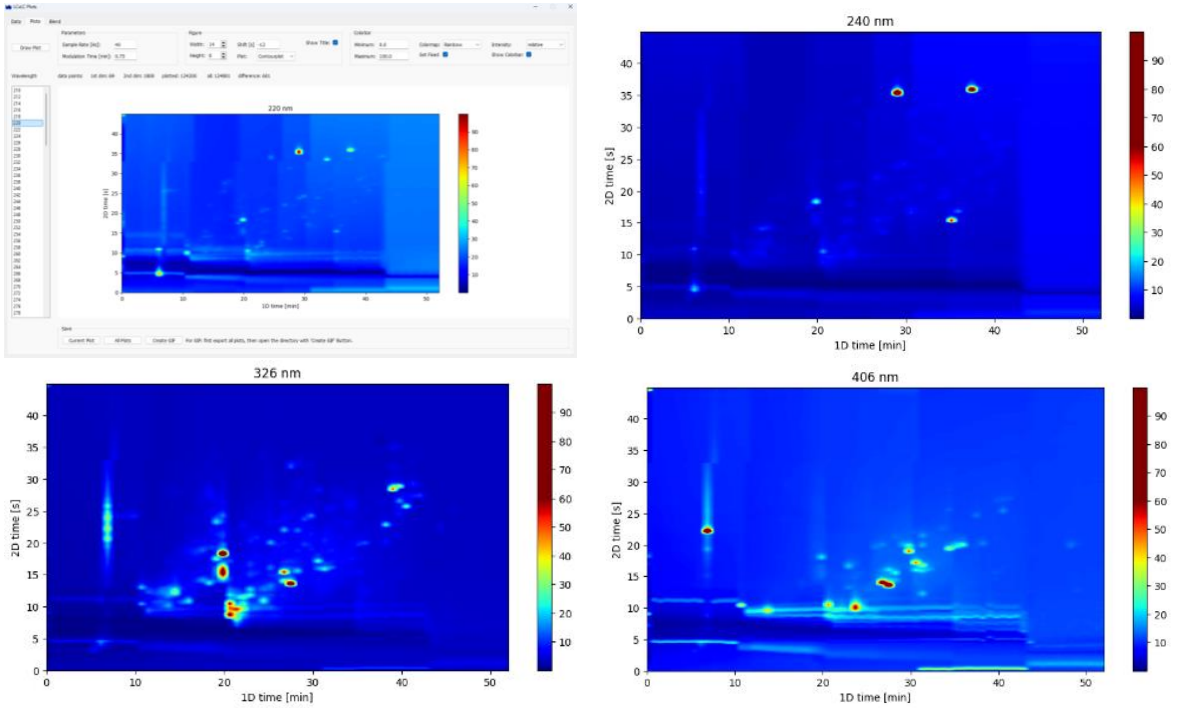


Fig. 1: LC × LC-DAD of the herbal liqueur Underberg at three different absorption wavelengths of the detector and the programm LCxLC Plots, with which these plots were created.

PSeaC – Application for feature combining in LC × LC data

Jaqueline Leddin, Sven W. Meckelmann

PSeaC stands for Peak Selection and Combining and is used in the processing of multidimensional chromatography measurements, such as LC × LC - MS or LC × LC - IMS - MS. After the separation in the first column, the peak is modulated several times and transferred to the second column for further separation. This leads to multiple signals for the same compound with a similar retention time difference according to the modulation time and their intensities follow a Gaussian curve shape. After preprocessing of these raw chromatogramms using MSDIAL, the detected signals are exported as features in a list with retention time, m/z values (averaged from the MS spectra) and intensity. To later perform statistical analysis across multiple samples, it is necessary to demodulate the signals using the developed PSeaC. Fig. 1 shows an example with nine signals. Using the m/z values and the modulation time. The individual signals are combined between the modulations and their intensities are sumed up. This results in a usable feature list with one entry for each detected compound. The algorithm is implemented in Python and was successfully tested with data representing different possible cases for a modulated compound (Fig. 2).

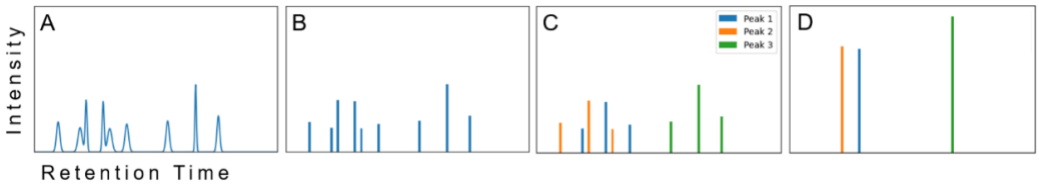


Fig. 1: Post-processing of artificial LC × LC data as an example of the algorithm steps: raw chromatogram (A), feature finding with MSDIAL (B), clustering (C) and summation of intensities (D) with PSeaC.

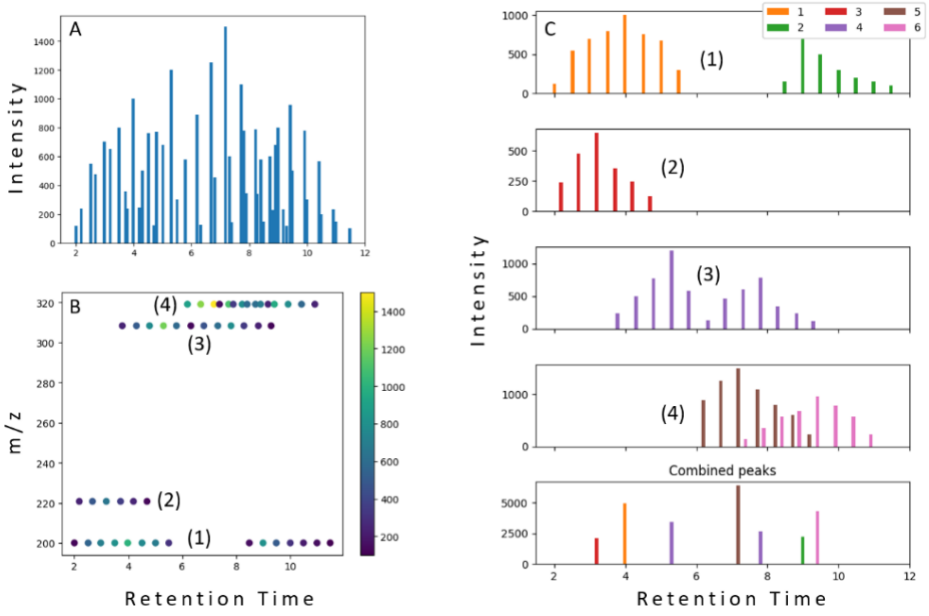


Fig. 2: Test of the algorithm with artificial data. All features of the list (A), overview of the cases (B), i.e. same m/z values and different retention times (1), different m/z values and same retention time (2), two separated peaks (3) and peaks running into each other (4). After successful combination by the algorithm, six clusters with a total of seven peaks are obtained (C).

Targeted metabolite identification and quantification for enzyme activity profiling

Constantin P. Krempe, Jonas Rösler, Sven W. Meckelmann

In 2022, the International Agency for Research on Cancer reported 20 million new cancer cases and 10 million cancer-related deaths worldwide. Dysregulations in important metabolic pathways such as the tricarboxylic acid cycle (TCA cycle) and glycolysis have been linked to cancer as well as other diseases such as Parkinson's disease. Identifying and quantifying metabolites specific to these pathways could enhance insights into disease mechanisms and inform treatment strategies. This ongoing study evaluates various analytical platforms—RP-LC-QqQ-MS, HILIC-QqQ-MS, and GC-QqQ-MS—for their ability to separate and detect pathway-specific metabolites from the TCA cycle and glycolysis. Particular attention was given to challenging isomeric compounds, including polyprotic carboxylates and sugar phosphates and to quantify these metabolites for kinetic studies in collaboration with the ISAS. Therefore, we´ll integrate enzyme quantification to determine flux within these pathways. An optimized GC method with a runtime of 31 minutes was developed and successfully applied to a mix of 30 glycolytic and TCA metabolites, including critical isomers such as citrate and isocitrate (Fig 2). The method achieves detection in the lower nmol l^{-1} range. The sensitivity and separation power are very promising also for low sample amounts. Combining these results with proteomic studies done at the ISAS could enable the determination of enzyme kinetics.

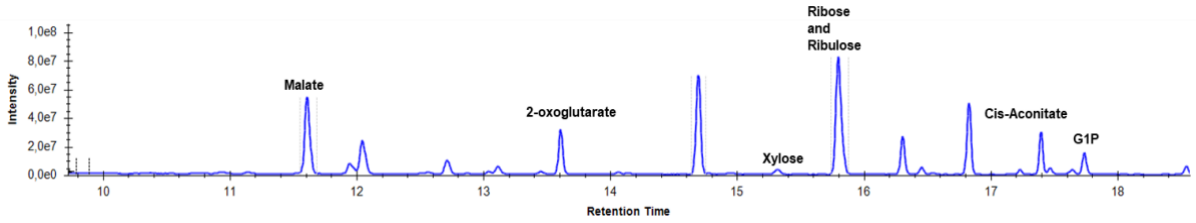


Fig. 1: GC-QqQ chromatogram of isomeric metabolites in full scan mode. The EIC of the *m/z* 73.0 equivalent to a TMS adduct is presented.

The method separates different (poly)protic carboxylic acids like *cis*-aconitate, malate, and 2-oxoglutarate. The pentoses Xylose and Ribose are distinguishable using this method but the ketopentose Ribulose is coeluting with Ribose. Therefore, another separation technique should be used to address this challenge.

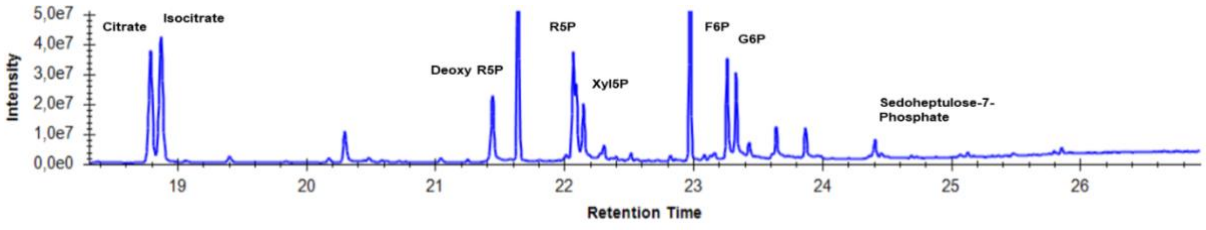


Fig. 2: GC-QqQ of isomeric metabolites in full scan mode. The EIC of the *m/z* 73.0 equivalent to a TMS adduct is presented.

The figure demonstrates the separation of citrate and *iso*-citrate as well as sugar phosphates like Deoxyribose-5-phosphate (Deoxy R5P), Ribose-5-phosphate (R5P), Xylulose-5-phosphate (Xyl5P), Fructose-6-phosphate (F6P), Glucose-6-phosphate (G6P).

Collaborative Project – Project Partner: Prof. Dr. Albert Sickmann (Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany)
Funded by: Agilent Technologies

Fast and robust metabolome screening in cancer research

Jonas Rösler, Constantin P. Krempe, Jaqueline Leddin, Sven W. Meckelmann, Alpaslan Tasdogan

Non-target metabolomics has become a fundamental technique in life sciences as it enables to cover a wide range of metabolites with comparable few resources. However, the quality of the generated data is highly dependent on the certainty of identification applied in the data processing. Most of the published methods for non-target metabolomics use open-source databases (e.g. Fiehn database) without matching of retention times, resulting in low data quality compared to standard target approaches such as LC-QqQ-MS. This can cause major problems in later data analysis and interpretation.

Furthermore, most HILIC methods applied for polar metabolites have long run times, posing low sample throughput, and high solvent consumption. In combination with the difficult identification and data analysis, these strategies are less promising for high throughput screening in large clinical cohort studies.

This work focuses on the development of a fast HILIC-HRMS method for generating high-quality metabolomics data for application in system medicine and biology.

Therefore, a repeatable (as shown in Figure 1) LC method was optimized to separate complex biological samples within eight minutes. Furthermore, a database of over 400 major metabolites was created, which includes exact mass, retention time, and MS/MS fragmentation pattern by analyzing authentic standards.

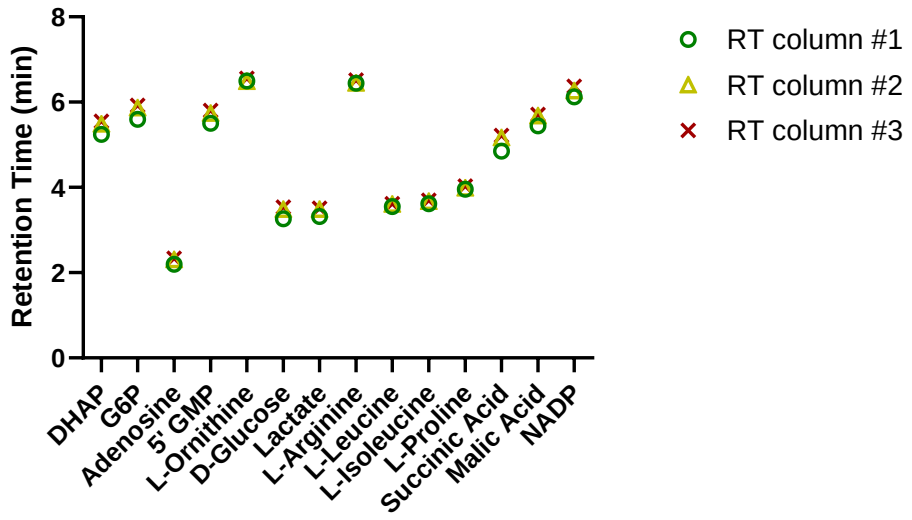


Fig. 1: Inter-column retention time repeatability on three different columns at three different days for a representative set of metabolites.

The utilization of this method enables not only a faster analysis time but moreover a drastically reduced effort in data analysis due to enhanced confidence of identification.

The method is applied in several studies regarding cancer research and shows its utility for the screening of clinical samples with high robustness.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm) and Agilent Technologies

Quantitative analysis of polyamine synthesis in intestinal stem cells

Jonas Rösler, Pia Wittenhofer, Sven W. Meckelmann

Fasting regimens have been shown to improve health, and enhance tissue regeneration across various organisms, including humans. However, the effect on the polyamine metabolism of fasting and subsequent post-fast refeeding is not well explored. This is of special interest as polyamines are key to understand protein synthesis and corresponding processes.

To investigate the role of polyamines in this context, we developed an LC-ESI-QqQ-MS method for the sensitive and selective quantification of polyamines after derivatization with dansyl chloride. Separation was achieved using a ZORBAX Eclipse Plus C18 RRHD column (50 × 2.1 mm, 1.8 µm) with water (A) and ACN/water (90:10) containing 0.1% formic acid (B) as eluents. This method enabled rapid quantification of key polyamines (**Fig 1**).

Moreover, we used LC-Orbitrap-MS, to demonstrat isotopic enrichment of the isotopologue of putrescine via labeled ornithine or arginine. The ratio of labeled to unlabeled analytes over time provided insights into the activity of the polyamine pathway and the urea cycle (**Fig. 1**).

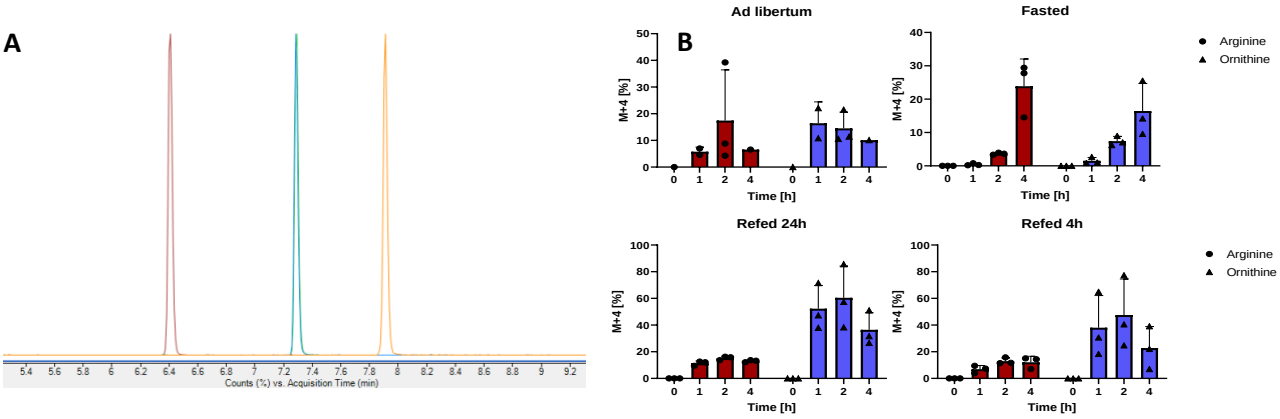


Fig. 1: A) RP Separation of Polyamines Putrescine (brown), Spermidine (blue) and Spermine (yellow). B) Kinetics of polyamine metabolism via isotope tracing for different nutrition. Cells were fed and measured with no treatment, after fasting and after fasting and refeeding for 4 and 24 hours.

[1] S. Imada, et al., Nature 633 (2024): 895-904

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen, Ömer H. Yilma, Koch Institute for Integrative Cancer Research at MIT, Cambridge, USA) and Agilent Technologies

Implementation of automated sample preparation and script assisted data analysis in the metabolome analysis

Jonas Rösler, Jost Guinand, Jaqueline Leddin, Sven W. Meckelmann, Alpaslan Tasdogan

The plenty of information generated in non-target metabolomics have established it as a standard tool in many scientific communities. Still the applicability for high throughput screening applications is rather limited due to the amount of time and work needed for the analysis. This is especially true for the sample preparation and data analysis of large sample cohorts.

The automatation of sample preparation steps has become a feasible possibility to reduce the demand on time and ressources in modern analytical method development. But unfortunately in metabolomic research the composition of the optained samples is very unstable, resulting in the need of a fast and gentle sample preparation to maintain the initial metabolite profile. As automated sample preparation procedures often need more time for the extraction of metabolites and can also break the cooling chain for the frozen samples, they are often not applicable, although the exact effect is not always known.

In order to optimize the workflow of metabolite analysis, we implemented an automated sample preparation to our established metabolomics workflow. The effect of an automated sample preparation was compared to a manual extraction and we are investigating the metabolic pahtways effected by the sample preparation and the corresponding severity.

To optimize the process of data analysis a script assisted data filtering is added to our workflow.

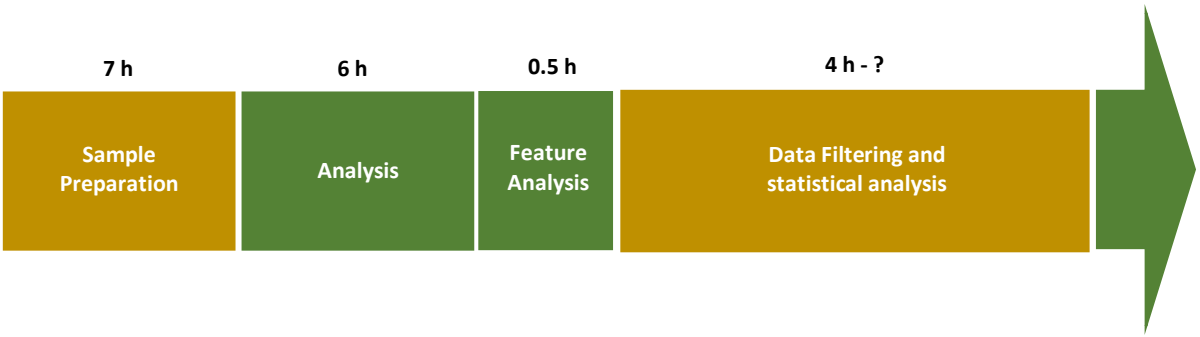


Fig. 1: Workflow for a LC-MS based metabolomics workflow with their estimated time within the whole workflow for a set with about 40 samples. The key steps for the optimization in this work are marked in yellow, showing the high potential for a more time and resource efficient setup.

Overall we could observe a severe reduction in the toal analysis time due to a shorter data analysis and a less ressources consuming sample preparation.

Yet, for the automated sample preparation a metabolic shift could be observed in time. Therefore the effect of an automated treatment is connected to the number of samples processed by the robot. The metabolic influence is rather low for small sample sets, while large cohorts can suffer from severe shifting.

The exact influence within the TCA, glycolysis and PPP is currently under investigation and will be characterized in further experiments.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm)

Changes within the lipidomic profile induced by different medication strategies in cancer therapy

Jonas Rösler, Luiza Martins Nascentes Melo, Sven W. Meckelmann, Alpaslan Tasdogan

Lipids and their metabolism are not just important for the cell structure as key parts of membranes, but are also directly connected to central metabolic functions in organisms. Besides Metabolomics, the lipidomic profile is therefore a key indicator in biological reasearch.

In our group a suitable workflow for lipidome analysis was established using an automated sample preparation followed by LC-Orbitrap-MS analysis. This method was succesfully applied to characterize the impact on the lipidome of different cancer treatment strategies for two types of cancer cell lines.

The treatment caused a clear metabolic effect on the cells, which can easily be considered in the PCA plot depicted in Figure 1. Both cell lines (A375 and SKMEL) have a distinct profile (WT in fair blue), which is severely influenced by the treatment (DT in red). Interistingly this shift is stable during the drug holiday (DH in green) for the A375 cell line, while the lipidome of the SKMEL cells undergo another severe shift induced by the recovery.

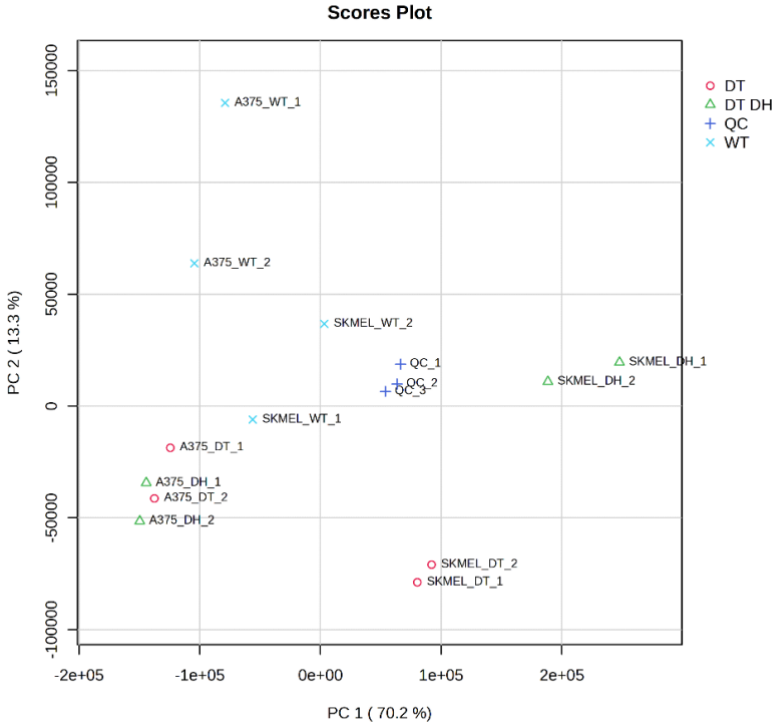


Fig. 1: Principle Component Analysis for the lipidome analysis of two different cell lines (A375 and SKMEL) as wild types (WT), under drug therapy (DT) and after a drug holiday (DH/DT DH).

Indicating a totally different response to a potential therapy, this effect is of high interest.

The exact effect of the treatment is under further investigation and will be also deepened with metabolomics data to fully characterize the metabolic influence of these treatment strategies, enhancing the understanding of its possible biological impact.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm) and Agilent Technologies

Metabolic effect in treatment strategies for heart disease

Jonas Rösler, Feyza Cansiz, Luiza Martins Nascentes Melo, Gabriele Allies, Sven W. Meckelmann, Alpaslan Tasdogan

Fatal heart dieseases, as also corresponding treatment strategies based e.g. on drugs, are known to induce a severe change to the local metabolome. To elucidate these mechanisms in heart disease and also during their treatment, metabolic investigations are of high importance. The treatment can for example induce severe side effects, which might also enhance or lower the disease burden. Especially for the analysis of heart samples a reliable and sensitive method is desired to generate meaningful data, as the sample size can be very small if limited to specific heart regions.

Our group has developed a fast metabolome screening method based on HILIC-Orbitrap-MS, which was subsequently utilized to observe the metabolic effect during the treatment of mice suffering from ischemia. Clear metabolic effects could be observed both in central carbon metabolism as visible in Figure 1 for the VIP score as also in the examplary boxplot, and also in the amino acid metabolism. The data indicated a metabolic effect of the drug treatment, which recovers the prior induced alteration from the ischemia.

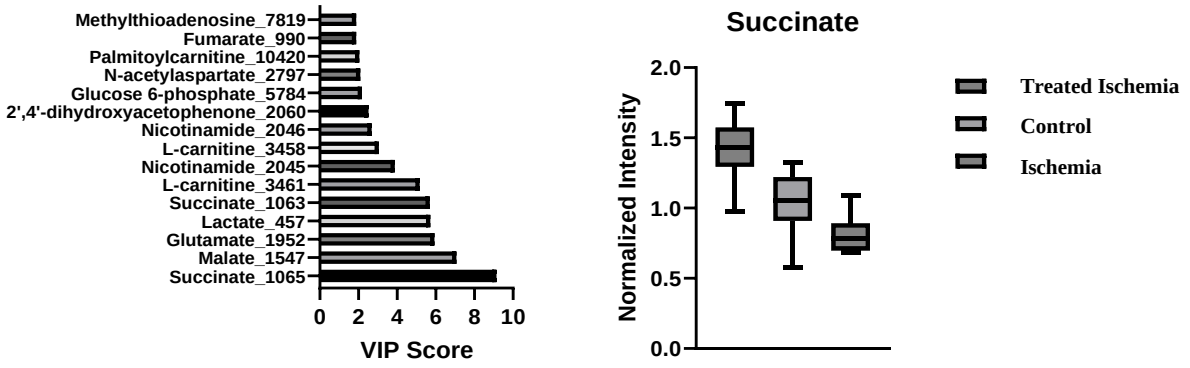


Fig. 1: Left: VIP scores for the comparison of the treated and untreated mice, both with disease burden. Right: Boxplot for succinic acid as an example for the metabolic shift during the treatment. Treated Ischemia and Ischemia group both suffered from disease burden, while the control group shows the metabolic levels for healthy mice.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen, Prof. Dr. Ulrike Hendgen-Cotta, CardioScienceLabs, Universitätsmedizin Essen

Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm) and Agilent Technologies

Effects on the metabolome in the central carbon metabolism for genetic deficient liver cells by HILIC-Orbitrap-MS

Jonas Rösler, Feyza Cansiz, Luiza Martins Nascentes Melo, Gabriele Allies, Sven W. Meckelmann, Alpaslan Tasdogan

The genetic and metabolic interactions in organisms are of special interest in all topics of life science, which aim to unreveal molecular mechanisms in complex biological systems. Often genetic alterations are applied in order to induce alterated metabolic behaviour and dependencies.

To investigate these differences our group has set up an metabolite screening method utilizing HILIC-Orbitrap-MS, which was subsequently applied to investigate the metabolic shift within the central carbon metabolism of liver cells after a genetic knock-out.

As seen in Figure 1, key metabolites of the TCA and glycolysis have been severely changed by the knock-out indicating a cell autonomous shift in its metabolism. This effect is especially interesting to characterize the metabolic influence of this genetic knock-out and will also be applied to support the data of a current paper revision.

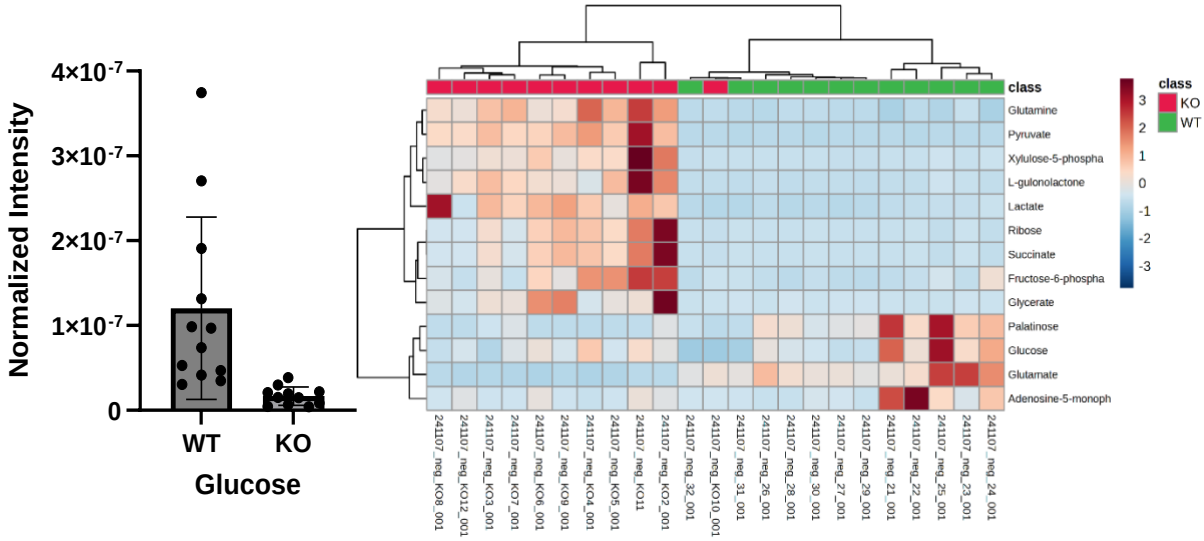


Fig. 1: Left: Glucose levels for wild type cells and knock-out liver cells. Right: Heatmap of important metabolites in the TCA and glycolysis for wild type and knock-out liver cells.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen, Prof. Dr. Matthias Heikenwälder, DKFZ Heidelberg

Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm) and Agilent Technologies

Dose dependent impact of polyamine inhibition on the global metabolome

Jonas Rösler, Feyza Cansiz, Sven W. Meckelmann, Alpaslan Tasdogan

Polyamines are important in many processes of cell proliferation and are well known to be disregulated in numerous types of cancer, making them a feasible target in cancer therapy. Suitable inhibitors as DFMO can be applied to suppress polyamine expression in cells, but their impact to the global metabolome and the influence of these alterations remain unknown.

By combining a RP based LC-QqQ method for the detection of polyamines and a HILIC-Orbitrap-MS method for global metabolomics, we could show the dose dependent effect of DFMO treatment for the polyamine expression in cells and its connection to the remaining metabolome.

Figure 1 clearly shows even low doses of DFMO to be sufficient for a severe reduction in the expression of polyamines. Higher doses do not have a further effect. The same pattern can be observed in the PCA of the global metabolomics measurements, as the control group (red) is the only group clearly standing out from the others. All DFMO treated cells group together, showing higher variation of the data for the very high doses, which indicate possible off-target effects. The lowest dose of DFMO (green) groups in the direction towards the control samples, also indicating further off-target effects of higher doses to the global metabolome.

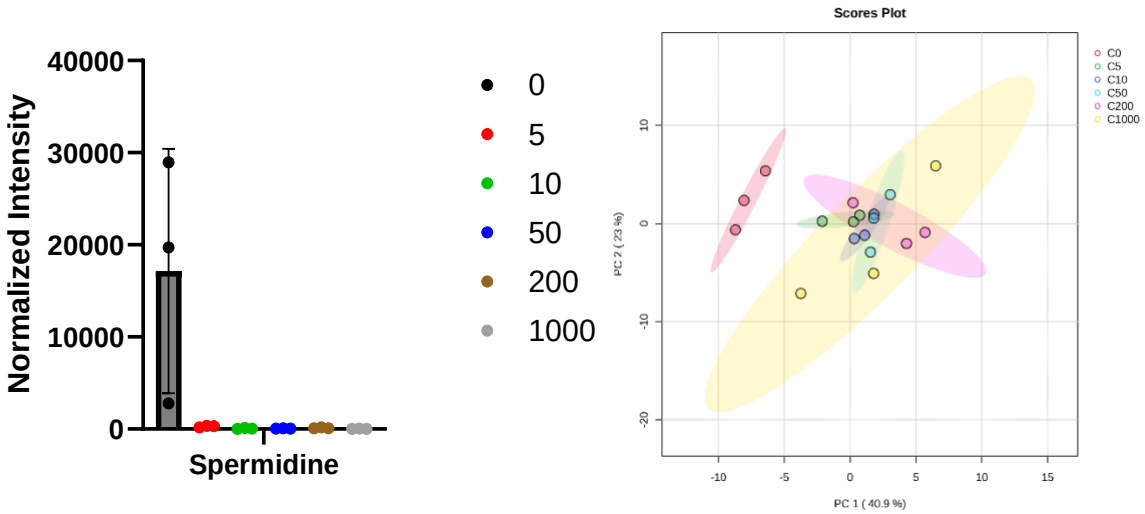


Fig. 1: Left: Normalized concentration of spermidine in cells depending on the dose of DFMO. This data is also representative for the polyamines spermine and putrescine. Right: Principle Component Analysis of the global metabolomics data in cells for different doses of DFMO.

This data shows the importance of the correct concentration for possible drug treatment in further experiments and its off-target effects in the metabolome. These effects are currently further investigated to elucidate the potential of DFMO treatment and its side effects in cancer therapy.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm) and Agilent Technologies

Metabolic differences of efficient and inefficient metastasizers in cancer disease

Jonas Rösler, Feyza Cansiz, Sven W. Meckelmann, Alpaslan Tasdogan

Metastasizing is for its mortality a major target in cancer research. It is well known, that some cancer cells efficiently metastasize to different organs, while others do not. During the process of metastasizing the cancer cells need to adapt their metabolism in order to survive the metastatic cascade (e.g. different pH and redox potential) and they must be able to adapt to their new microenvironment as soon as they arrive at the secondary side. This circumstances are raising the question, if there are metabolic alterations or potentials in the primary tumour boosting these cells ability to succesfully survive the process of metastasizing.

To answer this question, we applied HILIC-Orbitrap-MS to a dataset of in vivo sample from efficient and inefficient metastasizing cancer cells (primary tumour and multiple metastases). Here we could see interesting metabolic differences occuring between the metastases and the primary tumour, but more importantly between the efficient and inefficient metastasizers.

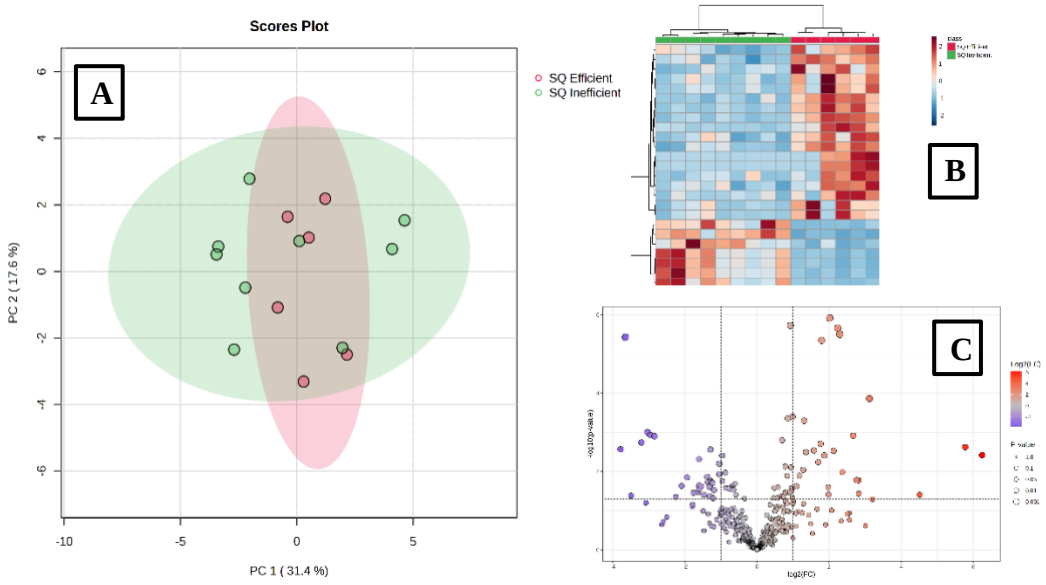


Fig. 1: A) Priciple Component Analysis of the efficient and inefficient metastasizers; B) Heatmap of the top 25 features based on t-test showing the clustering of efficient and inefficient metastasizers; C) volcano plot of efficient and inefficient metastasizers showing a big proportion of the data as significant different (FC > 2; p < 0.05).

The data is still under analysis, but yet it can be seen in Figure 1, that although the pattern in the PCA (A) might seem indifferent on the first sight, significant differences could be observed (C) in the metabolic profile of efficient and inefficient metastasizers, which can also be brought to specific metabolites in the heatmap (B). The pattern of the efficient metastasizers in the PCA seems to be much more distinct, indicating the possibility of a specific metabolic behaviour prone to enable the successful formation of metastases.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by:: A. T. was funded by an Emmy-Noether Award from the German Research Foundation (DFG, 467788900) and the Ministry of Culture and Science of the State of North Rhine-Westphalia (NRW-Nachwuchsgruppenprogramm) and Agilent Technologies

Doctoral Thesis Accomplished 2024

Dr. Maha Alhasbani

Coupling of thermogravimetry with mass spectrometry for the analysis of complex samples

Analyzing complex solid and liquid samples is a major challenge today. It is important to eliminate the cost of extraction methods and to perform the analysis in a short time. This was realized by coupling thermogravimetry and Orbitrap (TG-HRMS) via a photoionization source at atmospheric pressure. The direct use of the sample in the crucible also helps to avoid the absorption of VUV photons by a solvent. The two devices are connected by a transfer line. This consists of ceramic beads and corrugated steel tubes with a quartz glass capillary, which can be heated up to 350°C and is flexible.

To take advantage of thermogravimetry, once some of its physical properties are known, the sample is placed in the crucible, vaporized in the oven and the analytes are transferred through the fused silica capillary connected to the photoionization source at atmospheric pressure and the Orbitrap.

After optimizing the capillary diameter, installing the equipment, adjusting the nitrogen inlet and outlet and using temperatures between 50 and 300°C, the coupling was successful at a transfer temperature of 250°C.

In preliminary work, thermogravimetry was coupled with a quadrupole mass spectrometer and some drugs were successfully analyzed with the APPI used, whereby satisfactory qualitative results were achieved without additional sample preparation. However, quadrupole mass spectrometry is not suitable for analyzing more complex samples due to the low mass resolution without chromatographic pre-separation.

After combining thermogravimetry with the high-resolution Orbitrap mass spectrometer, which not only has a higher resolution but also better mass accuracy, the analysis parameters had to be optimized to increase sensitivity. Several complex samples were then analyzed.

The analysis of Arabica and Robusta coffee varieties was carried out to identify some differences between the two varieties and to detect blending and fraud. The coffee samples were successfully analyzed and showed mass errors of less than 1.0 ppm e.g. for caffeine. The analysis showed the presence of eight specific Arabica markers and 11 signals found only in Robusta samples, with 3 replicates demonstrating their presence. Subsequently, commercial samples were analyzed.

For the analysis of a reference urine sample, 45 compounds from different substance classes were identified based on the exact mass. Using standards, qualitative and quantitative results of creatinine and caffeine were obtained without extraction procedures or addition of reagents to the urine sample. The determination of creatinine using three different methods yielded an RSD of no more than 2.5%

and an LOD and LOQ of 0.97 mg/L and 3.24 mg/L, respectively. For caffeine, the RSD was less than 5.6%, while LOD and LOQ were 0.17 mg/L and 0.56 mg/L, respectively.

In addition, numerous signals were detected in blood samples but could not be assigned. Without sample preparation and a program to identify the metabolites, the analytical platform used could not provide satisfactory results.

The Maillard reaction was investigated by combining glucose with asparagine and glycine in a crucible and then carrying out the reaction. The mechanisms of the Maillard reaction, e.g. the formation of 2-ethyl-5-methylpyrazole, which belongs to the pyrazine group, were investigated and confirmed by the discovery of some intermediates. In addition, various food samples were analyzed for the Maillard reaction.

Based on the various applications of TG-HRMS, the coupling appears to be a possible analysis tool for quality control. However, further studies are required to prove the benefits.

Dr. Alexandra Pape

Improvement of atmospheric pressure ion sources for mass spectrometry

In the first project, a new nebulization system for a liquid chromatography – mass spectrometry application (LC-MS) utilizing an inverse low temperature plasma (iLTP) ion source was developed. Instead of a standard atmospheric pressure chemical ionization nebulizer, two commercial nebulizers were disassembled and re-configured to spray the liquid chromatograph eluent onto the ionizing plasma in front of the mass spectrometer inlet. Since both devices, a medical inhaler by Omron and a bottle humidifier by Daiso, demonstrated irregular vibration and nebulization properties as well as a lack of mechanical robustness, another ultrasonic spraying device was developed in cooperation with Hitachi High-Tech corporations. For this application, focusing cones were developed to transport the solvent into gas phase and to focus the spray onto the plasma region to ensure better ionization. A design of experiments was performed to find the best operating parameters, and calibration curves were established to compare both the standard nebulizer and the Hitachi ultrasonic nebulizer. In general, the standard nebulizer proved advantageous which leaves further room for improvement of the ultrasonic nebulization for mass spectrometry applications. Furthermore, the iLTP ion source was compared to a tube plasma ionization configuration. Even though the tube plasma ionization ion source was less robust, its sensitivity was higher than that of the iLTP ion source.

In a second project, a repeller electrode was inserted into an electrospray ionization (ESI) housing to improve the ion transfer from the electrospray capillary to the mass spectrometer inlet. Different repeller designs were manufactured and their position and applied voltage optimized. It was found that the *m/z* of the investigated molecules and the flow rate of the supplied liquid influenced the peak intensity and the required repeller voltage to reach a signal intensity maximum. Smaller repellers were discovered to give better results and a concave repeller with a diameter of 12 mm was found optimal. A design of experiments provided optimal spatial conditions and repeller voltage. However, the effect that the repeller had on the overall results at the applied liquid chromatography flow rate (450 µL min-1) was rather small in contrast to lower flow rates. A subsequent analysis of human plasma spiked with testosterone and reserpine also showed little improvement with the repeller electrode. It was furthermore discovered that the repeller electrode did not only aid the transfer of ions from the electrospray capillary into the mass spectrometer inlet, but that it was capable of ionizing molecules without any other ionization source present.

In the third project, the iLTP and a nanoESI ion source were coupled in a dual ion source design in front of a single quadrupole mass spectrometer. Due to the unshielded set-up, the experiments remained prone to environmental influences, but the parameters of both ion sources influencing the measurements were successfully investigated and subsequently optimized. The integration of a plasma ion source between ESI capillary and MS inlet showed a significant increase in the total ion current and the signal response of the test compound reserpine.

Dr. Martin Meyer

Application of chromatographic separation techniques for the analysis of polysaccharides in archaeal biofilms of *Sulfolobus acidocaldarius* and other biological materials

The interest in the analysis of saccharides has been growing continuously in recent years. It is highly relevant for modern system biology, biochemistry, as well as microbiology with their respective subdisciplines. Moreover, the analysis is also important in food and plant sciences as the interest in the analysis and identification of correlations regarding saccharides is growing. The analysis of saccharides confronts scientists with multiple challenges when it comes to investigating a sample containing different forms, whether it is mono-, oligo-, or polysaccharides, for their composition or other properties. Many different methods for analysis have been developed, optimized and applied over time and each of these methods offers its advantages and disadvantages in terms of separation performance, sensitivity or applicability. Care must be taken to adapt each method to the requirements of the particular issue at hand, as well as the sample to be analyzed.

For biological samples, the basic workflow in the determination of the saccharide fraction can be described by five steps: starting with the isolation and followed by extraction, hydrolysis, sample preparation and finally the analysis.

In this work, the main focus was on the identification of the monosaccharide composition of the exopolysaccharide in biofilms of the archaeal strain *Sulfolobus acidocaldarius (S. acidocaldarius)*. So far, little is known about the composition and size of archaeal polysaccharides, thus new essential insights are achieved with these studies. Therefore, a protocol for cultivation was first optimized that would yield the highest possible amount of biofilm biomass; this was achieved by static incubation of the archaea on floating modified PTFE membrane filters. These provided the highest mass of biofilm in general, but also the highest amount of carbohydrate produced per cell, compared to other incubation methods tested, whether submerged biofilms, cultures spread on solid media, or other filter materials.

In a further step, the methodology of carbohydrate analysis was investigated. For this purpose, first a comparison of four different chromatographic methods such as supercritical fluid chromatography, hydrophilic interaction chromatography, reversed-phase liquid chromatography, and gas chromatography, each coupled to mass spectrometry (MS), was performed. The methods were evaluated and compared in terms of separation performance and sensitivity for a set of 16 monosaccharides as components of microbial exopolysaccharides. Here, RP-LC-MS has shown to have both the highest separation performance and the best sensitivity after derivatization of the monosaccharides with 1-phenyl-3-methyl-5-pyrazolone (PMP) in multiple reaction monitoring (MRM) mode. It was possible to unambiguously identify 15 of the 16 monosaccharides by baseline separation or mass differences using this method, and the detection limit, depending on the substance, ranged from 10 to 200 nmol/L, corresponding to a mass concentration of 1 to 39 µg/L.

Having found a suitable method for determining the composition of polysaccharides (PS), this was applied to samples of *S. acidocaldarius* biofilms in order, on the one hand, to determine the composition of PS under defined incubation conditions, and on the other hand, in a further step, to investigate the influence of changes in the incubation conditions, here explicitly the change of the growth substrate. In the PS of *S. acidocaldarius* biofilms, pentoses (ribose), hexoses (mannose, glucose), amino sugars (galactosamine, glucosamine), deoxyhexoses (rhamnose) and acetylated species (*N*-Acetylglucosamine) were identified and quantified as structural components, resulting in the molar ratio 42:35:31:6:4:1:1. The influence of the nutrient medium supplementation was evident but replacement of glucose in the nutrient medium with xylose, maltose, or the absence of sugar as an additional carbon source in the medium changed only the concentrations of the monosaccharides and thus the ratio but not the number or identity of the monosaccharides present.

In a further step, the size of the PS from the biofilms was additionally determined by size exclusion chromatography (SEC). This resulted in only one PS fraction with a very narrow mass distribution for biological samples. The mean mass of the PS fraction was determined to be 71 kDa, with minimum and maximum of ~44 and ~92 kDa, respectively, corresponding to an average of 460 monosaccharide residues assumed to be a linear chain, based on the quantified composition.

Finally, the developed method was applied to analyze the monosaccharidic profiles of other biological samples, enabling the composition analysis of eight fractionated herbal liquors, two plant pectins, and human α1-acid glycoprotein to be determined. Thus, the developed, optimized and applied RP-LC-MS method is suitable not only for the analysis of PS from biofilms but also other biological samples.

Dr. Paul Görs

Development and optimization of mass spectrometry-based fatty acid analysis for systems biology, medicine and nutrition

Fatty acids fulfill various important roles in biological systems. They can serve as energy storage, hormones, building blocks of more complex lipids and assist in the intercellular transport of proteins. Therefore, sensitive methods for fatty acid analysis are required in various area. This includes the identification of illnesses, nutritional-physiological questions, the identification of food fraud, and evolutionary developments of organisms.

Due to this, a sensitive and selective method for analyzing fatty acid in different matrices is required. In the field of gas chromatographic analysis, electron impact ionization of fatty acid methylester is commonly used. An alternative is the derivatization using pentafluorobenzyl bromide which has been used in combination with various ion sources. The traditional used negative ion chemical ionization was compared with two modern atmospheric pressure sources. It was able to demonstrate that atmospheric pressure chemical ionization (APCI) is more sensitive and provides more uniform ionization of the analyzed fatty acids. The optimized and validated method was then used for two studies in the field of diagnostics. Significant differences in the fatty acid concentration of the analyzed tumor cells were identified.

The optimized GC-APCI-MS method was used to analyze fatty acids in archaea. Archaea are one of the three domains of life and their lipidome differs significantly from the other two domains. Until now, it was not clear whether archaea are able to synthe-size fatty acids. To target that, archaea were cultivated on different 13C-labeled media and the 13C-labeled fatty acids were analyzed. The ability to synthesize fatty acids could be proofed for archaea. Both the media and the species both have an high influence on the fatty acid synthesis in archaea. Caprylic acid (FA 8:0) caprinic acid (FA 10:0 and laurinic acid were identified and quantified in *Sulfolobus acidocaldarius*, while palmitic acid (FA 16:0) was the most dominant fatty acid in *Haloferax volcanii*.

The number and the position of double bonds play an important role in the physiological effects of fatty acids. While the number of double bonds can be determined from the mass-to-charge ratio of the ionized fatty acid, determining the position of these double bonds is much more challenging. The chromatographic separation of fatty acid isomers is often difficult and requires long analysis times. To simplify this, the GC-APCI-MS method has been modified using benzaldehyde as a reactant. This promotes a regiospecific in-source fragmentation, which can be used for double bond position determination. The performance of this method was demonstrated by the analyzis of fish oil. Several rare fatty acids such as hexadecatrienic acid (FA 16:3), hexadecatet-raenic acid (FA 16:4) or stearidonic acid (FA 18:4) were identified, quantified and the double bond position was confirmed by the regiospecific fragments. This method rep-resents a fast and simple approach, complementing the existing methods such as the Paternò-Büchi reaction and ozonolysis.

Master's Theses Accomplished 2024

Christopher Julian Jaeger
Charakterisierung und Anwendung einer probenvorbereitungsarmen Analysetechnik basierend auf Ultraschall Plasma Ionisation

Sarah Klaus
Development and application of an automated sample preparation for the analysis of polar metabolites

Matthias Miertz
Method development for the analysis of selected low molecular weight proteins using HPLC-MS/MS

Laila Orell
Effects of statins on cholesterol biosynthesis and the global lipidome in lung carcinoma cells

Lennard Warnecke
Charakterisierung und Identifizierung bioaktiver Bestandteile europäischer Heilpflanzen

Bachelor's Theses Accomplished 2024

Nadezhda Dimitrova
Charakterisierung und Identifizierung von antioxidativen Verbindungen aus A. Eupatorium, A. Archangelica, S. Nigra und S. Ebulus durch effektorientierte Analyse ihrer Fraktionen mit LC-MS sowie Elementverteilung durch ICP-OES

Friederike Jahr
Optimierung und Anwendung einer Ultraschall-basierten Plasmaionenquelle

Leonardo Nuredin
Einsatz der LCxLC zur Analyse von Heilpflanzen der Klostermedizin

Nico Pernberg
Optimierung und Validierung zur Polyamin- und Aminosöureanalyse mittels LC-MS

Claudia Meike Rzepinski
Aufbau einer gepulsten Elektrospray-Ionenquelle

Paula Schneyer
Charakterisierung und Anwendung einer Ultraschall-basierten Plasmaionenquelle zur Analyse von Zellen

Anna Maria Wegenaer
Spurenanalytik im Wasserstoff mittels unterschiedlicher Messverfahren

Scientific Publications 2024

Original Paper / Peer-reviewed

S. Löbbecke, A. Pape, L. Montero, F. Uteschil, J. F. Ayala-Cabrera, O. J. Schmitz, **Improving the reliability of phthalate esters analysis in water samples by gas chromatography-tube plasma ionization-high-resolution mass spectrometry (GC-TPI-HRMS)**, *accepted in Talanta*

Y. Kong, J. Riebe, M. Feßner, T. Schaller, C. Wölper, F. Stappert, S. W. Meckelmann, M. Krajnc, P. Weyrauch, O. J. Schmitz, C. Merten, J. Niemeyer, X. Hu, R. U. Meckenstock, **A CoA-transferase and an acyl-CoA dehydrogenase that convert 2-(carboxymethyl)cyclohexane-1-carboxyl-CoA in anaerobic naphthalene degradation,** *accepted in Environmental Microbiology*

D. Li, X. Du, W. Bai, O. J. Schmitz, **Sample preparation of *Hedyotis diffusa* Wild. for two-dimensional gas chromatography-time of flight mass spectroscopic analysis enhanced chemical profiling**, Pharmaceutical Science Advances (2024) 2:100056, doi.org/10.1016/j.pscia.2024.100056

S. D. Shelton, S. House, L. Martins Nascentes Melo, V. Ramesh, Z. Chen, T. Wei, X. Wang, C. B. Llamas, S. Sai Krishna Venigalla, C. J. Menezes, G. Allies, J. Krystkiewicz, J. Rösler, S. W. Meckelmann, P. Zhao, F. Rambow, D. Schadendorf, Z. Zhao, J. G. Gill, R. J. DeBerardinis, S. J. Morrison, A. Tasdogan, P. Mishra, **Pathogenic mitochondrial DNA mutations inhibit melanoma metastasis**, Science Advances (2024) 10:eadk8801, doi.org/10.1126/sciadv.adk8801

P. Wittenhofer, L. Kiesewetter, O. J. Schmitz, S. W. Meckelmann, **Investigation of the Cholesterol Biosynthesis by Heart-Cut Liquid Chromatography and Mass Spectrometric Detection**, Journal of Chromatography A (2024) 1738:465475; doi.org/10.1016/j.chroma.2024.465475

A. Pape, J. F. Ayala-Cabrera, F. Stappert, F. Uteschil, C. Thom, Y. Terui, S. Yoshioka, O. J. Schmitz, **Development of an ultrasonic nebulization system for an inverse low temperature plasma ionization source**, Journal of the American Society for Mass Spectrometry (2024) 35:2918-2925 doi.org/10.1021/jasms.4c00259

P. Wittenhofer, L. Montero, O. J. Schmitz, S. W. Meckelmann, **Automated green sample preparation for quantitative extraction of lipids in different sample matrices**, Green Analytical Chemistry (2024) 10:100128, doi.org/10.1016/j.greeac.2024.100128

S. Imada, H. Shin, S. Khawaled, S. W. Meckelmann, C. A Whittaker, R. Oliveira Corrêa, Y. Lu, G. Tie, D. Pradhan, G. Calibasi-Kocal, L. Martins Nascentes Melo, G. Allies, J. Rösler, P. Wittenhofer, J. Krystkiewicz, O. J. Schmitz, J. Roper, M. Aurelio Ramirez Vinolo, L. Ricciardiello, E. C. Lien, M. G. Vander Heiden, R. A. Shivdasani, C.-W. Cheng, A. Tasdogan, Ö. H Yilmaz, **Post-fast refeeding enhances intestinal stem cell-mediated regeneration and tumourigenesis through mTORC1-dependent polyamine synthesis**, Nature (2024) 633:895-904, doi.org/10.1038/s41586-024-07840-z

E. Sekera, A. Somogyi, Z. Takats, F. Stappert, C. Thom, O. J. Schmitz, C. Moeckel, B. Paizs, J. Sommertune, **Utilization of bis-MPA Dendrimers for the Calibration of Ion Mobility Collision Cross Section Calculations**, Journal of the American Society for Mass Spectrometry (2024) 35:1101–1109, doi.org/10.1021/jasms.3c00428

Z. Yao, K. Zhu, T. Gu, O. J. Schmitz, D. Li, **An active derivatization detection method for inline monitoring the isolation of carbohydrates by preparative liquid chromatography**, Journal of Chromatography A (2024) 1719:464730, doi.org/10.1016/j.chroma.2024.464730

A. K. Mallik, L. Montero, S. W. Meckelmann O. J. Schmitz, **Facile preparation of embedded polar group-containing pentafluorophenyl stationary phases for highly selective separations of diverse analytes**, Journal of Chromatography A (2024) 1717:464688, doi.org/10.1016/j.chroma.2024.464688

M. Rogava, T. J. Aprati, W.-Y. Chi, J. C. Melms, C. Hug, S.H. Davis, E.M. Earlie, C. Chung, S. K. Deshmukh, S. Wu, G. Sledge, S. Tang, P. Ho, A. D. Amin, L. Caprio, C. Gurjao, S. Tagore, B. Ngo, M. J. Lee, G. Zanetti, Y. Wang, S. Chen, W. Ge, L. Martins Nascentes Melo, G. Akkies, J. Rösler, G. T. Gibney, O. J. Schmitz, M. Sykes, R. J. Creusot, T. Tüting, D. Schadendorf, M. Röcken, T. K. Eigentler, A. Molotkov, A. Mintz, S. F. Bakhoum, S. Beyaz, L. C. Cantley, P. K. Sorger, S. W. Meckelmann, A. Tasdogan, D. Liu, A. M. Laughney, B. Izar, **Loss of *Pip4k2c* confers liver-metastatic organotropism**, Nature Cancer (2024) 5:433–447, doi.org/10.1038/s43018-023-00704-x

A. Pape, O. J. Schmitz, **Dielectric barrier discharge in mass spectrometry - an overview over plasma investigations and ion sources applications,** Trends in Analytical Chemistry (2024) 170:117420, doi.org/10.1016/j.trac.2023.117420

Misc. Publications

S. Hamman, S. W. Meckelmann, M. Maares, E. Varga (2024) **Trendbericht Lebensmittelchemie**, Nachrichten aus der Chemie 72:50-57

Poster Presentation

K. Wetzel, T. Tishakova, M. Häßler, L. Montero, O. J. Schmitz, **Chemical characterization and fractionation of bioactive compounds derived from European medicinal plants**, 10th analytica conference China, Shanghai, China, Novemver 2024

K. Wetzel, T. Tishakova, M. Häßler, L. Montero, O. J. Schmitz, **Chemical characterization and fractionation of bioactive compounds derived from European medicinal plants**, 34th International Symposium on Chromatography – ISC 2024, Liverpool, UK, October 2024

K. Wetzel, T. Tishakova, M. Häßler, L. Montero, O. J. Schmitz, **Chemical characterization of European medicinal plants by multi-2D LC x LC**, 18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Leuven, Belgium, May 2024

M. Häßler, K. Wetzel, L. Montero, J. F. Ayala-Cabrera, O. J. Schmitz, **Design of a Dual Ion Source coupled to HRMS to enhance the chemical characterization of comprehensive 2D-LC of European Medicinal Plants**, 18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Leuven, Belgium, May 2024

P. Wittenhofer, O. J. Schmitz, S. W. Meckelmann, **Comprehensive Analysis of Sterols and Oxysterols by Heart-Cut 2D-LC-MS,** 18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Leuven, Belgium, May 2024

J. Rösler, C. P. Krempe, J. Leddin, S. W. Meckelmann, A. Tasdogan, O. J. Schmitz, **Fast Metabolome Screening by HILIC-Orbitrap-MS**, analytica Munich conference, Munich, Germany, March 2024

A. Pape, J. F. Ayala-Cabrera, F. Stappert, F. Uteschil, C. Thom, S. Yoshioka, Y. Terui, O. J. Schmitz, **Development of a new nebulization system for LC-MS coupling employing an inverse low temperature plasma ion source**, 55th Annual Conference of the German Society for Mass Spectrometry (DGMS), Freising, Germany, March 2024

Invited Lectures / Oral Presentations

Prof. Oliver J. Schmitz

The challange of non-targeted analysis
Research Center for Eco-Environmental Sciences, Yancheng, China, November 2024

Do we still need chromatography to analyze complex samples?
Soochow University, Suzhou, China, November 2024

Do we still need chromatography to analyze complex samples?
Agilent Technologies, Santa Clara, USA, November 2024

Ultra-high-resolution MS and ever more powerful ion mobility mass spectrometers, and we are still talking about chromatography: why?
University of British Columbia, Vancouver, Canada, September 2024

Ultra-high-resolution MS and ever more powerful ion mobility mass spectrometers, and we are still talking about chromatography: why?
University of Münster, Münster, Germany, Juni 2024

Ultra-high-resolution MS and ever more powerful ion mobility mass spectrometers, and we are still talking about chromatography: why?
18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Leuven, Belgium, May 2024

Dr. Yassine Oulad El Majdoub

Orthogonal separation in comprehensive two-dimensional liquid chromatography (LCxLC) using Sil-Lys-2C18 as stationary phase in both dimensions.
34th International Symposium on Chromatography – ISC 2024, Liverpool, UK, October 2024

Comprehensive Analysis of Polyphenols in Wine Grape Pomace Using Two-Dimensional Liquid Chromatography
18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Leuven, Belgium, May 2024

Marvin Häßler

Switching the ion source during a LC run for deeper characterization of herbal medicinal plants
Agilent Food & Live Science Seminar, Universität Duisburg-Essen, Essen, Germany, March 2024

Jonas Rössler

Rapid metabolome screening in clinical research and analytics using LC-HRMS
Agilent Food & Live Science Seminar, Universität Duisburg-Essen, Essen, Germany, March 2024

Sebastian Löbbecke

Comparison of state-of-the-art analytical platforms for the analysis of growth promotors in livestock biofluids
34th International Symposium on Chromatography – ISC 2024, Liverpool, UK, October 2024

Investigation of growth promotors using tube plasma ionization with LC-MS and GC-MS
Agilent Food & Live Science Seminar, Universität Duisburg-Essen, Essen, Germany, March 2024

Alexandra Pape

Development of a new nebulization system for LC-MS coupling employing an inverse low temperature plasma ion source
55th Annual Conference of the German Society for Mass Spectrometry (DGMS), Freising, Germany, March 2024

Development of a new nebulization system for an inverse low temperature plasma ion source
34th Doctoral seminar Hohenroda, Hohenroda, Germany, January 2024

Katharina Wetzel

Boosting the separation power of LC × LC –one dimension more?
10th analytica conference China, Shanghai, China, November 2024

Green extraction and fractionation via preparative LC for the enrichment of bioactive compounds derived from European medicinal plants
Agilent Food & Live Science Seminar, Universität Duisburg-Essen, Essen, Germany, March 2024

Pia Wittenhofer

Analysis of Sterols in Cancer Cells by 2D-LC-MS
Agilent Dissolution Seminar: The path from formulation to analytical results, Universität Duisburg-Essen, Essen, Germany, September 2024

Heart-Cut 2D LC-MS as Tool for the Analysis of Cholesterol Biosynthesis in Pancreatic and Melanoma Cancer
18th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology, Leuven, Belgium, May 2024

Charaterization of the biosynthesis of cholesterol in cancer cells
Agilent Food & Live Science Seminar, Universität Duisburg-Essen, Essen, Germany, March 2024

Charaterization of the biosynthesis of cholesterol in cancer cells
34th Doctoral seminar Hohenroda, Hohenroda, Germany, January 2024

Awards

In January 2024, during the PhD seminar in Hohenroda Alexandra Pape was awarded with the 3rd Price for the best presentation.

Miscellaneous

Conference Organization

At analytica Munich, the world's leading trade fair for laboratory technology, analysis, biotechnology and analytica conference (April 9 to 12, 2024), we organized a session with 11 speakers from eight different countries at the analytica conference on April 9 with the title **"A dream comes true: Fantastic news from analytical chemistry"**.

Also the 10th analytica conference in Shanghai, China was organized in November 2024 together with my colleague Prof. Jin-Ming Lin (Tsinghua University, Bejing, China). The two-day conference was a complete success and was extremely well attended.

Special thanks also go to all invited international speakers, some of whom have attended this conference several times and contributed to its success.

In addition, two seminars together with Agilent Technologies were organized at the University of Duisburg-Essen. In March 2024 the Agilent Food & Live Science Seminar and in September 2024 the Dissolution Seminar: The path from formulation to analytical results.

TRC-Forum

The Teaching and Research Center for Separation, the TRC, is part of Agilent´s global network of world-class Centers of Excellence and besides research in the field of multidimensional chromatography, Ion mobility-mass spectrometry, ion source development, lipidomics and metabolomics we offer three-day-courses on different analytical separation techniques with a practical part. These courses are open for everyone (www.trc-separation.com).

In addition, a digital seminar, called TRC-Forum is organized. Next year we will continue these lectures.

GDCh Course

From May 11 to 13, 2025, we are offering a GDCh course on **Multidimensional chromatography for non-targeted analysis** at the University of Duisburg-Essen. The following contents are planned:

Theory

- Explanations of the different identification levels
- Theory of 2D gas chromatography
  - Heart cutting (GC-GC)
  - Comprehensive two-dimensional gas chromatography (GCxGC)
- Theory of 2D liquid chromatography
  - multiple heart-cutting
  - comprehensive two-dimensional chromatography (LCxLC)
- Theory of ion mobility mass spectrometry
  - FAIMS
  - TWIMS
  - TIMS
  - DTIMS
  - SLIM
  - Collision Cross Section and its benefits for identification
- Workflow: From the raw data to the result

Practical work

- GCxGC-EI-MS analysis of a complex sample
- LCxLC-ESI-qTOF-MS of a complex sample
- LC-ESI-IM-qTOF-MS of a complex sample

Scientific functions by Prof. Oliver J. Schmitz

- Permanent Scientific Committee of the International Symposium on Chromatography (ISC)
- Editorial Board member of Green Analytical Chemistry
- Editorial Board member of *Talanta open*
- Editorial Advisory Board member of Trends in Analytical Chemistry (TrAC)
- Editorial Advisory Board member of *LCGC International*
- Associate Editor-in-Chief of Journal of Analysis and Testing
- Advisory Board member of *Chromatographia*
- Editorial Board member of Journal of Pharmaceutical Analysis
- Editorial Board member of Vietnam Journal of Chemistry
- Editorial Board member of Chinese Journal of Chromatography
- Member of the advisory board of analytica Munich
- Member of the committee for the Ernst-Bayer-Price
- Member of the committee for the Eberhard-Gerstel-Price

Teaching

Chemistry (B.Sc. / M.Sc.)

- Lecture Analytical Chemistry I (in German, Prof. O. J. Schmitz and Dr. S. W. Meckelmann)
- Tutorial Analytical Chemistry I (in German, Dr. S. W. Meckelmann)
- Lecture Analytical Chemistry II (in German, Prof. O. J. Schmitz)
- Tutorial Analytical Chemistry II (in German, Dr. S. W. Meckelmann)
- Lecture Modern analytical methods for systems medicine (in German, Prof. O. J. Schmitz, Dr. S. W. Meckelmann and Prof. S. Heiles)
- Seminar Modern analytical methods for systems medicine (in German, Prof. O. J. Schmitz)
- Lecture Chemistry and analytics of food and their authenticity (in German, Dr. S. W. Meckelmann)
- Seminar Chemistry and analytics of food and their authenticity (in German, Dr. S. W. Meckelmann)
- Lecture Foodomics: Biochemistry of nutrition and analysis of functional foods (in German, Dr. S. W. Meckelmann)
- Seminar Foodomics: Biochemistry of nutrition and analysis of functional foods (in German, Dr. S. W. Meckelmann)

Water Science (B.Sc. / M.Sc)

- Lecture Analytical Chemistry I (in German, Prof. O. J. Schmitz and Dr. S. Meckelmann)
- Tutorial Analytical Chemistry I (in German, Dr. S. W. Meckelmann)
- Lecture Analytical Chemistry II (in German, Prof. O. J. Schmitz)
- Tutorial Analytical Chemistry II (in German, Dr. S. W. Meckelmann)
- Lecture Applied Analytical Chemistry (in English, Prof. O. J. Schmitz)
- Tutorial Applied Analytical Chemistry (in English, Prof. O. J. Schmitz)
- Lecture Modern analytical methods for systems medicine (in German, Prof. O. J. Schmitz, Dr. S. W. Meckelmann and Prof. S. Heiles)
- Seminar Modern analytical methods for systems medicine (in German, Prof. O. J. Schmitz)
- Lecture Chemistry and analytics of food and their authenticity (in German, Dr. S. W. Meckelmann)
- Seminar Chemistry and analytics of food and their authenticity (in German, Dr. S.W. Meckelmann)

Laboratory Technician Training

Instrumental analytical chemistry (in German, Prof. O. J. Schmitz)

Seminar

Analytical-chemical seminar (in German/English, Prof. O. J. Schmitz in cooperation with Prof. T. Schmidt and Prof. S. Heiles)

Practical Courses

- Practical course analytical chemistry (Prof. O. J. Schmitz and Dr. S. W. Meckelmann)
- Research practical courses (Prof. O. J. Schmitz and Dr. S. W. Meckelmann)

Knowledge Transfer (by Prof. O. J. Schmitz, in German)

- Mass spectrometry: A comprehensive overview of the most important detection method, Haus der Technik, Essen, Germany, March 2024
- HPLC-MS in der Non-Target und in der Spurenanalytik, Haus der Technik, Essen, Germany, May 2024
- Method school: HPLC for beginners (digital), Klinkner & Partner, June 2023
- Basic course LC-MS (digital), Provadis, May 2024
- Method school: HPLC for advanced users (digital), Klinkner & Partner, May 2024
- Basic course LC-MS (digital), Provadis, May 2024
- GC-MS: From the ion source to the mass analyzer, Haus der Technik, Essen, Germany, June 2024
- Chromatography Special Part 2 (stationary phases), Provadis, June 2024
- Chromatography Special Part 4 (difficult analytes), Provadis, November 2024