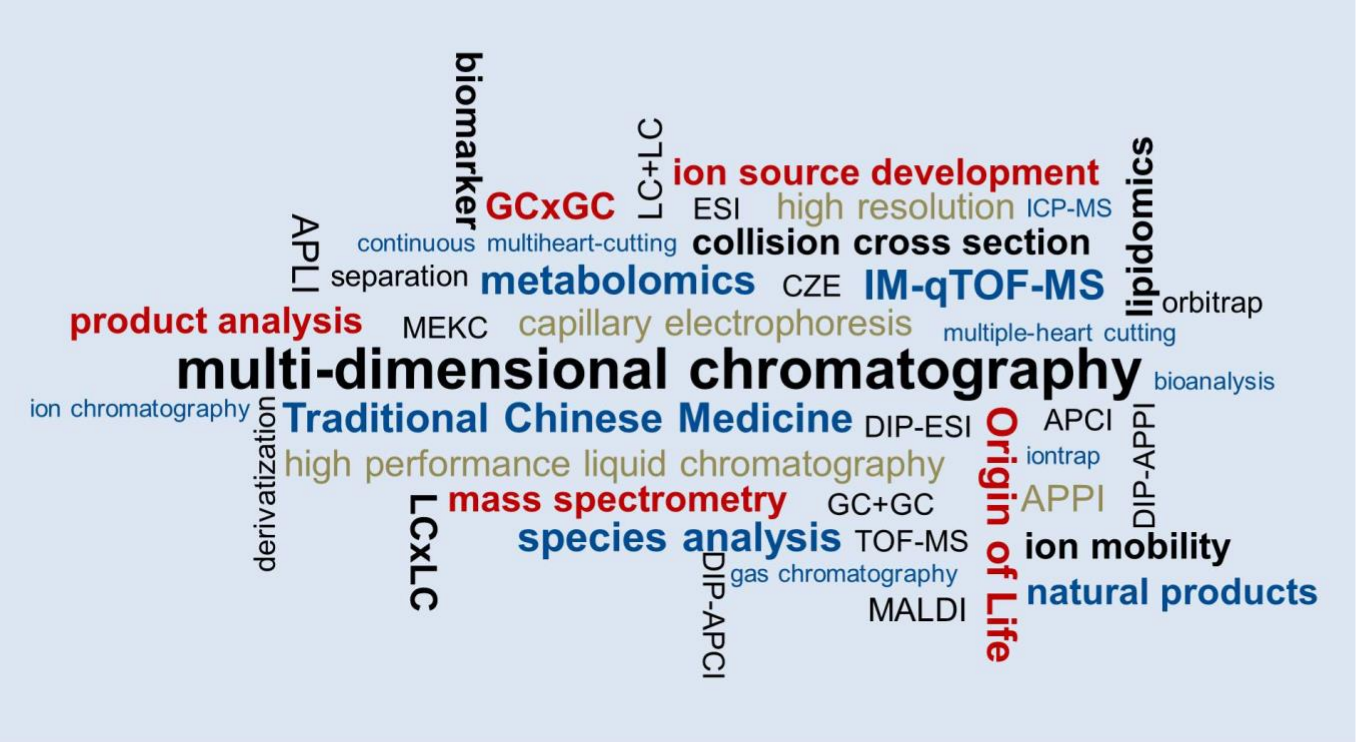
Applied Analytical Chemistry (AAC)

Annual Report 2025

AAC
applied
analytical
chemistry

Applied Analytical Chemistry

Annual Report 2025



University of Duisburg-Essen
Faculty of Chemistry
Applied Analytical Chemistry
Universitaetsstr. 5
45141 Essen
Germany

Table of Contents

The Year 2025 at a Glance

As in previous years, I am pleased to send you the annual report of Applied Analytical Chemistry (AAC) at the University of Duisburg-Essen, providing you with a concise overview of the work conducted at AAC over the past year.

AAC is part of the Faculty of Chemistry at the University of Duisburg-Essen (UDE) and has been active since September 2012. Our research focuses on the development of novel ion sources for mass spectrometry, the non-target analysis of complex samples using multi-dimensional separation techniques combined with ion mobility and high-resolution mass spectrometry, metabolomics/lipidomics, and industry-relevant analytical applications.

2025 was the thirteenth year of the Applied Analytical Chemistry research group at the University of Duisburg-Essen and from the perspective of our publications, the most successful to date. This year began with the end of my sabbatical at the beginning of January.

One of the most significant developments in 2025 was the renewal of long-standing collaborations as well as the establishment of several important and forward-looking partnerships with national and international research groups. I would like to highlight a few of these cooperation partners.

We continued our collaborations with Trent Northen from Lawrence Berkeley National Laboratory, David Chen and Huan Tao from the University of British Columbia, the companies ThyssenKrupp and BASF, as well as with Ralf Schäfer and Alexander Probst from the University of Duisburg-Essen. In addition, our successful partnership with Mobilion progressed further, culminating in the acquisition of their Mobie 3.0 instrument—after nine months of testing—making it the first next-generation ion mobility mass spectrometer of its kind in Europe.

Our highly productive collaboration with Prof. Altaslan Tasdogan from Essen University Hospital was also intensified this year and has already resulted in multiple high-impact publications in *Nature*, *Nature Cancer*, *Nature Cell Biology*, and other leading journals.

Teaching and Research
Center for Separation

In 2018, we established a partnership with Agilent Technologies. Through this collaboration, Agilent provides us with a wide range of analytical systems. As in 2021 and 2024, we had the opportunity in 2025 to upgrade these excellent instruments to the latest versions. This year, the system transition was completed with the replacement of the Infinity II systems with the new Infinity III systems. Much of the work presented in this report would not have been possible without this outstanding equipment. We would therefore like to express our sincere thanks to Agilent Technologies for their continued support.

Agilent Technologies

In 2025, we published 14 scientific papers in peer-reviewed journals and three in non-reviewed journals, with an additional five manuscripts currently under review. Our team also presented 12 posters and 12 lectures at national and international conferences.

Furthermore, one PhD, five master's, and eight bachelor's theses were successfully completed at AAC in 2025. Several projects were either initiated or continued, including analytical support for the Carbon2Chem cluster project, aimed at optimizing reaction conditions and catalysts for the conversion of methanol to sustainable aviation fuel (SAF), as well as investigations of the metabolome and lipidome in cancer research.

We wish you all the best, good health, happiness, and success in 2026.

Essen, December 17, 2025

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. Jaqueline Leddin
Dr. Yassine Oulad El Majdoub
Dr. Tatyana Tishakova

Ph.D. Students

University Duisburg-Essen	External
Marvin Häßler	Sarah Fasbender
Constantin Krempe	Anneke Niehuus
Sebastian Löbbecke	Simon Schastok
Priscilla Nhan	Nina Timmer
Jonas Rösler	
Cedric Thom	
Katharina Wetzel	
Pia Wittenhofer	
Ling Tang	

M.Sc. Students

Nils Becker, Jost Guinand, Constantin Krempe, Sebastian Löbbecke, Priscilla Nhan

B.Sc. Students

Andre Brandt, Aristotelis Charchantis, Lukas Ickerott, Dustin Linke, Karolin Orth, Erik Pösken, Alexander Rosellen, Tobias Thiel

Guest Scientists

Prof. Abdalla Ahmed Elbashir (King Faisal University, Saudi Arabia), Ana Paula Ramos Pereira (Federal University of Mato Grosso do Sul, Brazil), Dr. Taher Sahlabji (King Khalid University, Saudi Arabia)

Apprentices

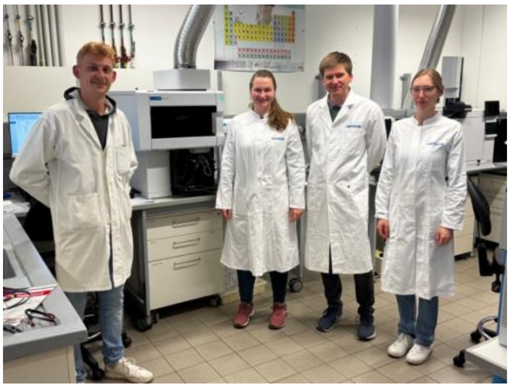
Ronja Becker, Jan Müller

Major News 2025

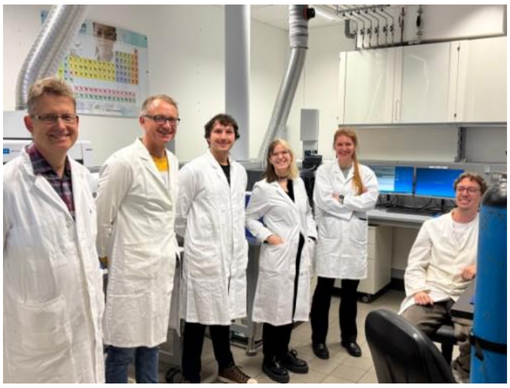
New Cooperation

During a delegation trip by the Faculty of Chemistry at UDE to University of Tsukuba, AIST and RIKEN in Japan in June, many cooperation talks took place and a cooperation between the AAC and the working group of Yuta Koda from the Department of Material Science, University of Tsukuba started shortly afterwards in the field of metabolomics.

Teaching Courses



The Teaching and Research Center for Separation (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 theoretical and practical part. These courses are open for everyone (www.trc-separation.com). This year, from April 9 to 11, our working group held a course at the Teaching and Research Center for participants from industry. The course covered theory on **ICP-OES/MS, GC, and LC**, and included practical experiments on our new Agilent equipment. In addition, a three-day course from the German Chemical Society (GDCh) on **Non-target analysis using multidimensional chromatography or ion mobility-mass spectrometry** took place for the first time from November 10 to 12. The course covered theoretical knowledge in the areas of LCxLC, GCxGC, ion mobility-mass spectrometry, and data evaluation in non-target analysis, which was then reinforced in three different laboratory experiments.



Another highlight of the year was, first, the visit and lecture by Dr. Markus Kamieth, UDE alumnus and CEO of BASF, on July 1. During his visit to his alma mater, we had the opportunity to introduce him to our research group and showcase our work. Second, we celebrated the birth of Florian's son, Piet. We extend our warmest wishes to the entire family.

Conference Organization

In February one seminar together with Agilent Technologies about Infinity III and GC were organized at the University of Duisburg-Essen. This was followed by the analytica conferences in Vietnam and the USA.

analytica Vietnam took place from April 2 to 4, and we organized an international conference in Ho Chi Minh City together with Prof. Viet Pham from Vietnam National University. It is wonderful to see that analytica Vietnam is gaining in importance and quality with each event and represents a real scientific highlight in the region.

A special highlight took place in September in Columbus, Ohio (USA), when the first analytica USA was held there. We organized the accompanying conference, and top-class speakers and scientists took part in this very first American analytica. Following the conference, two poster sessions were held, and the best three posters were selected and awarded prizes by an international committee of experts. Special thanks also go to all invited international speakers, some of whom have attended this conference several times and contributed to its success.

After analytica is before analytica. The program for the next analytica in Munich (Germany) has also been finalized. Fantastic speakers are expected at the Separation Science Session titled **The Future of Chromatography is Here – Are You Ready?** on Tuesday, March 24, 2026:

The Future of Chromatography Is Here – Are You Ready? **1st Part: Towards Sustainable HPLC: Are Narrower Columns the Solution?**			
10:00 – 10:30	Thorsten Teutenberg	IUTA, Duisburg, Germany	From filament to chromatogram: using additive manufacturing to democratise miniaturisation and promote sustainability in analytical laboratories
10:30 – 11:00	Caroline West	University of Orleans, France	Supercritical fluid chromatography as a sustainable analytical method
11:00 – 11:30	Frederic Lynen	University of Gent, Belgium	Microfluidic versus conventional LC–HRMS for HIV metabolomics: a comparative performance assessment
Coffee Break and Poster session			
The Future of Chromatography Is Here – Are You Ready? **2nd Part: The Cutting-Edge Advances You Need to Know**			
12:30 – 13:00	Gerd Desmet	Free University Brussel, Belgium	Next-Gen HPLC: A Column Technology Deep Dive
13:00 – 13:30	Giorgia Purcaro	University of Liege, Belgium	GC-Based hyphenated techniques for detailed characterization of complex samples
13:30 – 14:00	Kevin Schug	University of Texas at Arlington, USA	Molecular Encoding as a Tool to Enable Data Science-Assisted Analytical Measurements
14:00 – 14:30	Gauthier Eppe	University of Liege, Belgium	Good Resolutions for Bad Pollutants: Ion Mobility–HRMS to Resolve Complex Environmental Mixtures
Coffee Break			
15:00 – 15:30	J.F. Focant	University of Liege, Belgium	GC×GC-TOFMS for Medical Volatolomics
15:30 – 16:00	Luigi Mondello	University of Messina, Italy	Cryogenic Zone Compression after Conventional and Comprehensive Gas Chromatography to Enhance Sensitivity
16:00 – 16:30	Bob Pirok	University of Amsterdam, The Netherland	Significance of radial dispersion to effective modulation in two-dimensional liquid chromatography and its role in machine-learning based automated method development
16:30 – 17:00	Karine Faure	University of Lyon, France	LCxSFC coupling: A robust tool to aid understanding of renewable and circular industrial processes

Hero of the Year 2025

Jonas Rösler

During his PhD thesis, Jonas published seven articles in Nature, Nature Cancer, Nature Cell Biology, Science Advances, ACS Applied Materials & Interfaces, ACS Omega and ABC. These results in an incredible total impact factor of 125. In addition, one manuscript to Science is in revision and another one (with Jonas as first author) will be submitted very soon.

List of Projects 2025

(Abstracts of these projects within the next pages)

A Match Made in Heaven? – Coupling of LC × LC to Ion Mobility
Katharina Wetzel, Priscilla Nhan, Jaqueline Leddin

Is Bayesian Optimization a Useful Beginner Tool for LC × LC?
Katharina Wetzel, Nils Becker, Jaqueline Leddin

The Healing Properties of Nature – Hepatoprotectivity of European Herbal Remedies
Katharina Wetzel, Marvin Häßler

Development of a Trap-based LC+LC-IM-QTOF-MS Method for Non-target Analysis
Priscilla Nhan, Sven W. Meckelmann

Comprehensive Sterolomics: Expanding Analytical Horizons in Sterol and Oxysterol Metabolism for Cancer Progression
Pia Wittenhofer, Priscilla Nahn, Sven W. Meckelmann

Advancing Sterol Analysis in Cancer Cells Using Atmospheric Pressure Ionization Techniques
Pia Wittenhofer, Sven W. Meckelmann

Comprehensive Profile of the Unique Aroma of Khawlani Arabica Coffee Using HS-SPME Coupled to GC-EI-QTOF
Yassine O. E. Majdoub, Abdalla A. Elbashir

Labware Leachables as a Source of Artefacts in Untargeted Metabolomics with LC-SLIM-QTOF
Yassine O. E. Majdoub, Tobias Thiel, Taher Sehlabji

Targeted Metabolomics of the Tryptophan Pathway by LC-QqQ-MS for Cancer Research
Friederike Jahr, Constantin P. Krempe, Ronja Becker, Sven W. Meckelmann, Alpaslan Tasdogan

Metabolic Characterization of Adaptive Resistance Mechanismsin Melanoma under BRAF/MEK Inhibition
Friederike Jahr, Luiza Martins Nascentes Melo, Constantin P. Krempe, Jonas Rösler, Sven W. Meckelmann, Alpaslan Tasdogan

Comparative Metabolomics of Acral and Cutaneous Melanoma
Friederike Jahr, Flavia Carvalho Aguiar, Constantin P. Krempe, Jonas Rösler, Sven W. Meckelmann, Alpaslan Tasdogan

Receptor-Dependent Metabolic Shifts in Melanoma
Friederike Jahr, Feyza Cansiz, Jonas Rösler, Constantin P. Krempe, Sven W. Meckelmann, Alpaslan Tasdogan

Lipidomic Analysis of CRISPR-edited Organoids in Cancer Research
Friederike Jahr, Jonas Rösler, Constantin P. Krempe, Sven W. Meckelmann, Alpaslan Tasdogan

Fast and Robust Target Lipidomics of Sphingolipids
Constantin P. Krempe, Friederike Jahr, Ronja Becker, Alpaslan Tasdogan, Sven W. Meckelmann

Stable Isotope Tracing as an Analytical Tool for Investigating the Role of SFXN1 in Polyamine Synthesis
Constantin P. Krempe, Friederike Jahr, Sven W. Meckelmann, Alpaslan Tasdogan

Chemical Characterisation of Cannabis Sativa by GC-iLTP-SLIM-MS
Cedric Thom, Sebastian Löbbecke, Marvin Häßler, Sven W. Meckelmann

Separation of German Herbal Liqueur by LC-ESI-SLIM-MS
Cedric Thom, Sven W. Meckelmann

Characterization of the Human Lipidome
Cedric Thom, Sven W. Meckelmann

PSeaC – Algorithm for Feature Combining in LC × LC Data
Jaqueline Leddin, Sven W. Meckelmann

Analysis of Sustainable Aviation Fuels by Cryo-Modulated GC × GC-CI-(q)MS
Sebastian Löbbecke, Jaqueline Leddin, Karolin Orth, Florian Uteschil

Evaluation of Flow-Modulated GC × GC coupled to API-MS for Fuel Analysis
Sebastian Löbbecke, Jaqueline Leddin, Florian Uteschil

Analysis of Fuels by DI-iLTP-Orbitrap HRMS
Sebastian Löbbecke, Karolin Orth, Jaqueline Leddin, Florian Uteschil

Derivatization Strategies for the Determination of PFAS by GC-APLI-(Iontrap)MS
Ling Tang

Development of a Dual Ionization Source for Enhanced Metabolomics Analysis
Constantin P. Krempe, Lukas Ickerott, Friederike Jahr, Florian Uteschil

Dual Ion Source HRMS for Improved Chemical Characterization of European Medicinal Plants Using 2D-LC
Marvin Häßler, Katharina Wetzel, Cedric Thom, Sebastian Löbbecke, Florian Uteschil

Evaluation of the Ionization Efficiency of iLTP in Combination with Orbitrap MS
Marvin Häßler, Sebastian Löbbecke, Florian Uteschil

A Match Made in Heaven? – Coupling of LC × LC to Ion Mobility

Katharina Wetzel, Priscilla Nhan, Jaqueline Leddin

The separation of complex samples such as the herbal liqueur Underberg remains challenging due to the chemical composition, both in terms of various physicochemical properties or similar chemical structures. One possibility to overcome these obstacles is comprehensive two-dimensional liquid chromatography (LC × LC) where each fraction eluting from the first dimension is separated again in the second dimension of a complimentary retention mechanism. Another possibility is to hyphenate LC to ion mobility where the generated ions collide with a carrier gas in a drift tube. Due to different arrival times at the detector, collision cross section (CCS) values can be determined that vary based on the size, shape and charge of the analyte. As CCS values differ for isomeric compounds and can be mores specific than tandem mass spectrometry, ion mobility enhances separation and annotation efficiency for non-target approaches.

For the chemical characterization of Underberg, a LC × LC method with a PFP (Kinetex, 150 x 2.1 mm, 1.7 µm) column in the first and a C18 (Kinetex, 50 x 4.6mm, 2.6 µm) column in the second dimension was coupled to ion mobility and high resolution mass spectrometry (HRMS/MS) (Fig. 1). The data interpretation was conducted using MS-Dial for the spectral library matching to annotate tentative candidates. As features were reoccurring in the result list due to the modulation of the LC × LC method, feature lists were extremely long and repetitive. An in-house script called Peak Selection and Combining (PSeaC) was adapted that combines features of the same m/z and CCS values but differ in retention time equal to the modulation time. These features were combined to one feature and the signal intensities or areas summed. The combination of multi-dimensional separation techniques offer great opportunities but also challenges regarding the data visualization and interpretation.

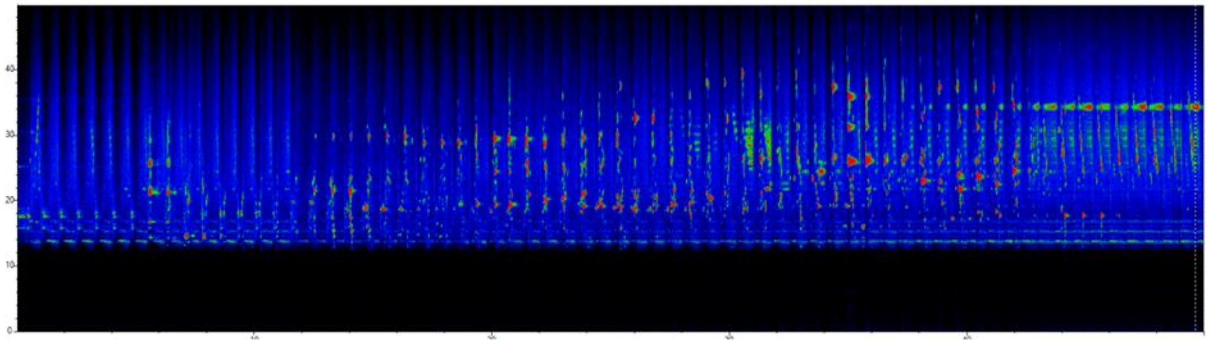


Fig. 1: Heatmap of a LC × LC-IM-MS/MS measurement of Underberg, drift time vs. retention time.

Collaborative Project – Project Partner: Marvin Häßler (University Duisburg-Essen, Essen, Germany)

Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143 and Agilent Technologies

Is Bayesian Optimization a Useful Beginner Tool for LC × LC?

Katharina Wetzel, Nils Becker, Jaqueline Leddin

The method development of comprehensive liquid chromatography (LC × LC) is time-consuming due to multiple, dependent variables that require expert knowledge and hinder applicability of such methods. A possible solution to downstream method development is Bayesian optimization that determines optimal parameters based on a limited amount of experiments. A regression model balances between the possibility to enhance already known regions (exploitation) and regions of high uncertainty (exploration). In collaboration with the British Columbia University, a previously developed app called BAGO for the gradient optimization of untargeted LC-MS analysis was applied for the separation of the herbal liqueur Underberg. First, gradients for various columns have been optimized for the first (^{1}D) and second dimension (^{2}D) before combining promising columns to LC × LC methods. Optimal gradients for the ^{1}D were obtained within 3 to 11 experiments while gradients for the ^{2}D proved to be difficult due to the short modulation times (<1 min). Despite this, promising LC × LC separations can be achieved even with full gradients in the ^{2}D, especially for columns with uncorrelated retention meachnisms (Fig. 2). Shift gradients can futher enhance the separation efficiency but require still expert knowledge. In conclusion, Bayesian optimization like BAGO enables fast gradient optimization for beginners and can be successfully applied even to two-dimensional LC separations.

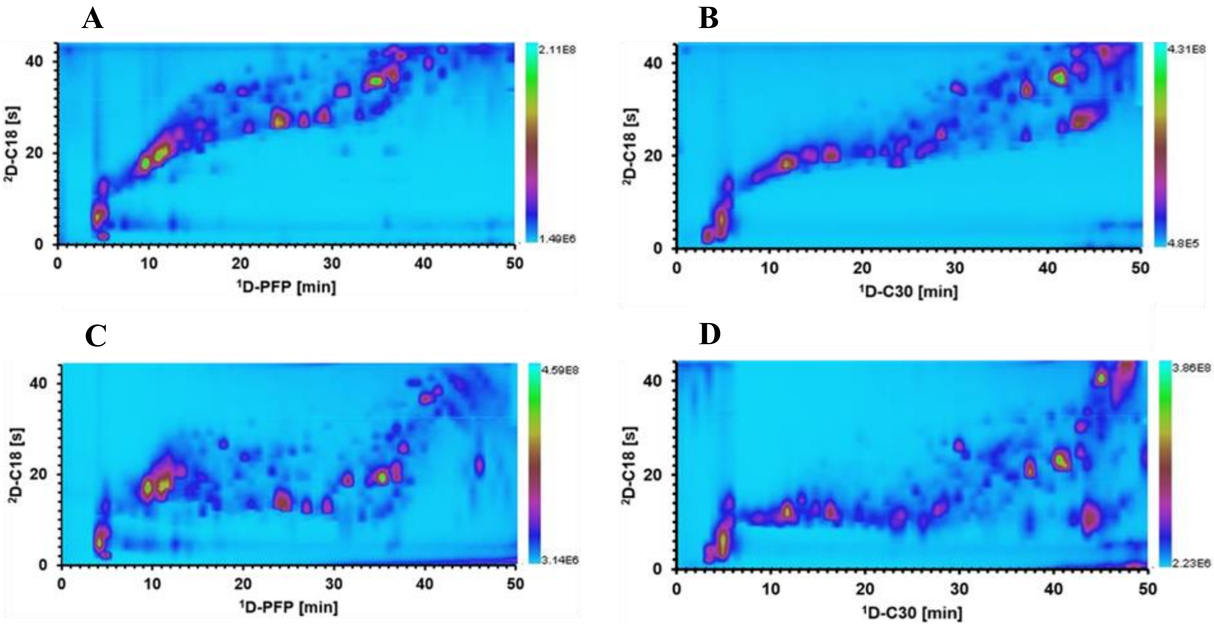


Fig. 2: TIC-HRMS-contour plots of Underberg measured with two columns cominiations, PFP × C18 or C30 × C18, with full or shift gradients in the ^{2}D. The ^{1}D gradient is optimized with BAGO. The full gradient ranged from 5 to 95% ACN in the ^{2}D while the sihft gradient is determined based on the full gradient measurements with the respective time points and mobile phase percentages of the elution range by a beginner.

A: PFP × C18 with a full gradient in the ^{2}D. B: C30 × C18 with a full gradient in the ^{2}D. C: PFP × C18 with a shift gradient in the ^{2}D. D: C30 × C18 with a shift gradient in the ^{2}D.

Collaborative Project – Project Partner: Tao Huan, British Columbia University, Canada

Funded by: Agilent Technologies

The healing Properties of Nature – Hepatoprotectivity of European Herbal Remedies

Katharina Wetzel, Marvin Häßler

Herbal medicine exceeds the use of conventional drugs by two to three times according to the world health organziation (WHO). Although, therapeutic practices has been passed down for centuries, there is little evidence about the healing effects of herbal plants. European herbal remedies known for their hepatoprotective activity were evaluted by *in-vitro* experiments with the human hepatoma cell line HepG2. The cells were cultivated containing a hepatic damage inducer in the medium and treated with flowers or leaves of *S. nigra* and *A. eupatoria*. After the incubation, the cell viability was measured to determine the amount of living cells remaining in the medium expressed in normalized absorption values. The flowers and leaves of *S. nigra* at a concentration of 15% (*v*/*v*) in medium were able to fully recover damaged cells to 100% of the normalized absorption of the undamaged control group (Fig. 3). The flowers of *A. eupatoria* did not only recover but exceeded the normlized absorption values, probably due to cell profilerating compounds in the extract. The leaves of *A. eupatoria* had at least a positive effect on the damaged cells compared to stressed cells which were not treated. These findings suggest that there is more to investigate about these plants with respect to their metabolic profiles. For example, rutin which was identified in the extracts of *S. nigra* had a positive effect on the cells. Therefore, more compounds found in the respective extracts should be investigated to further understand their hepatoprotective potential.

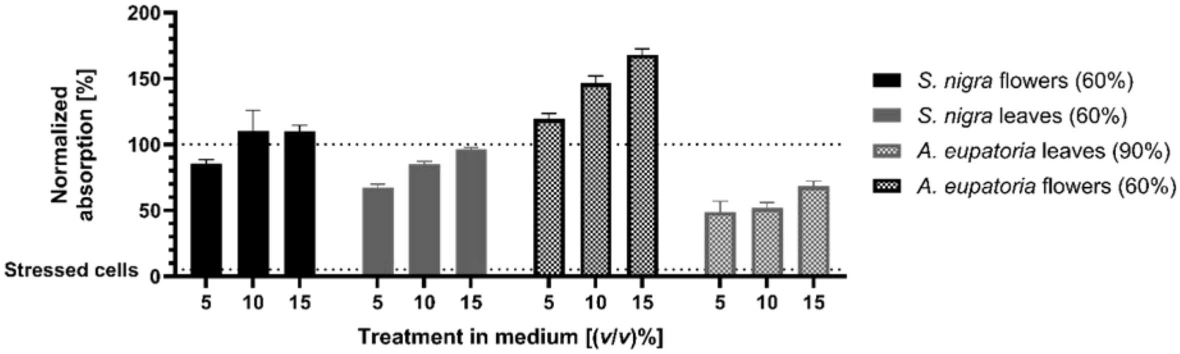


Fig. 3: Normalized absorption of cell viability assay of HepG2 cells after hepatic damage by 15% (v/v) ethanol in medium and treatment with flowers and leaves extracts of S. nigra and A. eupatoria. Normalized absorption values are starting at 0% equal to background measurements to 100% of the undamaged control group. The extraction of the plants was optimized before with either 60% or 90% (v/v) aqueous ethanol.

Collaborative Project – Project Partner: Marvin Häßler (University Duisburg-Essen, Essen, Germany)

Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143 and Agilent Technologies

Development of a Trap-based LC + LC-IM-QTOF-MS Method for Non-target Analysis

Priscilla Nhan, Sven W. Meckelmann

Herbal liqueurs are highly complex samples due to the large number of plant-based compounds. Their detailed chemical characterisation requires multidimensional analytical methodologies. Two-dimensional liquid chromatography (2D-LC) coupled to ion mobility quadrupole time-of-flight mass spectrometry (IM-QTOF-MS) offers separation in four dimensions based on compound polarity or chemistry, shape-to-charge and mass-to-charge (*m/z*) ratio. However, this method generates complex data, especially in comprehensive 2D-LC (LC × LC) setups. To reduce peak modulation and to facilitate multidimensional data interpretation, a hybrid 2D-LC method referred to as LC + LC was applied. Introduced by *Stephan et al.*[1], this method combines aspects of LC × LC and multi-heart cutting 2D-LC (mLC-LC) with a modulation time of four minutes. After careful optimisation of the first dimension (^{1}D) separation conditions on a PFP column (Kinetex, 150 x 2.1 mm, 1.7 µm) and second dimension (^{2}D) separation conditions on a C18 column (Kinetex, 50 x 2.1 mm, 1.7 µm), two modulation strategies were assessed to enhance analyte retention and detection sensitivity. A trap-based modulation system was developed, achieving sufficient separation performance with trapping column-assisted modulation, also referred to as stationary-phase-assisted modulation (SPAM), using C18 trapping columns (5 mm x 2.1 mm, 1.8 µm) and featuring an active dilution flow following the principles of active solvent modulation (ASM). Additionally, different ion mobility spectrometry (IMS) techniques were compared (Fig.4). Structures for lossless ion manipulations (SLIM) provided higher resolving power than drift tube ion mobility spectrometry (DTIMS), as demonstrated by improved isomeric separation. Non-target feature analysis was performed using MS-DIAL with tentative compound annotation based on spectral library matching.

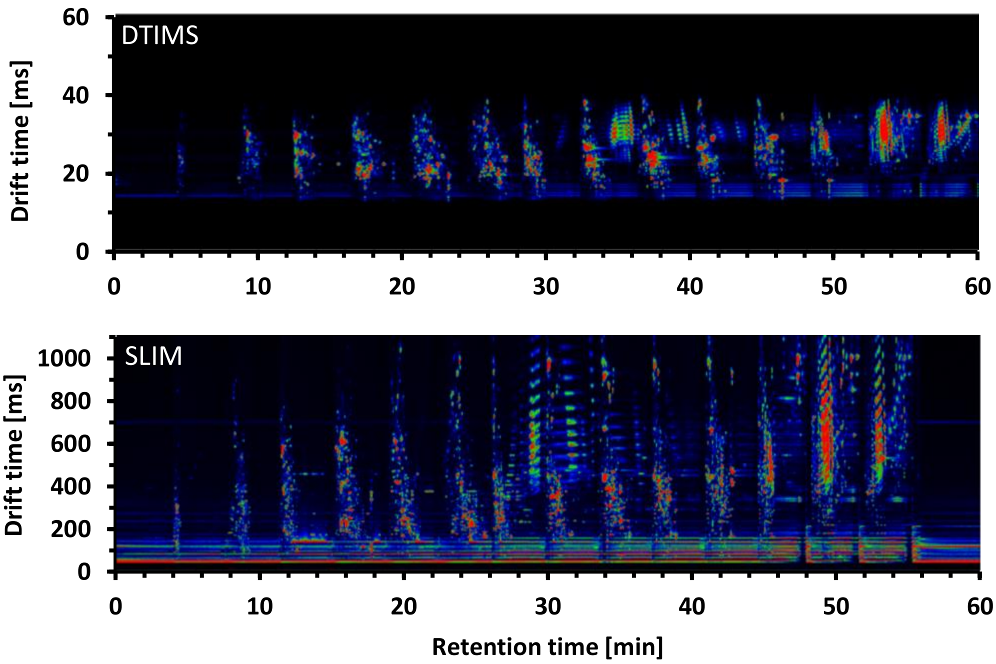


Fig. 4: Heat maps of an LC + LC-IM-QTOF-MS analysis of *Underberg* liqueur in positive ionisation mode with DTIMS and SLIM. DTIMS analysis was performed using nitrogen as drift gas, while SLIM analysis was performed with helium.

[1] Stephan, S.; Jakob, C.; Hippler, J.; Schmitz, O. J. A Novel Four-Dimensional Analytical Approach for Analysis of Complex Samples. Anal Bioanal Chem 2016, 408 (14), 3751–3759.

Collaborative Project – Project Partner: Mobilion
Funded by: Agilent Technologies

Comprehensive Sterolomics: Expanding Analytical Horizons in Sterol and Oxysterol Metabolism for Cancer Progression

Pia Wittenhofer, Priscilla Nahn, Sven W. Meckelmann

Cholesterol is probably the most abundant and extensively studied lipid in the last decades. However, the analysis of the all biosynthesis products is challenging because of the structural similarity, interferences by matrix compounds or the high concentration of cholesterol, the sensitivity as well as the reproducibility of existing method. Therefore, a heart-cut LC - LC method using triple quadrupole detection was developed to overcome these obstacles in a previous project with the title “A novel target approach to characterize the Biosynthesis of Cholesterol in Cancer Cells” and major results already published [1, 2]. It allows analyzing cholesterol and its biosynthetic precursors in one analysis with high sensitivity and reproducibility. The method was used to characterize sterols in melanoma and pancreas cancer cells indicating an adapted sterol metabolism to promote tumor progression and metastatic properties. However, important downstream metabolites were not in the scope of the project and therefore have not yet been incorporated to investigate oxysterol in one target LC-MS method. The developed LC - LC -MS method showed already promising indications that also oxysterols can be incorporated into the method (Fig. 5). This will require some adaptation regarding the i) chromatographic method, ii) mass spectrometry detection, and iii) sample preparation. To support this work, we successfully secured funding through a DFG research project.

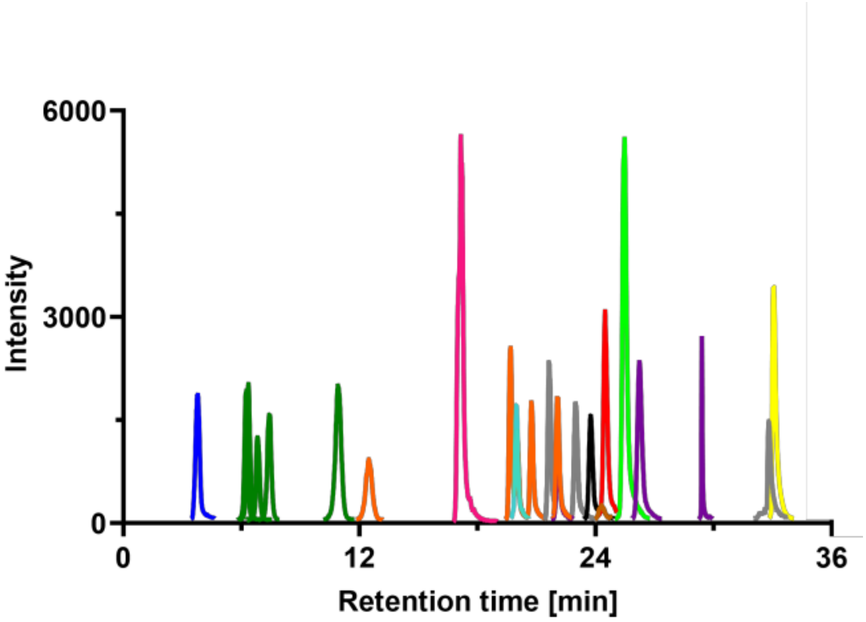


Fig. 5: Integration of oxysterols into the heart-cut 2D-LC method of sterols

[1] P. Wittenhofer, L. Kiesewetter, O. J. Schmitz, S. W. Meckelmann, Investigation of the Cholesterol Biosynthesis by Heart-Cut Liquid Chromatography and Mass Spectrometric Detection. J Chromatogr A 2024, 1738, 465475, 10.1016/j.chroma.2024.465475.

[2] 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, 10.1016/j.greeac.2024.100128.

Funded by: Deutsche Forschungsgemeinschaft DFG (Titel: „Umfassende Sterolomik: Erweiterung der analytischen Horizonte im Sterol- und Oxysterol-Stoffwechsel für die Krebsentwicklung“ 463367585) and Agilent Technologies

Advancing Sterol Analysis in Cancer Cells Using Atmospheric Pressure Ionization Techniques

Pia Wittenhofer, Sven W. Meckelmann

The quantitative analysis of cholesterol biosynthesis in cancer cells is hampered by extreme concentration differences between pathway intermediates and cholesterol, as well as pronounced matrix effects that compromise ionization efficiency. Here, we developed and evaluated a novel tube plasma ionization (TPI, Fig.6) source for liquid chromatography–tandem mass spectrometry (LC-MS/MS) and compared its performance to established atmospheric pressure ionization techniques, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Heart-cutting 2D-LC-QqQ-MS was used to separate 16 sterols involved in cholesterol biosynthesis, and all three ion sources were systematically characterized according to ICH guidelines. For the TPI source, plasma and source parameters were optimized using design-of-experiments approaches to maximize ionization efficiency across structurally similar sterols. Figures of merit (LOD, LOQ, linear range, and R^2) as well as signal stability and ion suppression were assessed in solvent standards and in complex matrices (human plasma, HepG2 cells, and beef liver). ESI showed substantial ion suppression, reduced linear ranges, and higher LOD/LOQ values, rendering it impractical for robust quantification of sterol biosynthesis intermediates in real samples. In contrast, APCI and the prototype TPI source exhibited comparable sensitivity and linear ranges over several orders of magnitude, with median LOQs of 30 nmol/L and upper LOQs up to 90,000 nmol/L for most sterols. Both sources provided stable signals over extended injection series and markedly reduced matrix effects compared to ESI. Application to biological samples demonstrated excellent agreement between APCI and TPI for sterol concentrations. Overall, TPI emerges as a promising alternative API source for routine sterol analysis in challenging cancer-related matrices, achieving performance comparable to APCI despite its prototype status.

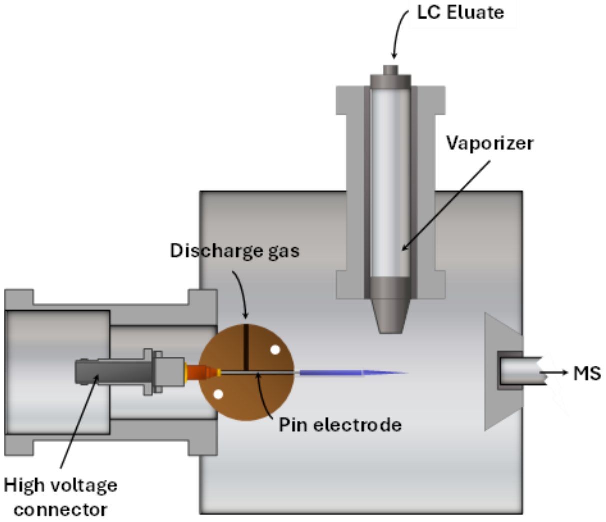


Fig. 6: Integration of oxysterols into the heart-cut 2D-LC method of sterols

---

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

Comprehensive Profile of the Unique Aroma of Khawlani Arabica Coffee Using HS-SPME Coupled to GC-EI-QTOF

Yassine O. E. Majdoub, Abdalla A. Elbashir

Khawlani Coffee Arabica was listed on the UNESCO in 2021 as part of the Intangible Cultural Heritage of Humanity. Khawlani coffee is recognised for its noted aroma, which originates from cultivation in the Jazan region of Saudi Arabia, at nearly 1700 m above sea level, on volcanic soil rich in minerals. Up to the present, the volatile profile of Khawlani coffee has not been characterized, and an investigation of the comprehensive volatile profile would help to preserve the authenticity of this heritage and also contribute to the hindrance of the increasing intentional misleading and substitution with other varieties in the market. To address this aim, the volatilomics of unroasted Khawlani coffee beans alongside three different roasting stages: light, medium, and dark were extracted by the headspace solid-phase microextraction (HS-SPME) with a triphasic 50/30 µm DVB/CAR/PDMS fiber and analyzed with gas chromatography coupled to electron ionization (EI) and quadrupole time-of-flight mass spectrometry (QTOF-MS) using a DB-5MS column (30 m × 0.25 mm × 0.25 µm) (Fig. 7A). Extraction conditions were optimized to achieve fast and efficient recovery of volatile compounds across all roast levels, where an optimal extraction and chromatographic separation were reached at 30 min and 37 min, respectively. Khawlani has shown in the principal component analysis (PCA) in (Fig. 7B) a distinctive volatile profile characteristic of specific chemical classes, including pyrazines, branched short-chain fatty acids, nitrogen heterocycles, besides esters and unsaturated acids, which appeared in noticeably higher abundance than in the benchmark coffees. Moreover, the strongest discrimination between Khawlani Arabica and the reference coffees was observed at the Medium roast level, where pyridines, pyrazines, and branched-chain fatty acids reached their maximum fold-differences relative to the benchmarks. The most intense volatile compounds in Khawlani coffee include 2,5-dimethylpyrazine, methylpyrazine, pyrazine, 3-methylbutanoic acid, 2-methylbutanoic acid, pyridine, methyl valerate, 2-pentenoic acid, 2-propenyl propanoate, and 2,5-dimethyl-1-pyrroline. This volatilomics study provides the first characterziation of volatilomics in Khawlani coffee, which supports future quality control studies, thus contributing to the protection of the cultural heritage of this coffee.

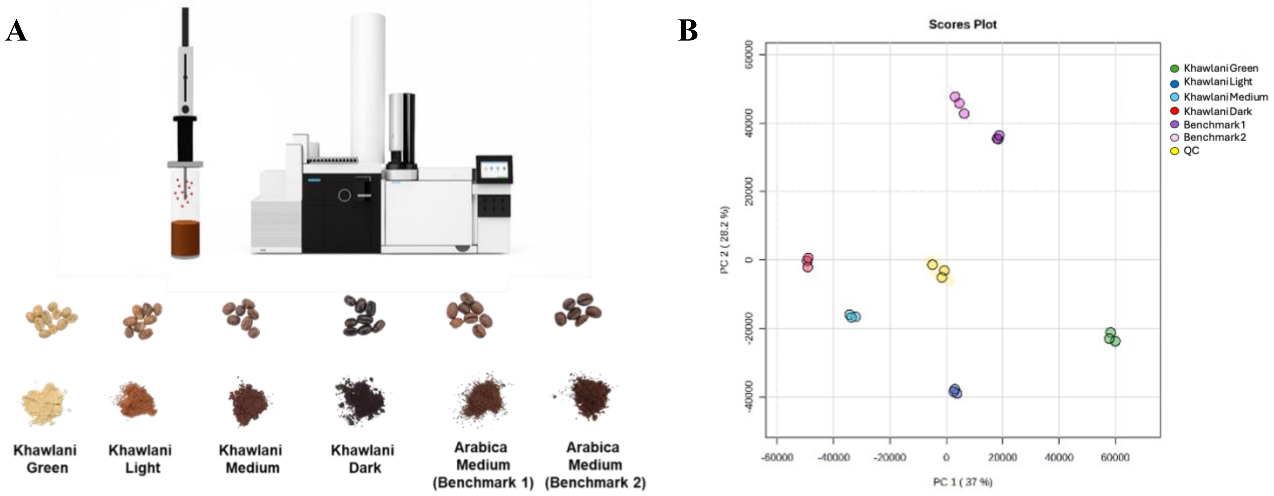


Fig. 7: Profiling of the unique aroma of different roasting levels of Khawlani Arabica coffee by GC-EI-QToF in comparison to two medium-roasted benchmarked Arabica coffee. A: HS-SPME–GC-QTOF for volatile analysis of Khawlani and benchmark Arabica coffee. B: Principal component analysis (PCA) of volatilomics in Khawlani coffee.

Funded by: Agilent Technologies

Labware Leachables as a Source of Artefacts in Untargeted Metabolomics with LC-SLIM-QTOF

Yassine O. E. Majdoub, Tobias Thiel, Taher Sehlabji

Untargeted metabolomics is always regarded as a powerful approach for an overall screening of biological compounds in complex matrices. In this study, metabolites from the rhizosphere of coniferous tree were extracted at 70-85 cm depth. To avoid leachables contamination, initial extraction was performed using glass bottles and without syringe filters, compared to workflows employing laboratory consumables during extraction (e.g. syringe filters, etc.). A 0.22 µm PTFE syringe filter was employed to assess the extent of filter-derived leachables. LC-SLIM-QToF analysis with He as buffer gas was employed to improve the resolving power of low molecular weight metabolites in the rhizosphere. To our knowledge, leachables detection using SLIM has never been reported before, which unveils the likelihood of high interference from leachables with untargeted metabolomics, resulting in high false positive assignments. Figure 8A demonstrates the metabolites profile derived from coniferous rhizosphere soil when extracted in glass labware without filtration with syringe filters, showing a distribution of biological molecular features characteristic of root-associated chemical diversity. In contrast, the PTFE syringe filter extract (Figure 8B) revealed a markedly different pattern, dominated by high-intensity signals not present in the unfiltered sample. These features represent filter-derived leachables, which overlapped with genuine metabolites in mobility and mass domains, posing a significant risk for false-positive annotation in untargeted workflows. Our findings evidence the necessity of avoiding any contact with likely leaching labware consumables, such as syringe filters, Eppendorf, and plastic syringe when working with high-sensitivity SLIM platforms.

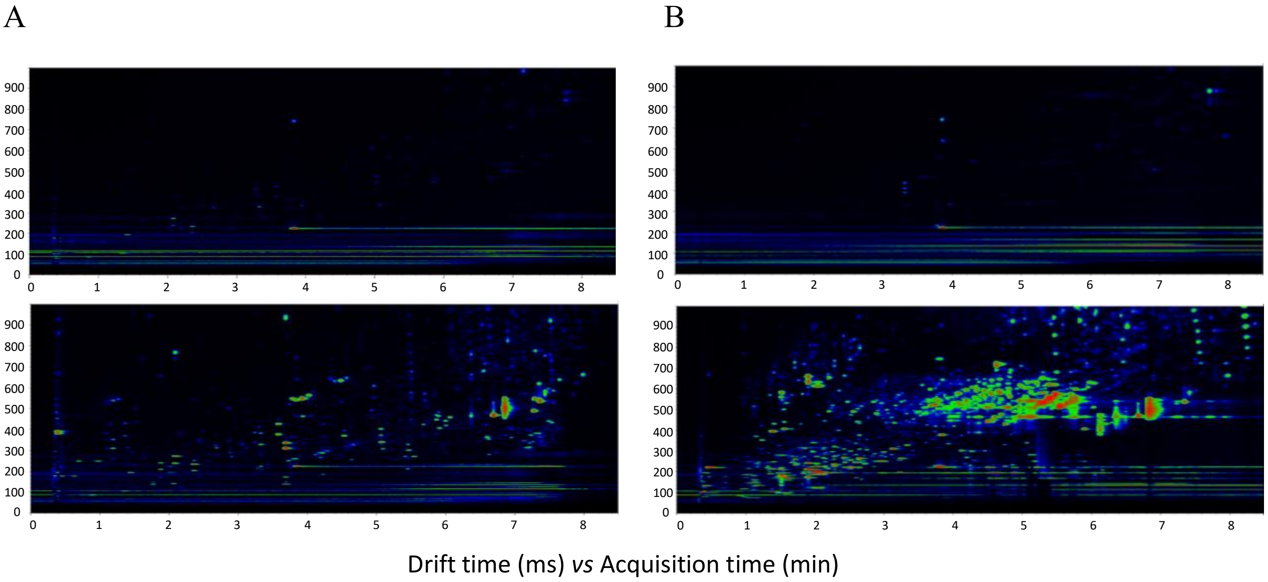


Fig 8. LC-SLIM-QTOF Heatmap of untargeted polar metabolomics in coniferous rhizosphere collected at 70-85 cm extracted using glass bottles and without syringe filters. A (top): Sample blank, A (bottom): Deciduous rhizosphere. B (top): the solvent blank, B (bottom): leachables from PTFE syringe filter, 0.22 µm.

Collaborative Project – Project Partner: Mobilion
Funded by: Agilent Technologies

Targeted Metabolomics of the Tryptophan Pathway by LC-QqQ-MS for Cancer Research

Friederike Jahr, Constantin P. Krempe, Ronja Becker, Sven W. Meckelmann, Alpaslan Tasdogan

The metabolic transformation of tryptophan is leading to a variety of important metabolites that are altered in diseases. Tryptophan and its downstream metabolites play a central role in immune regulation, inflammation and cellular signal transduction. Some metabolites are associated with increased malignancy and resistance to therapy in various types of cancer. This makes it an important target in understanding the tumour biology and to elucidate how changes in tryptophan metabolism contribute to cancer progression.

Given the biological relevance of this metabolic axis, a fast, reliable and matrix-compatible analytical method is essential to support ongoing studies on tumour models. Therefore a fast LC-QqQ-MS method for the quantitative analysis of important metabolites of the coresponding metabolic pathway has been developed.

Method development focused on establishing a robust method for the simultaneous quantification of 21 tryptophan metabolites. Building on an extensive column screening, a short 30 mm pentafluorophenyl (PFP) column was identified as the most suitable stationary phase that allows a sufficent separation of the chemicaly diverse metabolites. In combination with an optimized solvent gradient, this setup enables a separation of all compounds in a total run time of 1.8 minutes (Fig. 9). Despite the short analysis time, the chromatographic performance ensures that each analyte peak contains at least ten data points across its profile, supporting reliable quantification and robust peak integration. To ensure accurate detection under realistic conditions, a representative tumour matrix was used during method development. Currently the method is being validated prior to the application in various cancer models.

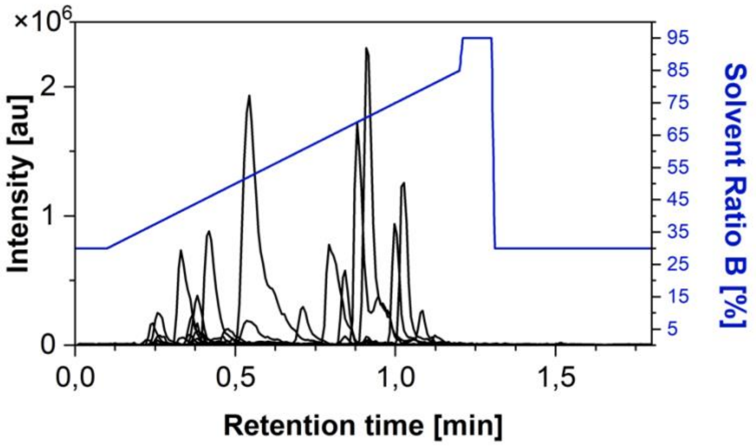


Fig. 9: Extracted ion chromatograms of tryptophan metabolites detected using the developed LC-QqQ method. The blue line represents the gradient of the solvent B used. The method enables rapid separation within a total run time of 1.8 minutes, thus allowing reliable quantification of several metabolites relevant to tumour metabolism.

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 Characterization of Adaptive Resistance Mechanismsin Melanoma Under BRAF/MEK Inhibition

Friederike Jahr, Luiza Martins Nascentes Melo, Constantin P. Krempe, Jonas Rösler,
Sven W. Meckelmann, Alpaslan Tasdogan

A key question in melanoma research is why tumors lose their initial sensitivity to target therapies such as BRAF and MEK inhibitors. These drugs, for example Dabrafenib and Trametinib, are designed to suppress a critical oncogenic signaling pathway that is frequently overactive in these cancers. Although patients often show strong initial responses, many melanoma cells eventually adapt and acquire resistance. Rather than relying solely on stable genetic mutations, these cells can reprogram their signaling pathways and alter their metabolism to survive.

For a riliable metabolomic characterisation, an HILIC–Orbitrap MS/MS method was established using over 400 authentic metabolits as standards. To study the metobolomic adaptation to the therapy, treated A375 human melanoma were comprehensively characterize. As shown in Figure 10, the data revealed significant metabolomic differences between melanoma cells treated with dabrafenib/trametinib-resistant cells (DT), cells exposed to triple therapy with dabrafenib/trametinib/moborcetinib (DTM) and the corresponding controls (DMSO). This allows to study the adaptive changes and determine whether disrupting the metabolic signaling axis is driving this process. This will help to overcome resistance and restore therapeutic efficacy.

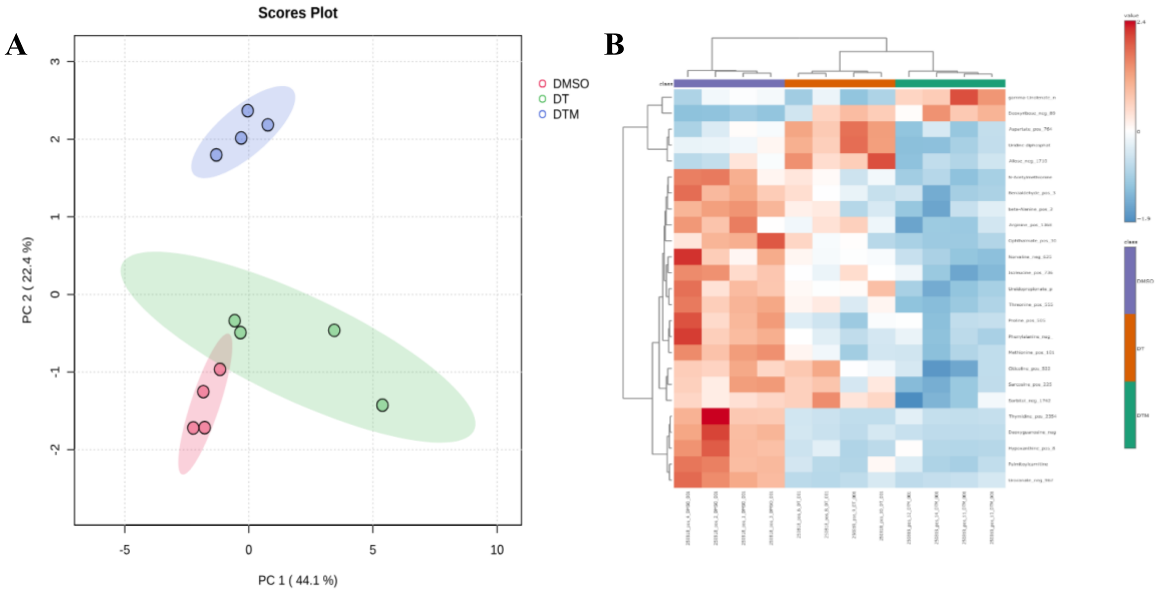


Fig. 10: A) Principle Component Analysis for the parental control cells (DMSO), dabrafenib/trametinib-resistant cells (DT) and triple terapy-treated cells (DTM) B: Heatmap of the top 25 features based on t-test showing the clustering od DMSO, DT and DTM.

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

Comparative Metabolomics of Acral and Cutaneous Melanoma

Friederike Jahr, Flavia Carvalho Aguiar, Constantin P. Krempe, Jonas Rösler,
Sven W. Meckelmann, Alpaslan Tasdogan

Acral melanoma differs clinically from cutaneous melanoma, as it arises on the palms, soles, and nail beds and often follows a distinct biological course. To better understand these differences, the metabolic profile of acral melanoma is being characterized using metabolomics analyses of patient-derived samples from primary tumors and lymph node metastases, as well as patient-derived xenograft (PDX) models generated from the same patients. To identify key metabolic distinctions between acral and cutaneous melanoma, metabolomics was also performed on corresponding melanoma cell lines. Together, these datasets provide a more comprehensive view of acral melanoma biology and support the identification of new therapeutic opportunities.

For this purpose, we established a rapid HILIC–Orbitrap MS–based metabolome-screening approach, which we subsequently applied to monitor metabolic changes. This workflow enables high-throughput and reproducible profiling of a broad spectrum of metabolites under standardized analytical conditions. It also allows direct comparison of patient-derived samples and cell lines, facilitating the identification of metabolic traits across model systems.

As shown in Figure 11A, the data reveal significant metabolomic differences between acral melanoma (AM007, 015a and 016) cell lines and the cutaneous melanoma cell line MaMel-61b. Figure 11B shows a Vulcano plot comparing acral melanoma with cutaneous melanoma. This shows that the metabolism of acral melanoma is downregulated.

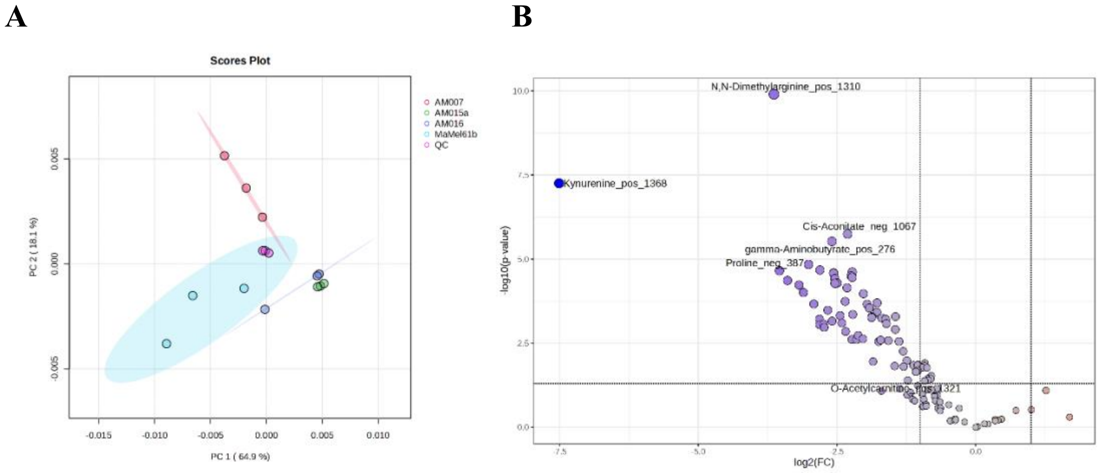


Fig. 11: A) Principle Component Analysis for cell lines of acral melanoma (AM) and cutaneous melanoma (MaMel-61b) B: volcano plot of global acral melanoma and cutaneous melanoma showing a big proportion of the data as significant different (FC > 2; p < 0.05).

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

Receptor-Dependent Metabolic Shifts in Melanoma

Friederike Jahr, Feyza Cansiz, Jonas Rösler, Constantin P. Krempe,
Sven W. Meckelmann, Alpaslan Tasdogan

Melanoma is among the most aggressive forms of cancer and its incidence continues to rise globally. A major challenge in clinical management is achieving effective treatment once the disease has spread to other organs and metastases have formed. Beyond targeting genetic signaling pathways, increasing attention is being directed toward addressing metabolic dysregulation in melanoma. In this context, the influence of a specific cell receptor on melanoma cell metabolism is being investigated to identify receptor-dependent metabolic alterations. These metabolic changes may offer valuable entry points for new therapeutic interventions and understanding receptor function could help pinpoint specific vulnerabilities in tumor cell physiology.

Using HILIC-Orbitrap- MS analysis, the metabolome of human cancer cells was examined as a function of receptor activity. For this purpose, an internal database with over 400 standards was established, enabling semi-targeted analysis. The goal is to render these cancer cells metabolically vulnerable and in combination with established therapeutic strategies, achieve a more targeted and effective treatment approach.

The PCA in figure 12 shows a clear shift from the wilde type group (WT) toward the gen knockout (A375shA and shD) samples, with one outlier in shD. Most knockout samples cluster tightly, except for shScr. Overall, the knockout conditions appear to show reduced metabolic activity, with shScr standing out as an exception.

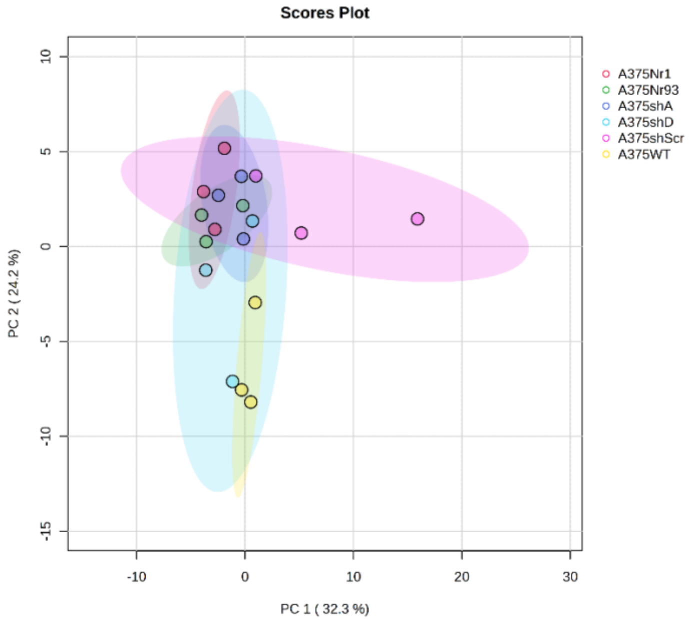


Fig. 12: Principle Component Analysis for wild-type (WT) cells and different knockdown variants, including A375shScr, A375shA, and A375shD.

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

Lipidomic Analysis of CRISPR-edited Organoids in Cancer Research

Friederike Jahr, Jonas Rösler, Constantin P. Krempe, Sven W. Meckelmann, Alpaslan Tasdogan

Lipidomics has become a key tool in cancer research, as lipids are much more than just structural components of biological membranes. Many lipid classes perform essential functions in signalling pathways that control cell growth, differentiation, stress response and programmed cell inactivation. This processes are often dysregulated in tumour cells.

Our group established a suitable workflow for lipidome analysis using an automated sample preparation followed by LC-Orbitrap-MS analysis.[1] Using an non-target lipidomics approach with automated sample preparation revealed various changes in the lipid methabolism of CRISPR-edited organoids with knockouts of the dihydroceramide desaturases DES1. The samples used in the experiment can be divided into four main groups. Des1 KO CT comprises organoids in which the DES1 gene was switched off using CRISPR. These samples received only the control treatment and therefore serve as knockout controls without additional stimulation. Des1 KO PA also describes DES1 knockout organoids, but these were additionally treated with palmitic acid to investigate the influence of increased fatty acid intake on the altered sphingolipid metabolism. Scrambled CT, i.e., control organoids with a non-target gRNA that only received the control treatment and Scrambled CT PA, consists of the same scrambled controls, but treated with palmitic acid. These groups are used to study the baseline condition and serve as control samples.

As shown in figure 13 the grouping of samples reflects genetic differences (DES1 knockout vs. scrambled) and the metabolic effects of DESI1 knockout and palmitic acid intake.

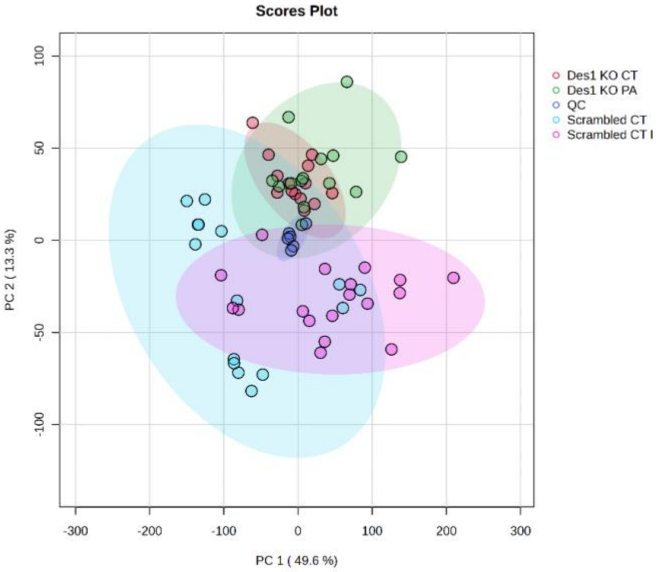


Fig. 13: Principle Component Analysis for the lipidome analysis DES1 KO CT (knockout, control treatment), DES1 KO PA (knockout, palmitic acid-treated), Scrambled CT (control organoids, control treatment), and Scrambled PA (control organoids, palmitic acid-treated).

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

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

Fast and Robust Target Lipidomics of Sphingolipids

Constantin P. Krempe, Friederike Jahr, Ronja Becker, Alpaslan Tasdogan, Sven W. Meckelmann

Sphingolipids are involved in structural membrane integrity and dynamic cellular signaling. Ceramides are known to be bioactive lipids controlling apoptosis, cell cycle arrest, and stress responses, whereas sphingomyelins are membrane components with regulatory roles in lipid rafts and signaling domains. Increased ceramide levels are often pro-apoptotic, and elevated sphingomyelin levels contribute to cancer cell survival, metastasis, and chemoresistance. Further, altered expression of sphingolipid-metabolizing enzymes has been linked to tumor aggressiveness, leading to poor prognosis across various cancer types, including breast, colon, and melanoma. This underlines the necessity for a fast, robust, and reliable analysis for high-throughput routine analytics of ceramides and sphingomyelins in the field of system medicine.

A specific characteristic of these lipids is the sphingosine backbone. If a fatty acid is linked to the sphingosine via an amide bond, the molecule is classified as a ceramide (Cer). Additionally, if a phosphocholine group substitutes the remaining unesterified hydroxyl group of the sphingosine backbone, the molecule converts to a sphingomyelin (SM). The development of the LC-QqQ-MS method included a column screening of 10 different reversed-phase HPLC columns, covering various modified C18 as well as more polar C14 phases, both with different linking groups and column dimensions. The evaluation of the column screening was based on retention times and peak width obtained from the 25 Cer and SM standards spiked into a plasma sample. The final method utilizes a C14-linked amide phase, which allows the separation of the ceramides and sphingomyelins according to the length of the fatty acids bonded to the sphingosine backbone in 10 minutes, as shown in Figure 14. After gradient optimization, MS parameters were optimized to gain sensitivity in a dynamic MRM mode. Therefore, ceramide- and sphingomyelin-specific transitions were established and optimized. The method allows the separation and accurate annotation of various species containing different fatty acids without interference. The method is currently evaluated according to the EMA Guidelines *ICH M10 on bioanalytical method validation*. LODs for ceramides and sphingomyelins are between 1 nM and 3 nM, with a linear range of up to 30 µM. Different extraction protocols are currently being investigated using NIST 1950 reference plasma as well as liver tissue samples.

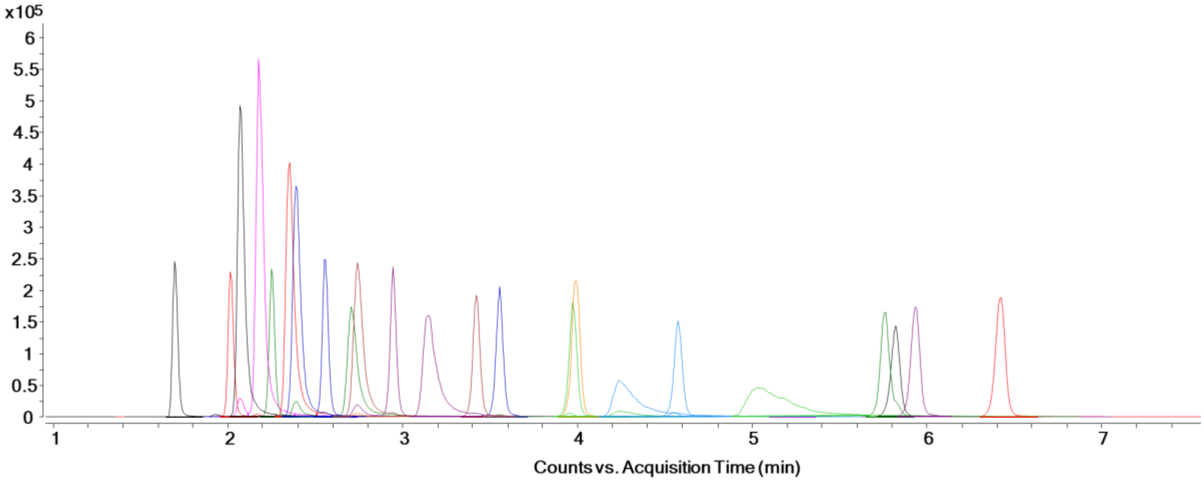


Fig. 14: Chromatogram of a 30 µM calibration solution including all 25 reference standards and 1 µM of each internal standard.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen
Funded by: A. T. was founded 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

Stable Isotope Tracing as an Analytical Tool for Investigating the Role of SFXN1 in Polyamine Synthesis

Constantin P. Krempe, Friederike Jahr, Sven W. Meckelmann, Alpaslan Tasdogan

Stable isotope tracing by LC-HRMS is a powerful analytical technique for monitoring specific biochemical pathways, enabling deeper insights into relative metabolic fluxes and supporting the generation of new hypotheses in clinical and translational research. This tool was used to study Sideroflexin-1 (SFXN1), which is a mitochondrial serine transporter essential for one-carbon metabolism, and preliminary LC-QqQ-MS data from SFXN1 knock-out models suggested a potential role in polyamine metabolism (Figure 15A).

To elucidate the metabolic function of SFXN1 and its involvement in polyamine biosynthesis, organoids were incubated with medium enriched in $^{13}C_5$-proline. Because the natural abundance of fully $^{13}C_5$-labeled proline is extremely low (0.0115%), feeding organoids with $^{13}C_5$-proline allows precise monitoring and comparison of relative fluxes between biological groups.

During the conversion of proline to ornithine, which subsequently enters the urea cycle, only enzymatic and non-enzymatic redox reactions take place, and thus, all five ^{13}C-carbon atoms remain labeled when $^{13}C_5$-proline enters this pathway. If ornithine is subsequently converted into putrescine, one carbon atom is removed during decarboxylation, resulting in $^{13}C_4$-putrescine. These four labeled carbons are retained during downstream conversion to spermidine and spermine. If ornithine does not enter the polyamine pathway, it is converted to citrulline and continues through the urea cycle.

Figure 15B shows the LC-HRMS results, demonstrating that SFXN1-KO organoids accumulate higher putrescine levels than wild-type. Moreover, KO organoids exhibit an accelerated conversion of proline to putrescine, indicating that loss of SFXN1 rewires polyamine metabolism and enhances flux through the putrescine biosynthetic pathway.

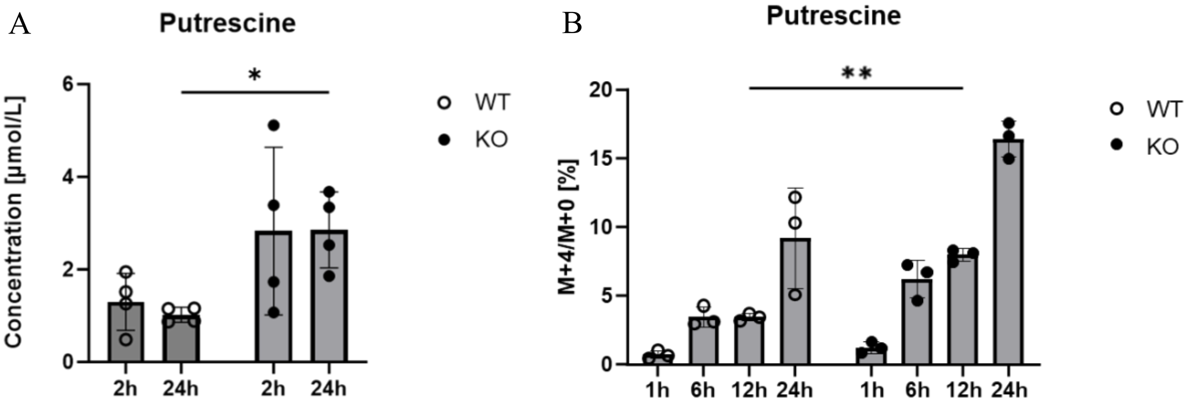


Fig. 15: A: Putrescine concentrations of wild type (WT) and SFXN1-KO (KO) liver tissue organoid samples at various times after refeeding. B: Tracer-Tracee-Ratios of Putrescine after various incubation times with $^{13}C_5$-Proline.

Collaborative Project – Project Partner: Prof. Alpaslan Tasdogan, Clinic for Dermatology at University Hospital Essen; Ömer Yilmaz, Department of Biology, The David H. Koch Institute for Integrative Cancer Research at MIT, MIT, Cambridge, MA, USA

Funded by: A. T. was founded 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

Chemical Characterisation of *Cannabis sativa* by GC-iLTP-SLIM-MS

Cedric Thom, Sebastian Löbbecke, Marvin Häßler, Sven W. Meckelmann

Plants of the genus *Cannabis* have been used for centuries for fiber, oils, and for their psychoactive effects. Cultivated and growing outdoors on every continent except Antarctica in a wide range of environments, it is one of the most widespread domesticated and cultivated plants. Its psychoactive effects led to the prohibition of Cannabis over the past centuries; however, cultivation continued. This uncontrolled and illegal market led to the existence of a vast amount of varieties with arbitrary names and unknown parental pedigrees, as well as the existence of completely different varieties with identical names. Over the last decades, Cannabis was legalised in several jurisdictions for recreational and medicinal use. Therefore, legal industrial production of cannabis has significantly increased. As Cannabis gains importance through legalisation there is a push to determine the chemical profile of each variety. However, the analytical characterisation is challenged by the complex matrix containing hundreds of terpenoids, cannabinoids, and secondary plant metabolites, many of which share isomeric structures and lead to peak overlap in conventional chromatography. Further, standard gas chromatography mass spectrometry (GC-MS) typically relies on electron ionization (EI), resulting in high in-source fragmentation and limited selectivity for isomers. To overcome these limitations and increase peak capacity, a multidimensional approach was established. A GC system was coupled to a Structures for Lossless Ion Manipulation (SLIM) based high resolution ion mobility-mass spectrometer (HRIM-MS) utilising an inverse low temperature plasma (iLTP) ion source. This setup introduces gas-phase mobility as an additional separation dimension. SLIM offers a 13-meter separation path, achieving unmatched high resolution. The iLTP source offers soft ionisation, preserving diagnostic ions. With this approach, molecular information is obtained with significantly higher quality compared to the standard EI approach. To mitigate undersampling of narrow GC peaks, nitrogen was used as carrier gas. For method evaluation, seven cultivars with varying commercial names were extracted (methanol, -20 °C, 24 h) and derivatized with BSTFA. The hyphenation of GC-SLIM-MS (Fig. 16) as a platform for metabolomic studys was successfully established further boosting the separation power of 1D-GC-API-MS. The method allows for a high-resolution metabolome characterization, addressing the need to differentiate cultivars with identical names but diverging metabolomic profiles.

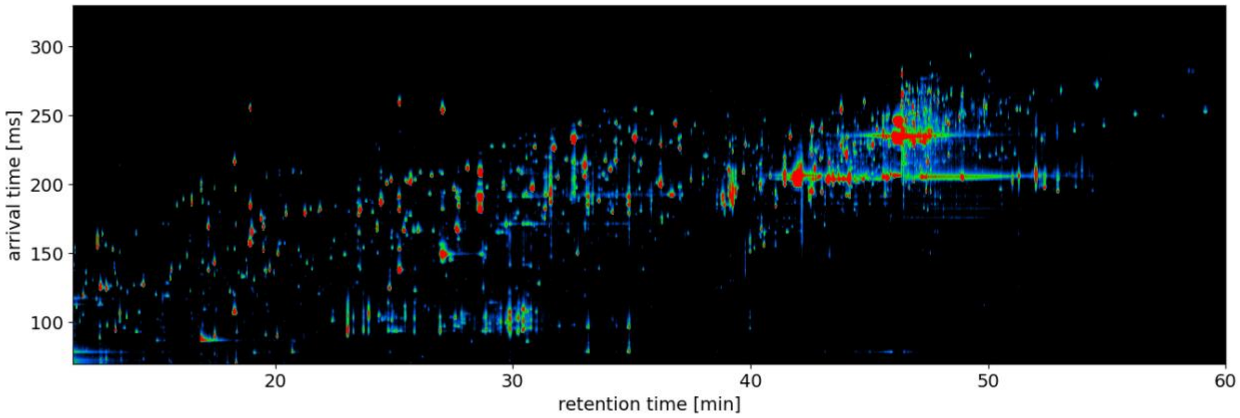


Fig. 16: Heatmap of GC-iLTP-SLIM-MS separation of a pooled cannabis extract with costume separation travelling wave profile. Retention time window of 11.5 min to 60 min and arrival time window from 70 ms to 330 ms are displayed.

Collaborative Project – Project Partner: Mobilion

Separation of German Herbal Liqueur by LC-ESI-SLIM-MS

Cedric Thom, Sven W. Meckelmann

Herbal bitter liqueurs have a long historical and social tradition in Europe as popular digestive beverages. They are composed of complex blends of herbs, roots, barks, and spices, with recipes that are often closely guarded. The botanicals are extracted by maceration in ethanol and are sometimes aged in wooden casks to further refine their flavor. This process results in a beverage with a highly diverse chemical profile of naturally occurring compounds, including terpenes, bitter principles, and polyphenols. This intrinsic complexity poses a particular challenge for analytical chemists, as many constituents co-elute or share similar structural features. Modern liquid chromatography (LC) workflows provide high reproducibility and robust retention mechanisms that enable systematic separation of chemically diverse analytes. However, due to the chemical diversity of these liqueurs, one-dimensional LC often lacks the separation power needed to resolve isomeric or closely related compounds. Therefore, a novel Structures for Lossless Ion Manipulation (SLIM) based high-resolution ion mobility–mass spectrometer (HRIM-MS) was coupled to an LC system to combine chromatographic separation with the enhanced resolving capabilities of SLIM-MS. The platform showed a high resolution and ion transmission even in the lower end of the mass range, thus proving itself as a powerful analytical platform for metabolomics studies of highly complex samples (Fig. 17).

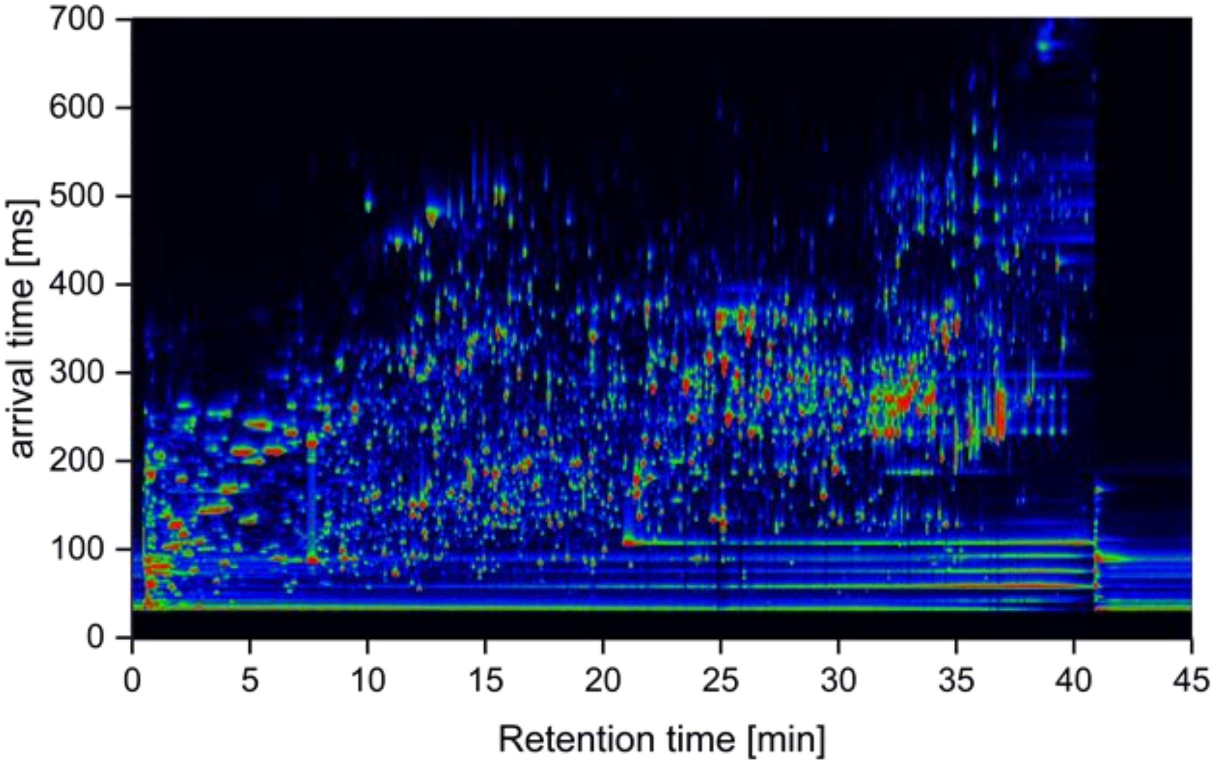


Fig. 17: Heatmap of LC-ESI-SLIM-MS measurement of a popular german herbal liqueur. Chromatographic separation was performed on a RP C18 phase and ion mobility separation with helium as a drift gas with a constant travelling wave.

Collaborative Project – Project Partner: Mobilion

Funded by: Agilent Technologies

Characterization of the Human Lipidome

Cedric Thom, Sven W. Meckelmann

The human plasma contains a highly complex lipidome, reflecting essential physiological processes such as energy metabolism, membrane homeostasis, and cell signaling. Alterations in the lipidome are strongly associated with diverse pathological processes, including cardiovascular disease, metabolic disorders, inflammation, and neurodegeneration. Given this biological relevance, comprehensive analytical characterization of the plasma lipidome is crucial but remains challenging due to its intrinsic structural diversity. It is composed of hundreds to thousands of lipid species spanning diverse structural classes, including glycerophospholipids, sphingolipids, glycerolipids, and sterols. These molecules often differ only subtly in acyl chain length, degree of unsaturation, or double-bond position, making their structural characterization analytically challenging. This intrinsic complexity poses a particular challenge for analytical chemists, as many lipid species coelute or share nearly identical mass-to-charge ratios. Reversed-phase (RP) liquid chromatography (LC) principally separates lipids based on hydrophobicity and thus resolves differences in acyl-chain length and saturation, but it offers limited discrimination between lipid classes. Conversely, hydrophilic interaction chromatography (HILIC) separates lipids according to headgroup polarity, yet struggles to resolve species with similar functional groups but different fatty acid compositions. As a result, both approaches leave many isomeric or structurally related lipids insufficiently separated.Therefore, a one-dimensional LC setup was coupled to a high-resolution ion mobility–mass spectrometry system based on Structures for Lossless Ion Manipulation (SLIM-MS), which provides an additional separation dimension based on the size, shape, and charge of the analytes, enabling the separation of isomeric compounds (Fig. 18). The additional separation followed by fragmentation enables more confident annotation of the human plasma lipidome. Non-targeted data analysis via MS-Dial annotated 457 unique lipids across both polarities.

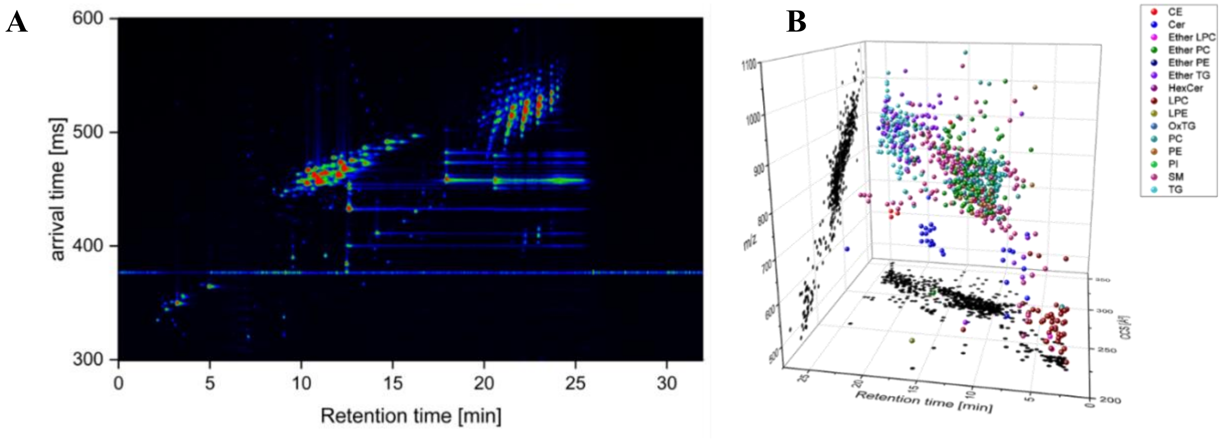


Fig. 18: A: Heatmap of human plasma extract separated by LC-ESI-SLIM-MS. Chromatogrpahic separation was performed on a RP C18 phase an ion mobility separation with Nitrogen as a drift gas with a variable travelling wave profile. B: 3D Scatterplot of m/z, retention time, and CCS values of annotated lipids .

Collaborative Project – Project Partner: Mobilion

Funded by: Agilent Technologies

PSeaC – Algorithm 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 for non-target experiments. After separation of compounds 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. Therefore, the signals need to be combined to reduce the data set without loosing information.

MS-DIAL processes the data for peak detection and alignment as one dimensional measurements. The exported feature list is then processed with PSeaC, a Python script for demodulation of LC × LC data, which is executed in a terminal.

After several clustering steps with respect to mass-to-charge ratio, collision cross-sections and retention time, the intensities of clustered signals are summed and a Gaussian fit algorithm provides information about the quality of the peak shape and numbers of modulations (Fig. 19). With theses information the user can remove contaminations and features with poor quality. This simplifies also identification of compounds and further statistical analysis.

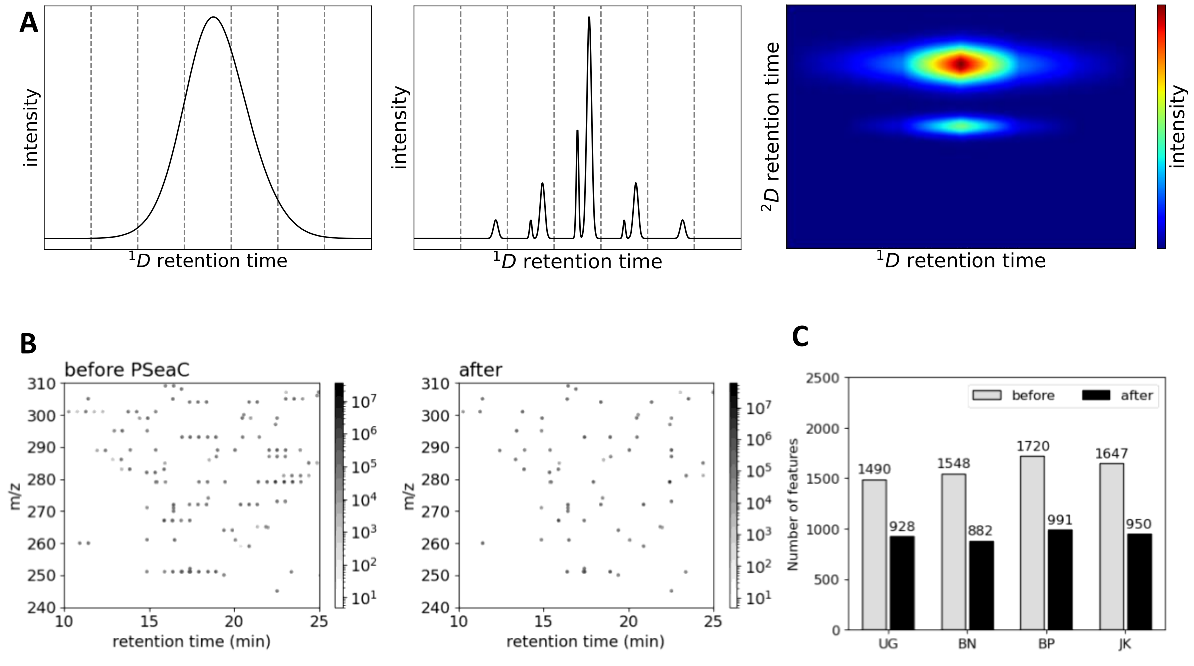


Fig. 19: A: Artificial example of twodimensional comprehensive liquid chromatography, with two compounds, which are separated on the second column. The difference in retention time corresponds to the modulation time. B: Process of a real sample with PSeaC, which shows the combining of the horizontal lines to one spot on the right side. C: Measurments of different herbal liquors with LC×LC-IMS-qToF and the reduction of features after using PSeaC.

Analysis of Sustainable Aviation Fuels by Cryo-Modulated GC × GC-CI-(q)MS

Sebastian Löbbecke, Jaqueline Leddin, Karolin Orth, Florian Uteschil

Sustainable Aviation Fuels (SAF) are a crutail short-term strategy for decarbonisation in the aviation sector. Different pathways exist to produce SAFs from biogenic and non-biogenic feedstocks. One emerging technology is the use of methanol as a multifunctional precorsor chemical directly synthesised from carbon dioxide and hydrogen. Methanol can be turned into light olefins using Methanol-to-Olefins (MtO) process technology. These light olefins then undergo further processes including oligomerisation and hydration. In the end a finished aviation fuel is obtained. This product consist of a large number of hydrocarbons which may be saturated, unsaturated, cyclic, aromatic or combination of these. Due to the large number of compounds found in fuels samples these are considered very complex and comprehensive analysis is challenging. For this purpose powerful analytical methods have to be applied to investigate the composition of SAF samples. One of those methods is two-dimensional gas chromatography offering superior separation power combined with mass spectrometry for compound group identification. The selection of columns and chromatographic conditions shows significant effects on the separation of analytes with GC×GC. The finalised method includes a combination of a 60 m polar DB-17ms column in the first dimension with a 3.5 m non-polar SLB-5ms column in the second dimension with Hydrogen used as carrier gas. Further, electron ionisation (EI) and chemical ionisation (CI) with methane and isobutane were compared. CI using isobutane showed significantly reduced fragmentation compared to EI and offers better sensitivity compared to methane. The analytical methods allows for detection of analytes in the low mg L^{-1} range with high precision (median RSD < 10%). However, this method also requires extensive data evaluation.

For this purpose a python-based script is applied. This script picks modulated peaks and clusters them into one feature (Fig. 20). The substance class is identified based on characteristic fragment ions in the mass spectra obtained for each feature. Aditionally, the ^{2}D retention time is used to affirm correct identification by the mass spectra. Further, the carbon number is estimated by the linear retention index of the ^{1}D separation. With this skript data evaluation is significantly simplified and automated in great extend reducing the analytical workload. These tools allow for extensive characterisation of complex SAF samples in a close to routine analysis approach.

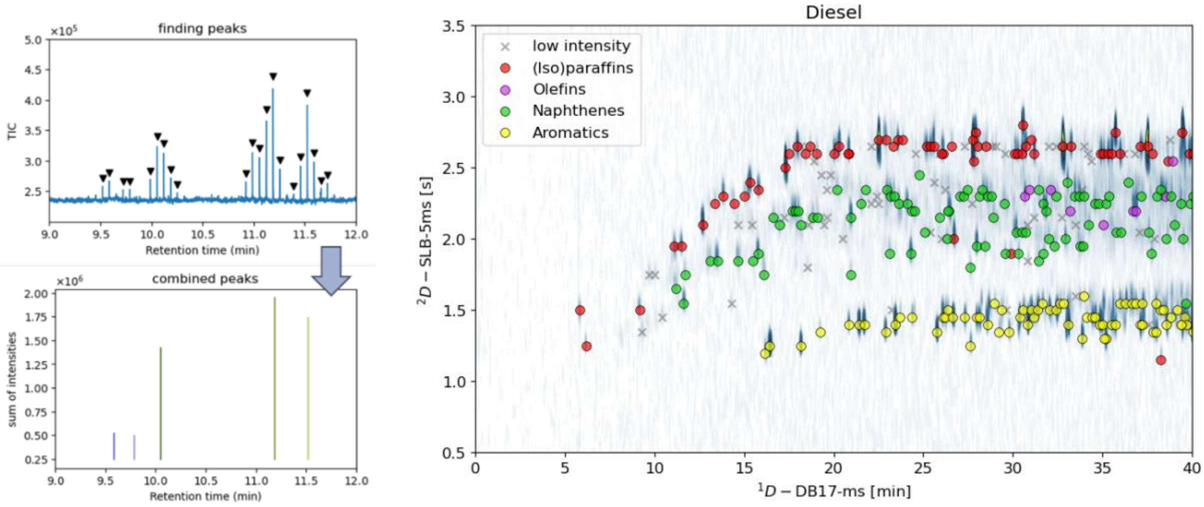


Fig. 20: A: Finding peaks and combining them with respect to modulation time of GCxGC. B: Section of a GC×GC-Chromatogram of Diesel with detected peaks and assigned substance class identified by fragment ions.

Collaborative Project – Project Partner: This Project was realised within the Carbon2Chem project frame together with ThyssenKrupp Uhde GmbH, Dortmund, Germany

Funded by: Federal Ministry of Research, Technology and Space (BMFTR)

Evaluation of Flow-Modulated GC × GC coupled to API-MS for Fuel Analysis

Sebastian Löbbecke, Jaqueline Leddin, Florian Uteschil

The chemical composition of fuels is closely linked to their physicochemical properties. Unsaturated compounds like Olefins (alkenes) negatively impact long-term storage stability and burning properties of the fuel. Especially when using the Methanol-to-Olefins (MtO) process to produce fuels, Olefins play a crucial role as they are required in certain steps of the process, but should not be present in the final product. However, Olefins are difficult to separate from Naphthenes (cycloalkanes) even with powerful GC × GC-separation. Further, they produce similar ions as Napthtenes with most ionisation sources. Therefore, atmospheric pressure ionisation sources are evaluated and coupled with flow-modulated GC × GC-qTOF. Both plasma based ionisation in form of Tube Plasma Ionsiation (TPI) and inversed Low Temperature Plasma (iLTP) as well as Atmospheric Pressure Photoionisation (APPI) are applied for this purpose. Previsous studies from our group have already proven plasma-based ionisation as a powerful tool in GC-MS and iLTP stands out in this study as well. It offers the unique ability to specifically ionise Olefins by $[M+NO]^+$ species while Naphthenes are ionised usually by $[M+H-H_2]^+$ species. Therefore, Olefins and Naphthenes, both with the molecular formula of C_nH_{2n}, can easily be differentiated by their mass spectra, even if coelution occurs (Fig. 21). This will allow for sensitive and selective screening of Olefins in fuel samples while also offering the possibility to detect other hydrocarbon groups present. A major challenge of the GC × GC-iLTP-qTOF methodology is however the difficult quantification by mass spectrometry as analytes have no universal response factor. For accurate quantification an extensive mix of native and isotopically labelled internal standards would be necessary. Alternatively, quantification can be done using flame ionisation detection (FID) as it's response factors are correlated to number of carbon atoms of the analytes. Therefore, a combination of FID for quantitative analysis and MS for qualitative analysis is required. This is established by splitting the flow after the second dimension between the MS and the FID. With this setup the advantages of both detection methods can be combined in a single run.

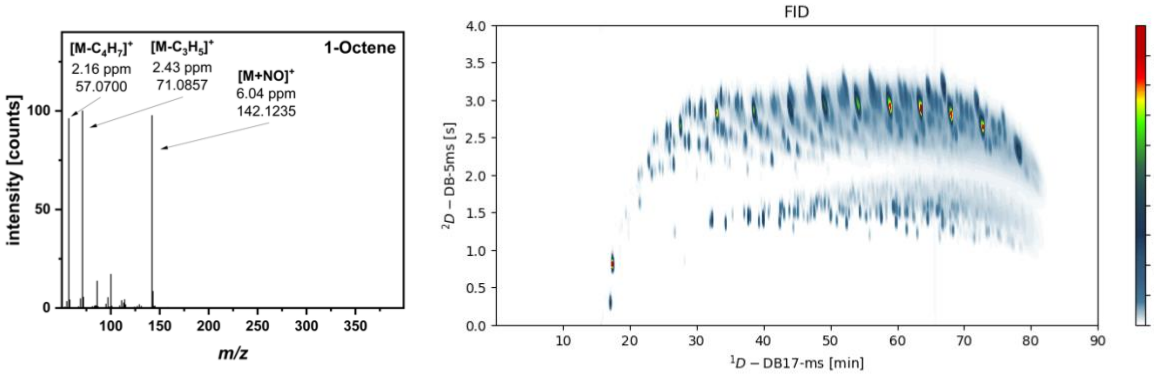


Fig. 21: A: Mass spectrum of 1-Octene with plasma-based ionisation (here: TPI). The ionisation as $[M+NO]^+$ species allows for easy differentiation from Naphthenes. B: Separation of a Diesel Oil sample with the GC×GC-qTOF/FID setup.

Collaborative Project – Project Partner: This Project was realised within the Carbon2Chem project frame together with ThyssenKrupp Uhde GmbH, Dortmund, Germany

Funded by: Federal Ministry of Research, Technology and Space (BMFTR) and Agilent Technologies

Analysis of Fuels by DI-iLTP-Orbitrap HRMS

Sebastian Löbbecke, Karolin Orth, Jaqueline Leddin, Florian Uteschil

Due to the complex composition of Sustainable Aviation Fuels (SAF) powerful analytical methods are required. In addition to GC-based methods direct-infusion high-resultion mass spectroscopy (DI-HRMS) are widely used in fossile fuel analysis to characterise heteroatomic species. However, synthetic fuels like SAFs usually only contain non-heteroatomic species without easy to ionise fuctional groups, making soft ionisation with low fragmentation an analytical challenge. For this purpose inverse low temperature plasma (iLTP) ionisation and atmospheric pressure ionisation (APPI) were coupled to an Orbitrap HRMS with direct sample infusion by a syringe pump. This methodology has the advantage of quick sample measurements (1 min compared to 90 min with GC × GC). However, the information depth obtained form a single samples is reduced as compounds are not separated. After a quick optimisation of plasma and ion source parameters the ionisation behaviour of hydrocarbons was evaluated. A variety of ionisation products were detected, including oxygenated adducts such as the $[M+O-H]^+$, $[M+O-3H]^+$, $[M+2O-3H]^+$ species, and fragment ions, indicating a high reactivity of ionised hydrocarbon mixtures. Interestingly, ions of high diagnostic value were usually oxygenated species. There are usually not oberved for GC-iLTP-MS experiments, possibly due to a concentration dependency of the ionisation reaction. To process the complex spectra obtained, Python-based scripts for automated data analysis were developed using two different formula-assignment strategies: (i) an all-possible-formula generator that calculates all chemically plausible molecular formulas for each adduct within a defined mass tolerance, and (ii) a Kendrick mass defect (KMD)-based method that classifies peaks into theoretical homologous series by adduct composition and derives structural parameters such as double bond equivalent (DBE) and elemental composition. Dedicated visualisation tools were used to assign peaks, plot KMD versus Kendrick nominal mass (KNM), and DBE versus carbon number, enabling rapid identification of homologous series within defined adduct and isotope classes (Fig. 22). For the most abundant peaks, the occurrence of the ^{13}C isotope peak was used to verify the identification. Both formula assignment strategies produced comparable molecular formulas for each peak and adduct. The KMD-based method, while more prone to misassignments between homologous series with similar KMD (e.g. ^{12}CH- and ^{13}C-series), enabled adequate assignment of the most abundant masses with substantially lower computational demand than the all-possible-formula generator. Overall, both approaches were found suitable for mapping the hydrocarbon space in SAF samples.

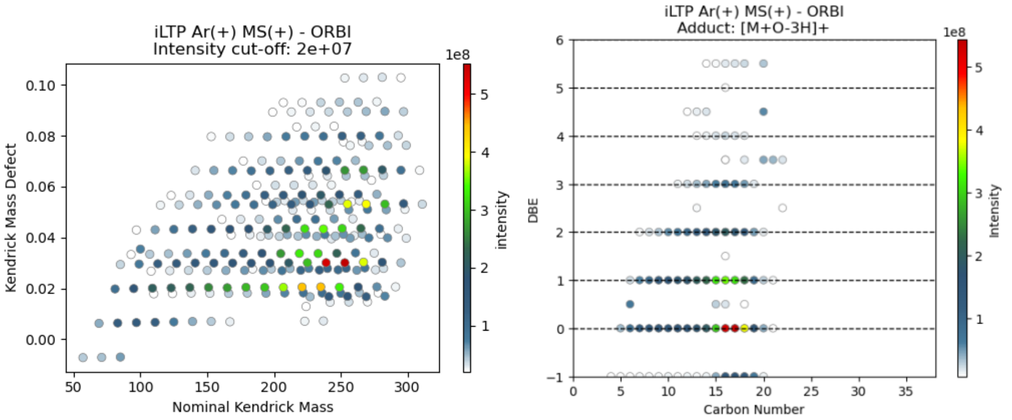


Fig. 22: Kendrick plots showing the characterisation of a fuel sample by (A) plotting Kendrick mass defect against Kendrick nominal mass and (B) double bond equivalent against carbon number.

Collaborative Project – Project Partner: This Project was realised within the Carbon2Chem project frame together with ThyssenKrupp Uhde GmbH, Dortmund, Germany

Funded by: Federal Ministry of Research, Technology and Space (BMFTR)

Derivatization Strategies for the Determination of PFAS by GC-APLI-(Iontrap)MS

Ling Tang

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organofluorine compounds. Since their discovery in the late 1930s, they have been widely used as surfactants, surface protectants, floor polishes, and fire-fighting foams due to their hydrophobic and oleophobic properties. However, the carbon-fluorine bond (~485 kJ/mol) is one of the strongest single bonds in nature. This inherent stability imparts exceptional resistance to thermal, chemical, and biological degradation, preventing effective decomposition and leading to their classification as “forever chemicals”. Once these persistent chemicals are released, environmental processes such as volatilization, atmospheric deposition, leaching, and runoff govern their redistribution across air, soil, water, and sediments. These pathways result in both short- and long-term exposure of aquatic and terrestrial ecosystems—as well as humans—to PFAS, which can further enter the food chain through bioaccumulation. Human exposure occurs mainly via dietary intake, inhalation of indoor air, consumption of contaminated drinking water and the dermal adsorption, which poses a significant risk to human health and may cause changes in development, lipid metabolism and endocrine system, and has carcinogenicity, immunotoxicity, hepatotoxicity and reproductive toxicity.

The conventional approach for PFAS analysis is chromatography hyphenated with mass spectrometry. The choice of separation technique is determined by the compounds' physicochemical properties: liquid chromatography (LC-MS) is applied to the majority of PFAS, whereas gas chromatography (GC-MS) is reserved for neutral and volatile subtypes. The number of peer-reviewed publications and established analytical methods for GC-amenable PFAS is substantially smaller than for those compatible with liquid chromatography, which have been extensively studied. Targeted methods using GC-MS and GC-MS/MS, typically with EI or positive chemical ionization (PCI), have been widely applied. Studies have also compared softer ionization techniques—such as negative chemical ionization (NCI) and APPI—with EI and PCI for analyzing FTOHs, FASAs, and FASEs.

In the present work, a soft ionization technique, atmospheric pressure laser ionization (APLI), was coupled with GC-(Iontrap)MS to quantify perfluorinated carboxylic acids (PFCAs) and fluorotelomer alcohols (FTOHs) using derivatization strategies (Fig. 23). These methods were applied to identify PFCAs and FTOHs in waterproof spray and firefighting foam, respectively. The instrument detection limits reached 2.5 nM and 0.1 nM for PFCAs and FTOHs, respectively.

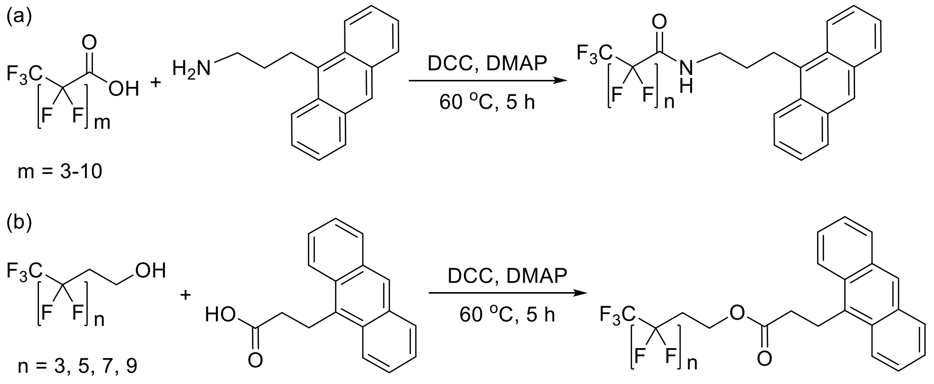


Fig. 23: Schematic of the derivatization reaction.

Funded by: China Scholarship Council

Development of a Dual Ionization Source for Enhanced Metabolomics Analysis

Constantin P. Krempe, Lukas Ickerott, Friederike Jahr, Florian Uteschil

The simultaneous detection of a broad range of metabolites, from highly polar to non-polar species, remains a major analytical challenge regarding comprehensive metabolomic studies. Conventional LC-ESI-MS workflows often struggle with the sensitivity for low-concentration analytes, non-surface-active compounds, or molecular structures that cannot be ionized in the liquid phase effectively. To address these limitations, a dual ionization concept combining nano-electrospray ionization (nESI) and inverse low-temperature plasma (iLTP) was investigated, and its potential was evaluated. The miniaturization achieved through nano-liquid chromatography (nano-LC) significantly reduces solvent consumption while improving sensitivity due to minimized radial diffusion and highly concentrated eluting bands. Coupled to a nano-ESI emitter, ionization efficiency increases further as smaller droplets and shorter distances to the MS inlet improve analyte generation and transmission. However, nESI remains limited to polar and moderately polar compounds. The incorporation of an iLTP source provides an orthogonal ionization mechanism capable of ionizing non-polar and medium-polar metabolites through plasma-induced reactions such as Penning ionization and proton transfer.

In a small setup utilizing a syringe pump, the ionization of 17-α-Methyltestosterone could be increased upon plasma activation within 1 second, while the ionization of 4-Chlorotestosterone decreased. Further experiments, including a nano-LC, revealed strong interactions between the electromagnetic fields generated by the nESI and the Plasma source, leading to poor ion transmission. This behavior results from complex electric field interactions between the nESI Taylor cone and the plasma jet, occasionally altering spray geometry and ion transmission, underscoring the need for electrical shielding to avoid perturbations (Fig. 24). Overall, the combined nano-LC–nESI–iLTP setup enables complementary ionization mechanisms, expanding analytical coverage across metabolite classes. While plasma-induced signal enhancement for unpolar analytes was clearly demonstrated, mitigation of spray–plasma interference remains essential for reliable routine operation. Future work will focus on redesigning the emitter–plasma geometry, implementing shielding concepts, and evaluating the dual source for real biological metabolomics samples.

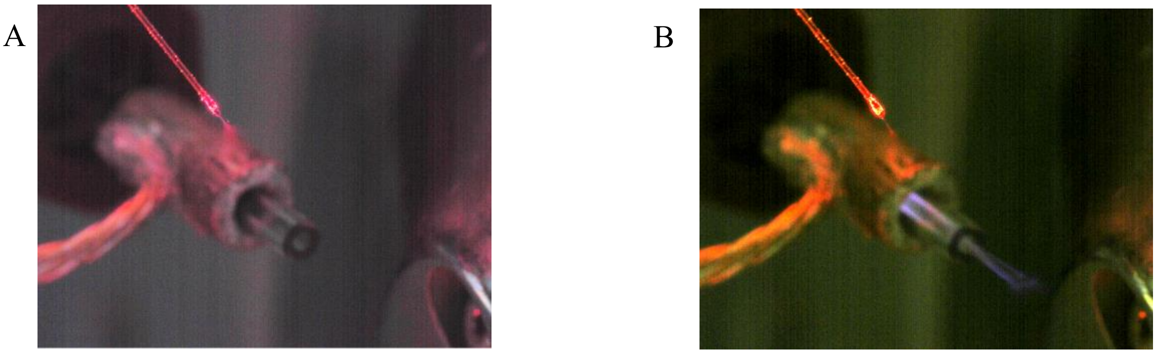


Fig. 24: DinoCam pictures of an nESI coupled to an iLTP, both produced in-house. A: Stable Taylor cone at optimized parameters without additional plasma post-ionization. B: Spray instabilities induced by the electromagnetic fluctuations produced by the nESI and iLTP source.

Dual Ion Source HRMS for Improved Chemical Characterization of European Medicinal Plants Using 2D-LC

Marvin Häßler, Katharina Wetzel, Cedric Thom, Sebastian Löbbecke, Florian Uteschil

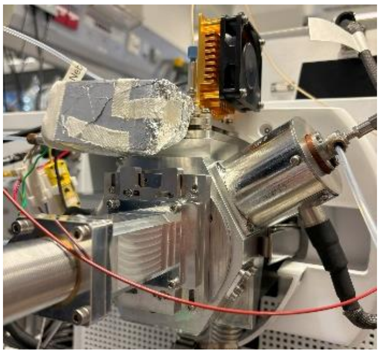


Fig. 25: Dual Ion source. In this configuration, the dual ion source is mounted onto a Agilent Tripple quad mass spectrometer. The two nebulizers get used separately by switching a valve during the run.

In liquid chromatography-mass spectrometry (LC-MS), high-throughput analysis is becoming increasingly important to minimize downtime and ensure efficient, cost-effective operations in the analytical laboratory. In response to this need, a dual ion source (figure 25) has been designed that provides two ionization modes under atmospheric conditions: electrospray ionization (ESI) and dielectric-barrier discharge (DBD)-based plasma. This dual ion source enables rapid switching between ionization modes during a chromatographic run. Therefore, substances with different ionization behaviors can be detected within one measurement if their retention times differ. We evaluated the performance of the dual ion source using single- and multidimensional liquid chromatography coupled with a quadrupole-time-of-flight (Q-TOF) mass spectrometer and a Structures for Lossless Ion Manipulations (SLIM) ion mobility (IM) mass spectrometer (figure 26) to analyze mixed standards and real samples. Notably, the dual ion source maintained strong ion signals and stable performance when switching ionization modes during a run. The instrumental limits of detection reached down to the sub-1 nM range for both ESI and DBD. This work describes the design and construction of the dual ion source, which provides both electrospray and plasma-based ionization that can be switched during a chromatographic run.

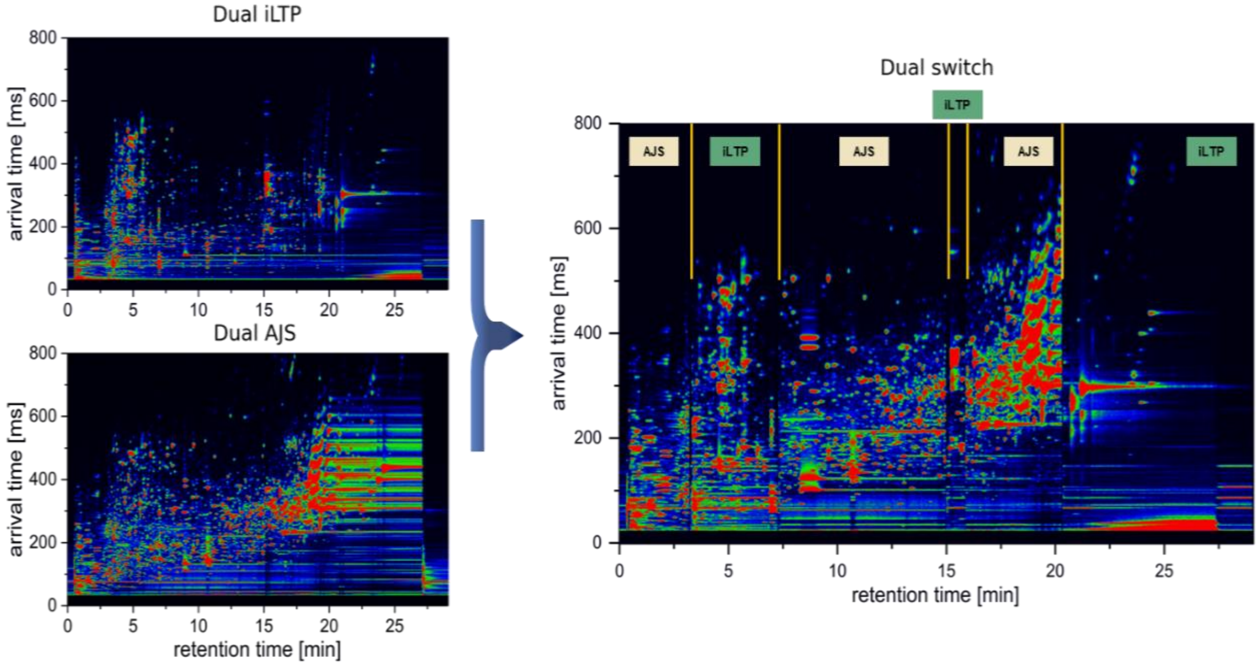


Fig. 26: LC-PFP separation of a herbal liqueur was coupled to a custom-built dual-source setup coupled with a SLIM IMS-MS system, enabling targeted ion source switching at 3.3, 7.3, 14.8,15.6 and 20 minutes to selectively apply TPI and AJS for specific regions in the sample

---

Collaborative Project – Project Partner: Mobilion

Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143 and Agilent Technologies

Evaluation of the Ionization Efficiency of iLTP in Combination with Orbitrap MS

Marvin Häßler, Sebastian Löbbecke, Florian Uteschil

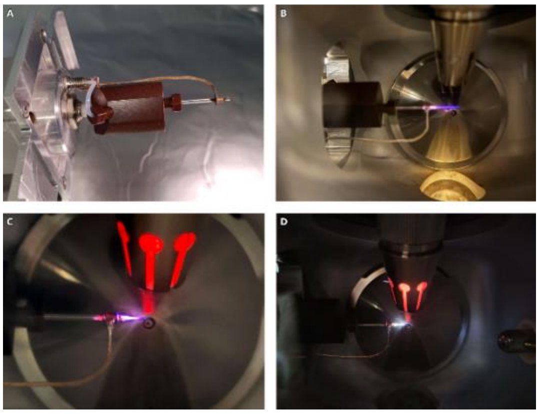


Fig. 27: iLTP source. A: iLTP source removed. B: iLTP source in operation with positive polarization. C: iLTP source in operation with negative polarization as pink plasma. D: iLTP source in operation with negative polarization as white plasma.

This study evaluated the ionization efficiency of an inverse low-temperature plasma (iLTP) source coupled to an LC-Orbitrap-MS system and compared its performance to electrospray ionization (ESI) in both positive and negative polarity modes. For negative iLTP operation, two plasma states, Pink Plasma and White Plasma, were distinguished based on pulse width-dependent changes and optimized separately (Fig. 27). The analytical focus was on derivatized sugars separated by RP-C18 chromatography and native sugars separated by HILIC chromatography. Both ionization techniques exhibited distinct strengths. ESI provided high precision and accuracy and yielded markedly higher signal intensities for derivatized sugars with RP. In contrast, the iLTP source achieved lower limits of detection and quantification for several native sugars. Particularly for hexoses such as glucose and galactose, both negative plasma modes offered substantially lower detection limits than ESI (Fig. 28). However, negatively charged ESI performed better for rhamnose, lactose, xylose, and fucose, indicating no universal trend favoring any of the ion sources. Signal intensities were strongly influenced by plasma and source operating parameters, underscoring the importance of targeted optimization. The results highlight the broader potential of plasma-based ionization sugar analysis with mass spectrometry. Moreover, the demonstrated sensitivity gains motivated to explore iLTP integration with gas-chromatographic workflows and high-resolution Orbitrap detection. Therefore the next step is the GC-plasma-Orbitrap coupling for advanced sugar analysis.

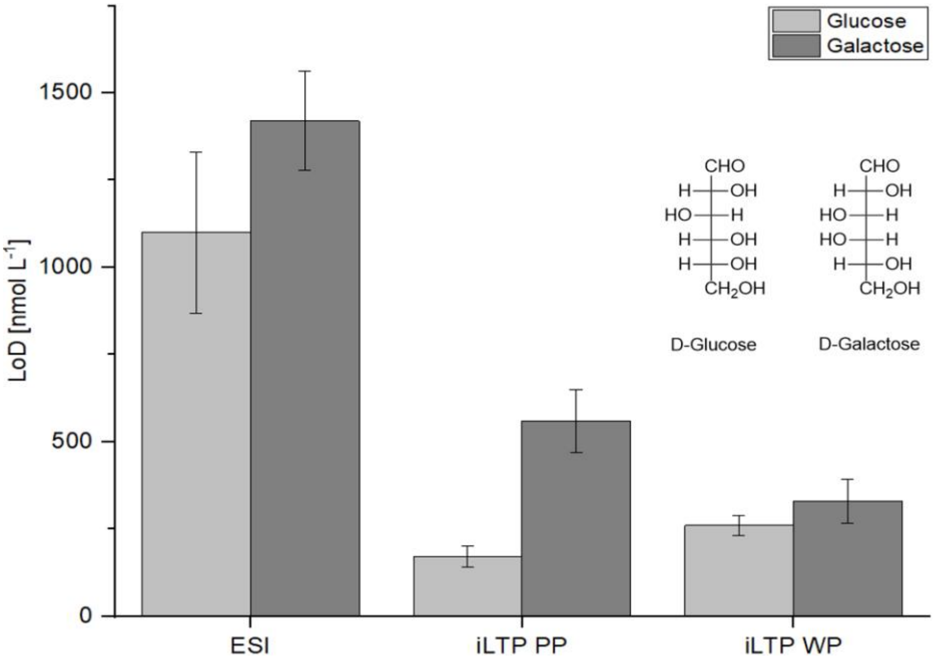


Fig. 28: Comparison of hexoses LoD. Determined limits of detection (LoD) for glucose and galactose, measured using the three ionization methods ESI (neg.), iLTP (neg. PP), and iLTP (neg. WP)

Doctoral Thesis Accomplished 2025

Dr. Pia Wittenhofer

A Novel Target Approach to Characterize the Biosynthesis of Cholesterol in Cancer Cells

The development and progression of diseases such as cancer is key to the development of personalized medicine. As a result, the demand for complex lipidomic analysis of relevant biological samples is growing. Analytical workflows are required that address various aspects, including sample collection, extraction, clean-up, as well as final instrumental analysis and data evaluation. In these workflows, economic and sustainable considerations are becoming increasingly important to facilitate the later transition of methods to applications in systems biology or medicine. The emphasis is already progressively placed on automation, minimizing errors and risks for both operators and the process.

In this work, a complete workflow for sterol analysis was designed. At the beginning, a green, automated sample preparation was developed and compared with two commonly used extraction methods. The global lipidome was considered and different influences, such as concentration and matrix differences are investigated. Extracting the entire lipidome presents a challenge due to the wide range of polarity. The most well-known and commonly used extraction protocol, developed by Bligh-Dyer, utilizes chloroform, methanol, and water. This method achieves high recovery rates for polar lipids, such as glycolipids and phospholipids, and non-polar lipids, like triglycerides and sterols. Due to the difference in density, the organic phase forms beneath the aqueous phase. However, this protocol is only suitable for robotic extraction to a limited extent because of potential matrix carry over. Alternatively, the protocol of Matyash *et al*. (solvents are MTBE /MeOH) is often used, as it has similar recovery rates and is less harmful to humans and the environment in comparison. The combination of ethyl acetate and ethanol also offers a green extraction alternative. The MTBE, ethyl acetate/ethanol and n-hexane protocols from Hara *et al*. (for later sterol analysis) was automated and optimized for possible comparability. For the optimization, the required amount of solvent, the number of extraction steps, insertion depths into the vials and evaporation programs were adjusted. In addition, the extraction protocols were compared with each other for different matrices (matrix and concentration effects). The extraction protocol with ethyl acetate has the same and, in some cases, better recovery rates than the MTBE protocol for the entire lipid spectrum. Only for the non-polar lipids, the hexane protocol shows better recovery rates.

The next step was the instrumental analysis. For this purpose, a two-dimensional heart-cut LC method was developed for the quantification of the cholesterol biosynthesis in cancer cells. The method was designed to overcome the challenges of large concentration differences and the associated strong ion suppression, as well as the structural similarities of the analytes, within a single approach. To reduce the ion suppression due to large concentration differences and to separate efficiently analytes with structural similarities, a suitable column was first determined through column screening, ensuring that all substances with the same m/z value are baseline-separated. This was achieved by using a 30 mm and a 100 mm PFP column. As a result, the separation of cholesterol was enabled, which has a concentration at least 1000 times higher than the precursor molecules in the samples, from lathosterol via a heart-cut after gradient optimization, ensuring a very good separation for accurate quantification. The cholesterol and coeluting T-MAS were cut and focused on a trapping column. After completing the detection of the first dimension separation, the fraction was injected on a second column for further separation. To be able to direct both LC flows into the MS and, therefore, detect all compounds, an additional 6-port valve was integrated for this purpose. This enables the ion suppressions to be kept as small as possible due to the large difference in concentration between the cholesterol and the precursor molecules, thus achieving better sensitivity to investigate all sterols. The method was subsequently validated, and NIST 1950 human plasma reference material was analyzed to assess its suitability. In addition, various other sample materials (e.g., tissue, cells) were analyze demonstrating the broad range of possible biological samples that can be analyzed using the developed method. Finally, the method was applied to analyze cancer cells treated with statins at varying concentrations and over different time periods, aiming to identify changes in the cholesterol biosynthesis pathway.

The issue of the large concentration difference between cholesterol and its precursor molecules places high demands on ionization and detection. Therefore, a comparison of ion sources was conducted to determine the most sensitive method with the highest linearity. For this project, an LC-TPI ion source was used, which is to be compared in terms of performance with the commercially available ESI and APCI sources. In addition to validation, adduct formation, matrix effects in the form of signal stability over a long measurement period and ion suppressions were also examined in more detail. Finally, the most sensitive method was used to examine cells for lipidomic changes after inhibition with statins. Adduct formation was considered first. With the ESI source, $[M+H]^+$, $[M+H-H_2O]^+$ and $[M+NH_4]^+$ adducts were formed, whereas with APCI and TPI mainly only $[M+H-H_2O]^+$ was formed. This already shows that sensitivity was lost when only one transition was used in ESI. Due to the high matrix content, interferences and strong ion suppression occur. To study the ion suppression, a constant flow of internal standard (IS) was introduced into the ion source after the separation column, and both the IS signal and the cholesterol signal were monitored after injecting a sample. With ESI, the IS signal was strongly suppressed during cholesterol detection. In contrast, with APCI, the IS signal remains stable, while with TPI, there was a slight intensity loss. A similar trend was observed when analyzing ion source stability over a 26-hour period. The intensity of the cholesterol decreases most significantly over time with ESI, APCI and TPI lose comparatively less intensity. During validation, the most sensitive LOD and LOQ values were obtained for APCI and TPI and for ESI the LOD values were between 10 to 333 times higher for the sterols and the linear range was significantly lower. This problem in particular reflects the lower possibility of quantification in the samples using the ESI source.

As oxysterols were presumably involved in the development and progression of cancer and metastasis in addition to sterols, the method was extended to include both sterols and oxysterols. For this purpose, an isocratic step was inserted before the actual analysis, as the oxysterols were more polar than the sterols. This allows around half of the oxysterols to be separated from each other. Using a further heart-cut (both heart-cuts were focused on the trap column and separated together on the ^{2}D separation column), a separation of almost all substances (24- and 25-hydroxycholesterol coelute, same *m*/*z* ratio) could be achieved. LOD and LOQ values of 30 nmol/L could also be achieved with the method for most substances, and thus sufficient sensitivity was achieved to detect oxysterols in the samples.

Master's Theses Accomplished 2025

Nils Becker
BAGO (Bayesian optimization) for untargeted LC-MS – a simple tool for LCxLC beginners?

Jost Guinand
Implementation and validation of automated sample preparation with LC-MS for characterizing the metabolome in biological samples

Constantin Krempe
Enzyme Activity Profiling through metabolite analysis by GC-MS and LC-MS

Sebastian Löbbecke
Development of a method for the analysis of hydrocarbons in complex samples

Priscilla Nhan
Development of a trap-based LC+LC-IM-QTOF-MS method for non-targeted analysis

Bachelor's Theses Accomplished 2025

Andre Brandt
Mass spectrometric non-target analysis of microalgae and subsequent data evaluation using MS-Dial and Sirius

Aristotelis Charchantis
Evaluation of the ionization efficiency of various extracts using a dual ion source

Lukas Ickerott
Nano-LC-HRMS with optional plasma post-ionization for metabolomic studies

Dustin Linke
Fundamental investigations into the optimization of a tube plasma ion source for mass spectrometric analysis of complex samples

Karolin Orth
Method Development for Hydrocarbon Analysis by GCxGC-MS

Erik Pösken
Metabolic studies of nitrogen-resistant algae using HPLC-MS

Alexander Rosellen
Evaluation of the ionization efficiency of iLTP in combination with Orbitrap MS

Tobias Thiel
Extraction of various soils and their analysis using LC-IM-MS

Scientific Publications 2025

Original Paper / Peer-reviewed

K. Wetzel, P. Nhan, T. Tishakova, M. Häßler, T. Niedenthal, L. Montero, O. J. Schmitz, **The benefits of multi-^{2}D LCxLC compared to LCxLC for the analysis of European herbal remedies**, *accepted in Analytical and Bioanalytical Chemistry*

S. Löbbecke, F. Stappert, F. Uteschil, M. H. Blokland, O. J. Schmitz, A. Arrizabalaga-Larranaga, J. F. Ayala-Cabrera, **High-throughput analysis of growth promoters by LC-MS/MS using a novel tube plasma ionisation source**, Microchemical Journal (2025) https://doi.org/10.1016/j.microc.2025.115603

P. Wittenhofer, J. F. Ayala-Cabrera, L. Orell, F. Uteschil, S. W. Meckelmann, O. J. Schmitz, **Sterol analysis in cancer cells using atmospheric pressure ionization techniques**, *accepted in Analytical and Bioanalytical Chemistry*

M. Häßler, K. Wetzel, L. Warnecke, T. Niedenthal, L. Montero, J. F. Ayala-Cabrera, O. J. Schmitz, **Assessment of the differentation fo *Sambucus nigra* plants using a multi-target and suspect screening by LX-HRMS and ICP-OES**, Analytical and Bioanalytical Chemistry (2025) https://doi.org/10.1007/s00216-025-06112-7

T. Friedenauer, S. Krdhishte, W. L. M. Narz, T. Bessel, L. K. Tintrop, M. Vosough, A. Salemi, C. Thom, S. W. Meckelmann, M. Spellauge, A. Sommeryns, C. Rehbock, T. C. Schmidt, O. J. Schmitz, H. P. Huber, S. Barcikowski, **Ultrashort-pulsed laser diffusion enhancement in liquids for cold extraction of coffee in an analytically validated low-fluence regime**, Adv. Photonics Res. (2025) 2500115

Y. Oulad El Majdoub, A. K. Mallik, L. Montero, J. Rösler, F. Stappert, J. Leddin, S. W. Meckelmann, O. J. Schmitz, **Development of an L-Lysine deriverd stationary phase for both RP and HILIC separations of complex samples - proof-of-concept in both dimensions of LCxLC**, Analytical and Bioanalytical Chemistry (2025) https://doi.org/10.1007/s00216-025-06060-2

A. A. Elbashier, O. J. Schmitz, **Development and validation of capillary zone electrophoresis method for simultaneous analysis of clarithromycin, omeprazole, and tinidazole in pharmaceutical dosage form**, Analytical Methods (2025) https://doi.org/10.1039/D5AY00437C

A. Mallik, T. Anik, J. Roesler, S. Meckelmann, O. J. Schmitz, **Isoniazid-modified silica for hydrophilic interaction chromatography and as adsorbent for dye reoval in wastewater samples**, ACS Omega (2025) 10:22779–22788

N. A. Samak, F. Götz, K. Adjir, T. Schaller, M.Häßler, O. J. Schmitz, J. Fax, G. Haberhauer, A. Surmeneva, R. U. Meckenstock, **Characterization of 2-phenanthroyl-CoA-reductase, an ATP-independent type III aryl-CoA reductase involved in anaerobic phenanthrene degradation,** Applied and Environmental Microbiology (2025) 0:e00166-25

C. Schmerling, C.Schroeder, X. Zhou, J. Bost, B. Waßmer, S. Ninck, T. Busche, L. Montero, F. Kaschani, O. J. Schmitz, J. Kalinowski, M. Kaiser, S.-V. Albers, C. Bräsen, B. Siebers, **The identification of an unusual glycerol-3-phosphate dehydrogenase in *Sulfolobus acidocaldarius* elucidates the evolution and functional diversity of glycerol metabolism enzymes across Archaea,** Communications Biology (2025) 8:539

K. Wetzel, T. Tishakova, M. Häßler, T. Niedenthal, J. F. Ayala-Cabrera, L. Montero, O. J. Schmitz, **Which extraction technique is the best for LC ×LC analysis of bioactive compounds from European medicinal plants: Conventional or sustainable extraction techniques?**, Green Analytical Chemistry (2025) 12:100233

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)**, Talanta (2025) 285:127388

A. Minetti, O. Omrani, C. Brenner, F. Cansiz, S. Imada, J. Rösler, S. Khawaled, G. Allies, S. W. Meckelmann, N. Gebert, I. Heinze, N. Rahnis, J. Lu, K. Spengler, M. Rasa, E. Cirri, R. Heller, Ö. Yilmaz, A. Tasdogan, F. Neri, A. Ori, **Polyamines sustain epithelial regeneration in aged intestines by modulating protein homeostasis**, Nat Cell Biol (2025) doi: 10.1038/s41556-025-01804-9

N. H. Schebb, N. Kampschulte, G. Hagn, K. Plitzko, S. W. Meckelmann, S. Ghosh, R. Joshi, J. Kuligowski, D. Vuckovic, M. T. Botana, Á. Sánchez-Illana, F. Zandkarimi, A. Das, J. Yang, L. Schmidt, A. Checa, H. M. Roche, A. M. Armando, M. L. Edin, F. B. Lih, J. J. Aristizabal-Henao, S. Miyamoto, F. Giuffrida, A. Moussaieff, R. Domingues, M. Rothe, C. Hinz, U. S. Das, K. M. Rund, A. Y. Taha, R. K. Hofstetter, M. Werner, O. Werz, A. S. Kahnt, J. Bertrand-Michel, P. Le Faouder, R. Gurke, D. Thomas, F. Torta, I. Milic, I. H. K. Dias, C. M. Spickett, D. Biagini, T. Lomonaco, H. Idborg, J.-Y. Liu, M. Fedorova, D. A. Ford, A. Barden, T. A. Mori, P. D. Kennedy, K. Maxey, J. Ivanisevic, H. Gallart-Ayala, C. Gladine, M. Wenk, J. M. Galano, T. Durand, K. D. Stark, C. Barbas, U. Garscha, S. L. Gelhaus, U. Ceglarek, N. Flamand, J. L. Griffin, R. Ahrends, M. Arita, D. C. Zeldin, F. J. Schopfer, O. Quehenberger, R. Julian, A. Nicolaou, I. A. Blair, M. P. Murphy, B. D. Hammock, B. Freeman, G. Liebisch, C. N. Serhan, H. C. Köfeler, P. J. Jakobsson, D. Steinhilber, M. H. Gelb, M. Holčapek, R. Andrew, M. Giera, G. A. FitzGerald, R. C. Murphy, J. W. Newman, E. A. Dennis, K. Ekroos, G. L. Milne, M. A. Gijón, H. W. Vesper, C. E. Wheelock, V. B. O'Donnell, **Technical recommendations for analyzing oxylipins by liquid chromatography-mass spectrometry, Science Signaling** (2025) 18(887): eadw1245. doi: 10.1126/scisignal.adw1245

Misc. Publications

K. Wetzel, O. J. Schmitz, **Boosting the Separation Power of LCxLC**, LCGC International (2025) May Issue

F. G. Strathmann, O. J. Schmitz, **Adressing PFAS Challenges with Modern High Resolution IMS-MS Methods**, Current Trends in Mass Spectrometry (2025) 23(1):20-25

O. J. Schmitz, **Chromatography Free: It's Closer Than You Think**, The Analytical Scientist (2025) January Issue

Poster Presentation

K. Wetzel, T. Tishakova, M. Häßler, L. Montero, O. J. Schmitz, **Chemical characterization of European medicinal plants by multi-2D LC × LC**, 8th analytica Vietnam, Ho Chi Minh City, Vietnam, April 2025

C. Thom, S. W. Meckelmann, O. J. Schmitz, **Identification of human plasma lipidome: A comparison of LC-SLIM-MS and LC-DTIM-MS**, 8th analytica Vietnam, Ho Chi Minh City, Vietnam, April 2025

C. P. Krempe, J. Rösler, S. W. Meckelmann, A. Tasdogan, O. J. Schmitz, **Why a retention time and m/z database make sense for metabolomics**, 54th HPLC, Bruges, Belgium, Juni 2025

S. Löbbecke, F. Stappert, F.Uteschil, M. H. Blokland, O. J. Schmitz, A. Arrizabalaga-Larrañaga, J. F. Ayala-Cabrera, J. **Comparison of modern analytical platforms for residue control of growth promoters in biological samples**, 54th HPLC, Bruges, Belgium, Juni 2025

K. Wetzel, M. Häßler, T. Tishakova, L. Montero, O. J. Schmitz, **Multi-2D LC × LC and more for a comprehensive analysis of European herbal remedies**, 54th HPLC, Bruges, Belgium, Juni 2025

C. Thom, S. W. Meckelmann, O. J. Schmitz, **Combination of HPLC and SLIM: An extremely powerful analysis platform**, 54th HPLC, Bruges, Belgium, Juni 2025

M. Häßler, K. Wetzel, C. Thom, F. Uteschil, s. Löbbecke, L. Montero, J. F. Ayala-Cabrera, O. J. Schmitz, **Dual ion source HRMS for improved chemical characterization of European medicinal plants using 2D-LC**, 73th ASMS, Baltimore, USA, Juni 2025

S. Löbbecke, F. Stappert, F.Uteschil, M. H. Blokland, O. J. Schmitz, A. Arrizabalaga-Larrañaga, J. F. Ayala-Cabrera, **Novel strategies for the analysis of growth promoters in biological samples**, ExTech 2025, Mülheim an der Ruhr, Germany, September 2025

K. Wetzel, T. Tishakova, M. Häßler, L. Montero, O. J. Schmitz, **The optimization of sustainable extraction techniques for the multi-2D LC × LC HRMS analysis of European medicinal plants**, ExTech 2025, Mülheim an der Ruhr, Germany, September 2025

L. Tang, F. Uteschil, O. J. Schmitz, **Derivatization strategy for the determination of alkylamines (C2-C8) by GC-APLI-(Iontrap)MS in plastic bottles**, ExTech 2025, Mülheim an der Ruhr, Germany, September 2025

S. Löbbecke, K. Orth, O. J. Schmitz, **Modern ionisation strategies for GC-MS based hydrocarbon analysis**, analytica USA, Columbus (Ohio), September 2025

Y. Oulad El Majdoub, A. K. Mallik, L. Montero, J. Rösler, F. Stappert, J. Leddin, S. W. Meckelmann, O. J. Schmitz, **Development of SiL-Lys 2C18 for the Analyses of Real and Complex Samples both in 1D and 2D-LC**, analytica USA, Columbus (Ohio), September 2025

Invited Lectures / Oral Presentations

Prof. Oliver J. Schmitz

Breaking Boundaries in Separation Science: LCxLC-IM-qTOF-MS
International Mini-Symposium and Sino-German Research Cooperation Meeting, Tübingen, November 2025

Revolution or complement? Can ion mobility mass spectrometry replace chromatography?
Chromatography round table (online), November 2025

Breaking Boundaries in Separation Science: LCxLC-IM-qTOF-MS
1th analytica USA conference, Columbus, USA, September 2025

Increase speed and/or confidence in metabolomics
University of Victoria, Canada, August 2025

Boosting the separation power of LCxLC
54th HPLC, Bruges, Belgium, April 2025

First experiences with next-generation ion mobility mass spectrometry
8th analytica Vietnam, Ho Chi Minh City, Vietnam, March 2025

Breaking Boundaries in Separation Science: Coupling Ion Mobility Mass Spectrometry and comprehensive Two-Dimensional Liquid Chromatography
Anakon 2025, Leipzig, Germany March 2025

Less is more: Data reduction for coupling LCxLC with ion mobility mass spectrometry
39th Agilent Forum Analytics, Vienna, Austria, February 2025

Development of a MULTI-2D LC×LC-ESI/TPI-DUAL SOURCE-QTOF-MS for the analysis of complex samples
16th Multidimensional Chromatography Workshop, Liège, Belgium, February 2025

Katharina Wetzel

Boosting the separation power of LC × LC – one dimension more?
35th Doctoral seminar Hohenroda, Hohenroda, Germany, January 2025

Multi-2D LC × LC and more for a comprehensive analysis of European herbal remedies
54th HPLC, Bruges, Belgium, April 2025

Cedric Thom

Combination of HPLC and SLIM: An extremely powerful analysis platform, 54th HPLC, Bruges, Belgium, Juni 2025

Awards

I In January 2025, during the PhD seminar in Hohenroda, **Katharina Wetzel** was awarded the 2nd Prize for Best Oral Presentation. She also received the 3rd International Poster Prize at the 8th analytica Vietnam in April 2025, held in Ho Chi Minh City.

In addition, **Karolin Orth** and **Lukas Ickerott**, who both completed their bachelor's theses in our research group, were recognized by the Faculty of Chemistry at the University of Duisburg-Essen for their outstanding academic achievements.

Congratulations to all!

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)

- Basic course LC-MS (digital), Provadis, March 2025
- Advanced LC-MS (digital), Provadis, March 2025
- LC-MS course in Schmalenberg, Germany, Provadis, March 2025
- Master course GC-MS, Haus der Technik, Essen, Germany, April 2025
- HPLC-MS in Non-Target and trace analysis, Haus der Technik, Essen, Germany, April 2025
- TRC course about ICP-OES/MS, GC and LC, University of Duisburg-Essen, Germany, April 2025
- Method school: HPLC for beginners (digital), Klinkner & Partner, May 2025
- Method school: HPLC for advanced users (digital), Klinkner & Partner, May 2025
- Chromatography Special Part 2 (stationary phases), Provadis, June 2025
- Chromatography Special Part 4 (difficult analytes), Provadis, October 2025
- LC-MS course in Aachen, Germany, Provadis, October 2025
- Basic course LC-MS (digital), Provadis, November 2025
- German Chemical Society (GDCh) course about Non-target Analysis with multi-dimensional chromatography or ion mobility-mass spectrometry, University of Duisburg-Essen, Germany, November 2025