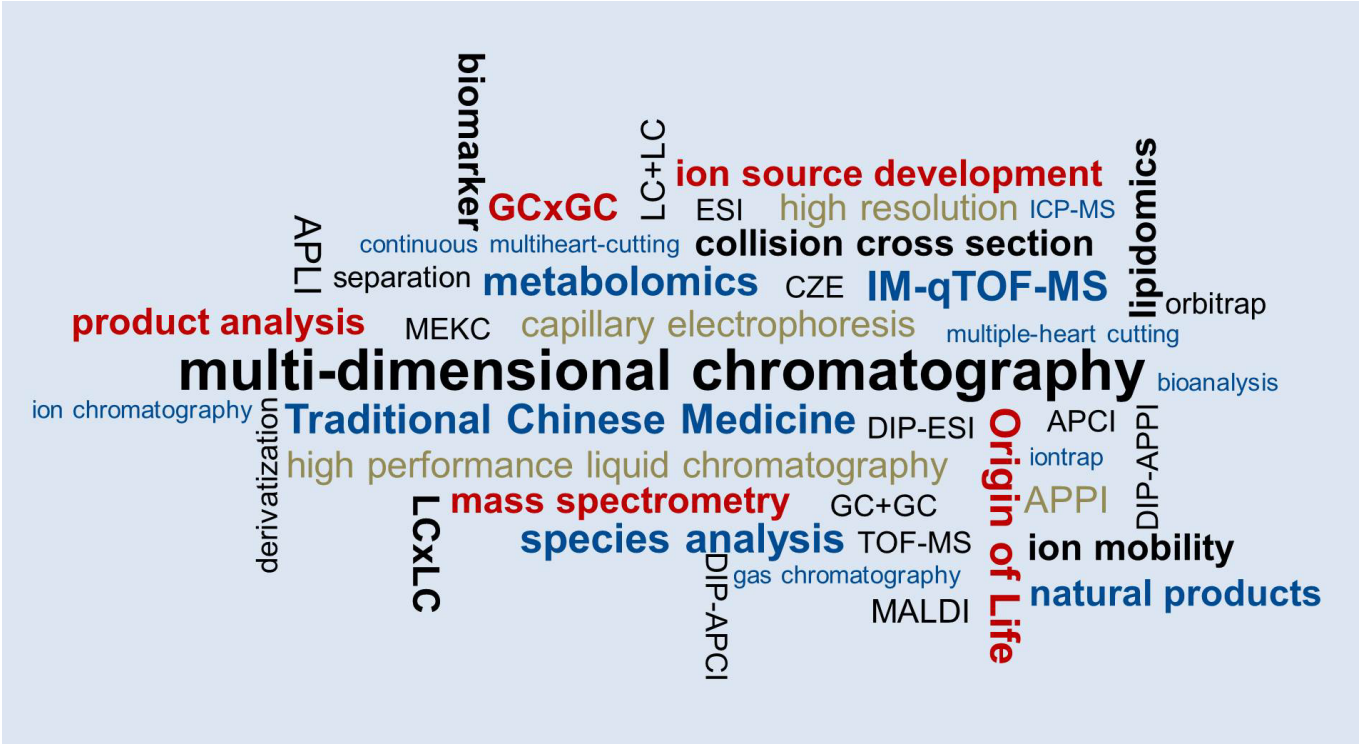
Applied Analytical Chemistry (AAC)

Annual Report 2023

AAC
applied analytical chemistry

Applied Analytical Chemistry

Annual Report 2023



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Applied Analytical Chemistry
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45141 Essen
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Applied Analytical Chemistry

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

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

2022 was the eleventh year of the Applied Analytical Chemistry research group at the University of Duisburg-Essen and the most successful to date.

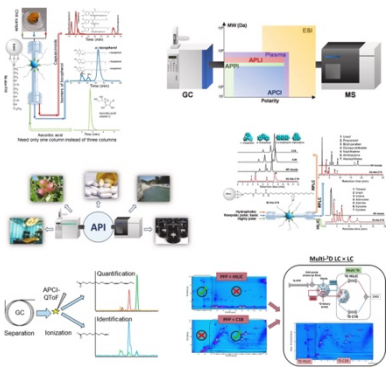
The most important thing in 2023 was that we renewed old collaborations and started some important and forward-looking collaborations with different groups. I would like to highlight some cooperation partners here. First, we started a collaboration with Jennifer Krone and coworker from MOBILion (USA) about hyphenation of SLIM, the German Aerospace Center (DLR) and the European Space Agency (ESA) on various exciting topics and with Prof. Jin-Ming Lin from Tsinghua University in Beijing about single-cell analysis.

TRC
united in separation

Teaching and Research Center for Separation

In 2018, we entered into a partnership with Agilent Technologies. In the course of this cooperation, Agilent provides us with a variety of analytical systems. And in 2021 we had the opportunity to exchange these fantastic instruments for the latest versions. Without this outstanding equipment, much of the work in this report would not have been possible. Therefore, a big thank you to Agilent Technologies at this point. In addition, we also want to mention our long-standing and extremely successful collaboration with Hitachi-High Tech (Japan), which will continue in 2023/24. Thank you very much for your trust in us.

In 2023 we managed to publish 14 scientific papers in peer-reviewed journals, three further manuscripts are in the review process. One book chapter (in Liquid Chromatography, Third Edition, Fundamentals and Instrumentation, Handbook in Separation Science) was published and 17 posters and nine lectures at international conferences were given.



In addition, four PhD, two master's, and one bachelor's theses were completed in 2023 in AAC and several projects, funded by DFG and industry were started or continued, e.g. development and optimization of a new ion source (Tube Plasma Ionization, TPI), an ion source for single-cell metabolome analysis, a new Multi 2D-LCxLC-MS platform for complex samples, and investigation of the metabolome/lipidome in cancer research.

Of course, the year 2023 for the AAC team was also mainly about a lot of work around the 51st HPLC, organized together with Prof. Michael Lämmerhofer (University of Tübingen) and the GDCh.

Since the first HPLC conference in 1973, the HPLC symposium series has established itself as the world's leading conference for chromatographic analysis techniques and their coupling with mass spectrometry. The event was a great success with 1268 registered participants from 46 different countries and was held at the Congress Center Düsseldorf (CCD) as a face-to-face event.

First of all, I would like to thank my colleague Prof. Michael Lämmerhofer and the GDCh (especially Kerstin Kattwinkel) for the great cooperation during the preparation for HPLC 2023 over the last seven (!) years. I would also like to take this opportunity to thank the sponsors and exhibitors once again, without whose financial commitment the HPLC would not have been so successful. But even without the active help of Michael´s and my colleagues, the HPLC would not have been so successful. Therefore, once again: THANK YOU SO MUCH!

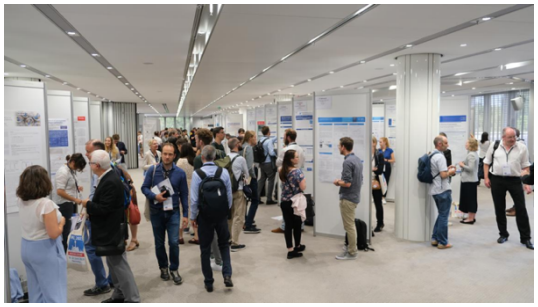
Here some facts about HPLC 2023:

Michael Lämmerhofer and me started with the preparation of this symposium in 2015, checking for possible conference centers and host cities. A bid book for HPLC 2021 in Düsseldorf, Germany (June 20-24, 2021) was submitted in May 2016. The decision for HPLC 2021 in Düsseldorf was made by the permanent scientific committee during the HPLC 2016 conference in San Francisco.

In March 2020, the corona virus pandemic hit the world and also Europe. Initially, it was hoped that it will be over until June 2021. However, in October 2020 the chairmen and the GDCh together decided to postpone the symposium for 2 years, which was made possible by the chairman of HPLC 2023 (Prof. Gert Desmet) who also postponed for 2 years (now HPLC 2025 in Bruges, Belgium).

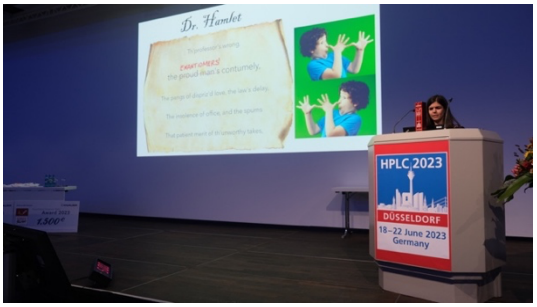
The new date was finally June 18-22, 2023. By then, the pandemic was over. China was the last country in the world to stop the pandemic restrictions somewhere in January 2023. Unfortunately, it was a bit late; many Chinese registered participants could not get a visa anymore and had to cancel their participation. For this reason, there were only a few Chinese participants at HPLC 2023. Otherwise everything went smooth without effect of the corona pandemic.

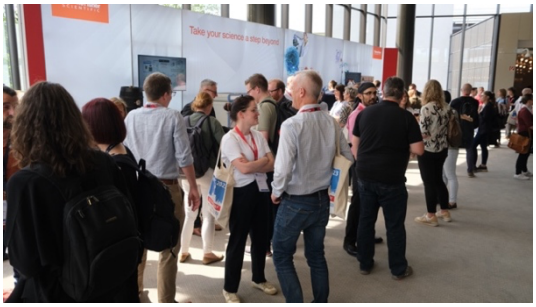
There were a total of 202 scientific presentations in the form of plenary lectures, keynote lectures and presentations by congress participants, which were offered in four parallel sessions. In the plenary lectures, renowned personalities presented the latest topics of current research in the field of separation techniques. Keynote lectures served to present new technologies and application areas in more detail.



506 poster contributions were used to give congress participants the opportunity to present their latest results and discuss them with other participants during the event.

In order to contribute to science communication, an HPLCtube and a Science Slam were organized. Here, young scientists presented their research topics to the public and the press in an outstanding manner within a given time frame via film or lecture. The focus here was on communicating scientific content in a popular and entertaining way. The best contributions were awarded prizes by a jury.





The latest technical, instrumental and methodological developments in the field of separation techniques were presented in a large and very well-attended industrial exhibition and at industrial seminars. The presence of industry representatives was also used to offer a job fair for young scientists, who were thus able to establish direct contact with potential future employers.

Especially for newcomers in the field of separation techniques, a training and further education program was offered, consisting of 12 half-day and one full-day workshop for the introduction to special techniques, as well as seven tutorial lectures, which focused on current topics.

In addition to all the science at the 51st HPLC, there was also enough time for networking and some fun.

I want to take this opportunity to thank the entire AAC team and all co-workers for their excellent work in the lab in 2023 as well as Agilent Technology and Hitachi High-Tech and the many collaborators in and outside the University of Duisburg-Essen for pleasant and efficient collaborations.

In case you see possibilities for future collaborations, I would be happy to discuss them with you.

We wish you all the best, good health, happiness, and success for the year 2024.

Essen, December 17, 2023

Applied Analytical Chemistry – Staff

Regular Staff

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

Post-Docs

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

Ph.D. Students

University Duisburg-Essen

Maha Alhasbani	Katharina Wetzel
Janosch Barthelmes	Pia Wittenhofer
Paul Görs	Ling Tang
Yildiz Großmann	Cedric Thom
Marvin Häßler	External
Martin Meyer	Tingting Li
Alexandra Pape	Dominik Mähler
Kristina Rentmeister	Anneke Niehuus
Jonas Rösler	Simon Schastok

M.Sc. Students

Christopher Jaeger, Sarah Klaus, Constantin Krempe, Sebastian Löbbecke, Kiana Mellinghaus (external), Laila Orell, Cedric Thom, Lennard Warnecke

B.Sc. Students

Anne Neugebauer, Leonardo Nuredin, Nico Pernberg, Claudia Meike Rzepinski

Guest Scientists

Assoc. Prof. Dr. Abul Khayer Mallik (AvH-fellower), Dr. Taher Sahlabji (King Khalid University, Saudi Arabia)

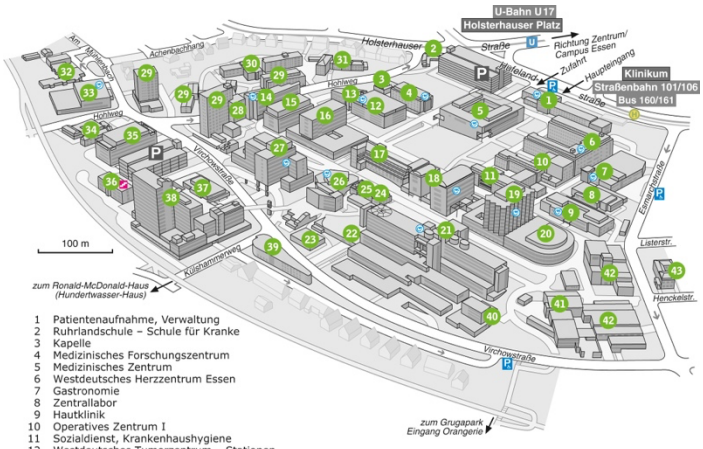
Apprentices

Tom Marcel Maxion, Leonie Nufer

Major News 2023

Mass Spectrometry Center for Omics

In 2023 we were able to continue our successful cooperation with the group of Prof. Alpaslan Tadogan from the Clinic for Dermatology at University Hospital Essen (Germany), the group of Prof. Ömer H. Yilmaz from MIT (USA) and the group of Prof. Benjamin Izar from the Columbia University on metabolomics.



1 Patientenaufnahme, Verwaltung
2 Ruhrlandschule – Schule für Kranke
3 Kapelle
4 Medizinisches Forschungszentrum
5 Medizinisches Zentrum
6 Westdeutsches Herzzentrum Essen
7 Gastronomie
8 Zentrallabor
9 Hautklinik
10 Operatives Zentrum I
11 Sozialdienst, Krankenhaushygiene
12 Westdeutsches Tumorzentrum – Stationen
13 Knochenmarktransplantation
14 Westdeutsches Tumorzentrum – Ambulanz
15 Westdeutsches Tumorzentrum – Forschungsgebäude
16 Strahlenklinik, Nuklearmedizin
17 Kinderklinik
18 Frauenklinik
19 Augenklinik, HNO-Klinik
20 Augenklinik/HNO-Klinik – Operationstrakt
21 Operatives Zentrum II
22 Notaufnahme
23 Studienzentrum Bildgebende Verfahren
24 Dekanat
25 Zentrale Informationstechnik (ZIT)
26 Audimax, Medizinische Fachbibliothek
27 LVR-Klinikum Essen (Psychiatrie/Psychotherapie)
28 Gästehaus
29 Schwesternwohnheime
30 Schulen für Medizinalfachberufe
31 DRK-Schwesternschaft
32 Kindertagesstätte
33 Westdeutsches Protonentherapiezentrum Essen
34 Sozial-Pädiatrisches Zentrum, Westdeutsches Zentrum für Infektiologie
35 Robert-Koch-Haus
36 Institut für Zellbiologie (Tumorforschung)
37 Mensa
38 Institutsgruppe I
39 Lehr- und Lernzentrum
40 Institute für Pathologie und Neuropathologie
41 Apotheke
42 Haustechnik
43 Betriebsarzt, Druckerei, Sicherheitstechnik

In order to further expand our expertise in the analysis of metabolome/lipidome with proteome and make the best possible use of it, we are planning to set up an MS Center for Omics.

It is planned to organize this center together with the recently appointed Professor Kathrin Thedieck, our long-term cooperation partner Prof. Alpaslan Tasdogan, Dr. Sven Meckelmann and myself. In 2024, we will join forces to drive this joint project forward and hopefully be ready for use soon.

Some important publications

Together with colleagues from Italy and Spain, we have recently published an article on comprehensive two-dimensional liquid chromatography (LCxLC) as a powerful separation method in Nature Reviews Methods Primers:

Luigi Mondello, Paola Dugo, Paola Donato, Miguel Herrero, Lidia Montero, Oliver J. Schmitz, *Comprehensive two-dimensional liquid chromatography.* Nat Rev Methods Primers 3, 86 (2023). https://doi.org/10.1038/s43586-023-00269-0
This primer should be a useful tool for all scientists interested in LCxLC.

Nature Reviews Methods Primers is part of Nature's portfolio of scientific journals and was launched in 2021 as a digital journal dedicated to the publication of analytical, statistical, computational, theoretical and applied methods in all disciplines of physics and the natural sciences (Impact Factor 39.8 and ranked 45th out of 21,762 journals assessed in Journal Citation Reports).

Another paper in the Nature family was published together with Prof. Tasdogan and colleagues from the USA in Nature Cancer (*in printing process*) on *A genetic-metabolic axis confers liver-metastatic organotropism*. This work demonstrate a rare example of metastatic organotropism through co-option

of physiological metabolic regulation, and proposes therapeutic avenues to abrogate these mechanisms. In this project we generated and analyzed metabolomics data.

In addition, a manuscript on "*Post-fast refeeding enhances intestinal stem cell-mediated regeneration and tumorigenesis through mTORC1-dependent polyamine synthesis*" is in the revision process at Nature and can be viewed as a preprint at Research Square (doi: 10.21203/rs.3.rs-2320717/v1). In this project we carried out the polyamine analyses.

Hero of the Year 2023

Dr. Sven W. Meckelmann

In 2023, Sven published a book chapter and 8 papers with a total impact factor of 97.

There is also another paper under revision at Nature and several other high-quality manuscripts in progress.

Besides his excellent publications Sven is very successful in working on projects. He is also working on a DFG proposal and is endeavouring to establish long-term cooperation with DLR and ESA.

List of Projects 2023

(Abstracts of these projects within the next pages)

Development of a β-alanine-derived unique stationary phase for the separation of various kinds of analytes with very high selectivity
Abul K. Mallik, Lidia Montero, Jonas Rösler, Sven W. Meckelmann

Monitoring the effect of statins on cholesterol biosynthesis by means of 2D-LC-QqQ-MS
Pia Wittenhofer**,** Sven W. Meckelmann

Investigation of polyphenols in wine grape pomace using comprehensive two-dimensional chromatography (LCxLC)
Yassine Oulad El Majdoub, Florian Stappert, Lidia Montero, Marvin Häßler, Taher Sahlabji

Comprehensive two-dimensional liquid chromatography (LC × LC) using identical stationary phase in both dimensions
Florian Stappert, Yassine Oulad El Majdoub, Lidia Montero, Abul K. Mallik

Green automated lipid extraction for non-targeted lipidomics
Pia Wittenhofer, Sven W. Meckelmann

Using high-resolution demultiplexing (HRdm) to increase the separation power in ion mobility spectrometry (IMS)
Florian Stappert, Sven W. Meckelmann

Optimization of conventional and emergent extraction techniques for *Sambucus nigra*
Katharina Wetzel, Tatyana Tishakova, Lidia Montero

Comprehensive chemical characterization of selected herbal remedies
Katharina Wetzel, Tatyana Tishakova, Leonardo Nuredin, Lidia Montero

Fast metabolome analysis by HILIC-Orbitrap-MS
Jonas Rösler, Constantin P. Krempe, Jaqueline Leddin, Sven W. Meckelmann, Alpaslan Tasdogan

Kinetic studies on polyamine metabolism by isotope tracing mass spectrometry
Jonas Rösler, Pia Wittenhofer, Gabriele Allies, Sven Meckelmann, Alpaslan Tasdogan

Characterisation of the metastatic liver organotropism
Jonas Rösler, Alpaslan Tasdogan, Sven W. Meckelmann

Characterization of metabolites in *A. Niger* under reduced gravitation
Sven W. Meckelmann, Yassine Oulad El Majdoub, Jonas Rösler, Marvin Häßler

Application of Bioinfomatics to support data analysis in metabolomics and lipidomics
Jaqueline Leddin, Jonas Rösler, Constantin Krempe, Sven W. Meckelmann

Inorganic examination of Mars similar deposit from Lüneburg Sole by ICP-OES for space research
Yassine Oulad El Majdoub, Leonie Nufer, Sven W. Meckelmann

Semi-quantification of E&L found in polymeric materials of medical devices
Anneke Niehuus, Sarah Oßwald, Sascha Reinschmiedt, Denise Sievers, Sven Meckelmann

Anaerobic degradation of the three-ring polycyclic aromatic hydrocarbon phenanthrene, from lab to field
Nadia A. A. Samak, Marvin Häßler, Sven W. Meckelmann, Rainer U. Meckenstock

Elucidating fatty acid double bond positions through GC-APCI-MS and in-source fragmentation patterns
Paul E. Görs, Sven W. Meckelmann

Metabolic labeling reveals fatty acid presence and biosynthesis in Archaea
Paul E. Görs, Sven W. Meckelmann

Lipidomics analysis of YUMM cells reveals mechanism of ferroptosis
Jonas Rösler, Sven W. Meckelmann, Alpaslan Tasdogan

Use of different extraction methods coupled with thermal desorption-gas chromatography / Q-TOF mass spectrometry to analyse polycyclic aromatic hydrocarbons
Isil Gazioglu

Development and application of an automated sample preparation for the analysis of polar metabolites
Sarah Klaus, Florian Stappert, Sven W. Meckelmann

Derivatization of non-aromatic compounds with ionization marker for GC-APLI-MS
Ling Tang, Florian Uteschil

Development in the novel design of the housing for a multi-ion-source
Marvin Häßler, Florian Uteschil, Juan Ayala Cabrera

Potential of tube plasma ionization for the determination of estrogenic compounds
Sebastian Löbbecke, Juan Ayala-Cabrera, Florian Stappert, Lidia Montero, Florian Uteschil

Evaluation and characterization of LC-QqQ-MS using ESI, APCI and TPI as ion sources for the analysis of sterols
Pia Wittenhofer**,** Sven W. Meckelmann

New tools in cancer metabolomics – ion source development for single cell analysis
Jonas Rösler, Florian Uteschil, Sven W. Meckelmann, Alpaslan Tasdogan

Ultrasonic assisted low temperature plasma ionization for direct sweat analysis
Jonas Rösler, Christopher Jaeger, Sven W. Meckelmann

Improvement of the detection capabilities by inserting a repeller electrode into an electrospray ionization ion source housing
Alexandra Pape, Florian Uteschil, Florian Stappert

Pulsed electrospray ionization
Florian Uteschil

Development of a β-alanine-derived unique stationary phase for the separation of various kinds of analytes with very high selectivity

Abul K. Mallik, Lidia Montero, Jonas Rösler, Sven W. Meckelmann

The reduction of operating costs in any laboratory is very important and the analysts are also concerned. There are many approaches to reducing the operating cost in high-performance liquid chromatography laboratories. One of the way is to reduce the number purchasing column for the analysis of different analytes. For example, one column may be applicable for the separation nonpolar, polar, basic, and highly polar analytes.

Here we designed and synthesized a stationary phase (Sil-Ala-C12, Fig. 1) in such a way that balanced polar and non-polar parts of the phase took part in multiple interactions with the analytes in different separation modes and mobile phase conditions. The column showed better separation abilities than the conventional commercial columns. Although the hydrophobic interaction was less here, the synergistic effect of two types of polar groups and alkyl chains created a special driving force for the separation of various types of challenging analytes. As a result, Sil-Ala-C12 was able to baseline separation of tocopherol isomers (β- and γ-isomers) and capsaicinoids (nordihydrocapsaicin and capsaicin). A single Sil-Ala-C12 column could be used for the analysis of tocopherols, capsaicinods, and ascorbic acid in chili peppers (Fig. 1), whereas pentaflurophenyl (PFP) or C30, C18, and HILIC columns, respectively, usually required. Therefore, by developing this type of stationary phase, the column purchasing cost is possible to reduce greatly.

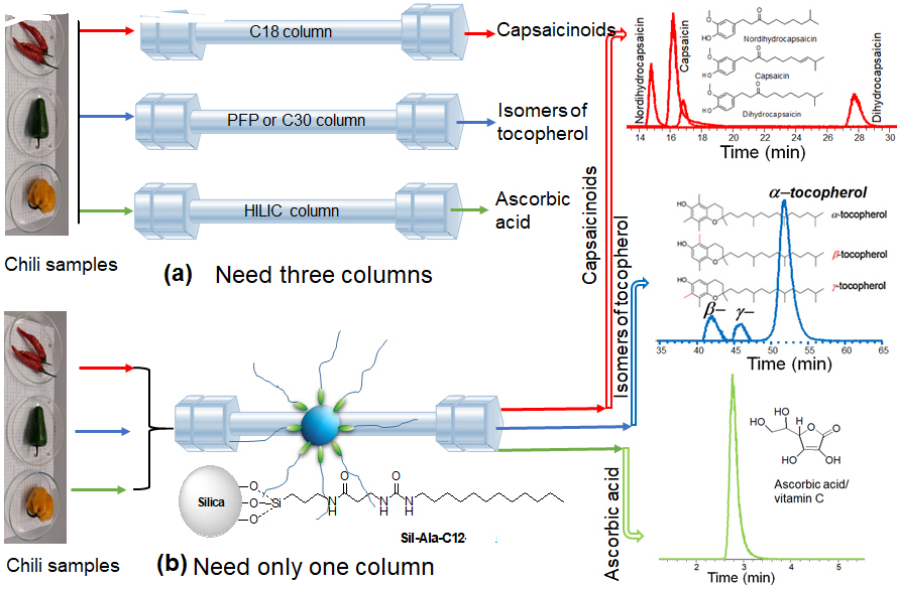


Fig. 1: Three in one. Generally, it needs three different columns for the analysis of capsaicinoids, vitamin E (tocopherols), and vitamin C (ascorbic acid) in chili peppers. However, our developed only Sil-Ala-C12 can serve the purpose of three or more columns (extracted ion chromatograms are given in the Fig. 1).

Funded by: Alexander von Humboldt Foundation

Monitoring the effect of statins on cholesterol biosynthesis by means of 2D-LC-QqQ-MS

Pia Wittenhofer, Sven W. Meckelmann

Current studies have shown that cholesterol levels play a role in various cancer diseases, often with an increased cholesterol content observed at the cellular levels. As statins have been known to inhibit cholesterol biosynthesis by inhibition of the HMG-CoA reductase they are discussed as a possible therapy. However, clear evidence is currently missing in the literature on how they affect cancer treatment and progression. To address this, we developed a novel targeted approach to characterize the cholesterol biosynthesis using a 2D heart-cut LC-LC method which uses QqQ-MS for a sensitive detection. Initially, a one-dimensional separation is performed using a PFP phase, which effectively separates all critical sterols with similar m/z ratios. Given the significant concentration differences between cholesterol and its precursors, a trap-based modulation was used to remove the cholesterol peak from the first dimension, followed by a separate analysis of the other sterols. The heart cut was achieved using a C18 column as a trap, allowing to focus of both cholesterol and simultaneously eluting dimethylcholestadienol. The separation of these two substances is performed using an EC-C18 column allowing the detection of all precursors in the biosynthetic pathway with high sensitivity. To confirm, that the method is capable of monitoring the effect of statins on biosynthesis, we incubated human epithelial lung adenocarcinoma cells with different statins. Different incubation times of lovastatin and pitavastatin at a concentration of 10 µM showed the lowest concentration of lathosterol after 16 and 24 hours. Dose-dependent experiment confirmed an effective concentration at 10 to 25 µM of the corresponding statins. This indicates, that the developed method is capable of monitoring the biological effect on the cholesterol biosynthesis in cancer cells. Currently, the method is applied to monitor pancreatic cancer cells and melanoma cells.

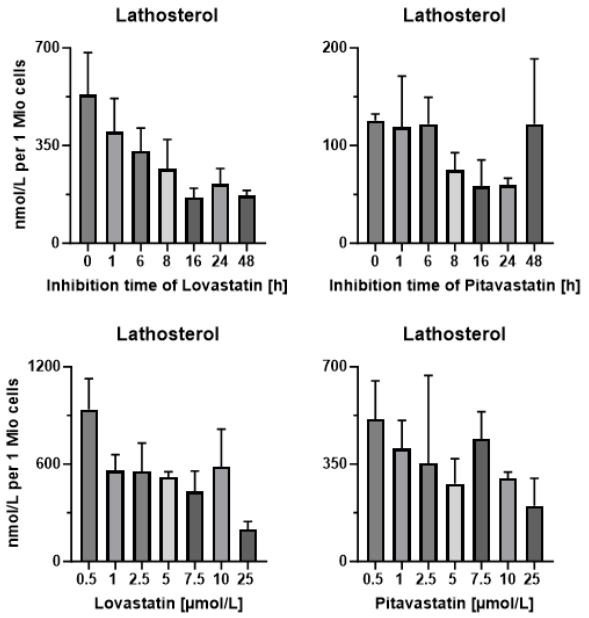


Fig. 1: Top row: sterol concentration in human epithelial lung adenocarcinoma cells incubated with lovastatin and pitavastatin at a concentration of 10 µM respectively. Bottom row: sterol concentration after a dose dependent treatment of lovastatin and pitavastatin incubated for 16 and 24 hours.

Collaborative Project – Project Partner: Prof. Barbara M. Grüner, (Cell Plasticity and Metastasis, University Hospital Essen, Germany); Prof. Annette Paschen (Molekulare Tumorimmunologie, University Hospital Essen, Germany)
Funded by: Deutsche Forschungsgemeinschaft (DFG) - ME 5800/1-1 and SCHM 1699/30-1

Investigation of polyphenols in wine grape pomace using comprehensive two-dimensional chromatography (LC × LC)

Yassine Oulad El Majdoub, Florian Stappert, Lidia Montero, Marvin Häßler, Taher Sahlabji

Several wine industries discard grape pomace, which is composed of grape seeds, skins, pulp, and stems, resulting in a complex matrix that contains a wide range of bioactive compounds. Among the constituents are phenolic compounds, flavonoids, anthocyanins, procyanidins etc. In the present work, a comprehensive two-dimensional liquid chromatography (LC × LC) method was developed to ensure a thorough investigation of all available bioactive components.

The 2D-LC technique employs in the first dimension a pentafluorophenyl (PFP) column coupled to a C18 column in the second dimension to improve the orthogonality of the separation. Additionally, a four-port, two-position switching valve was connected to two sampling loops for fraction collection.

The online 2D-LC setup was coupled to a diode array detector (DAD) and mass spectrometry (QToF) for the identification of the bioactive compounds.

The present 2D-LC method has proven to be a very effective method of separation, as evidenced by its significant orthogonality. More than 80 phenolic compounds belonging to phenolic acids, e.g. benzoic and cinnamic acids and their derivatives as well as various flavonoids, e.g. Procdyanidin and its derivatives were identified.

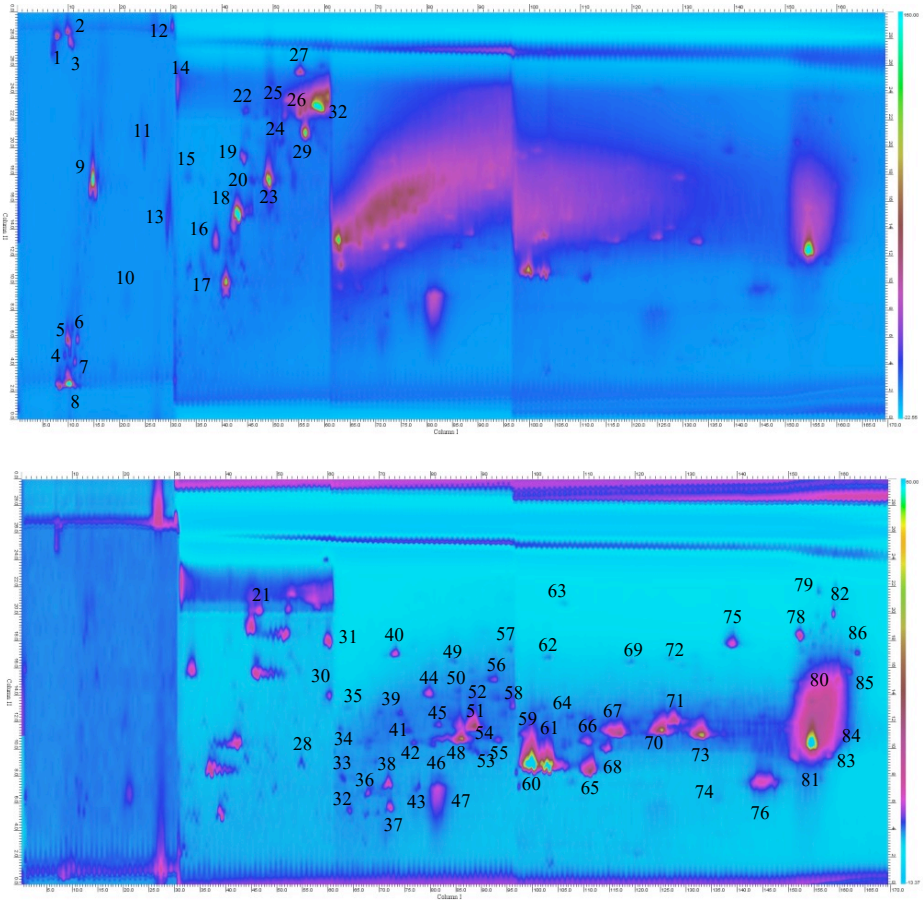


Fig. 1: 2D-LC RPxRP plot of polyphenols in wine grape pomace. (upper plot) which was acquired at 280 nm shows in its first part all phenolic acids, hydroxybenzoic and hydroxycinnamic acids. Whereas (lower plot) which was acquired at 330 nm shows all available procyanidin compounds and its derivatives.

Comprehensive two-dimensional liquid chromatography (LC × LC) using identical stationary phase in both dimensions

Florian Stappert, Yassine Oulad El Majdoub, Lidia Montero, Abul K. Mallik

In comprehensive two-dimensional liquid chromatography (LC × LC) two columns with different separation modes are coupled to achieve orthogonal separation with increased separation power compared to each column alone. This enables the separation of complex matrices which include compounds in a wide range of polarity. The orthogonality of the separation in both dimensions is usually achieved by using different stationary phases (SPs) with different separation modes (e.g. HILIC × RP). A challenge in developing an LC × LC method lies behind the different measurement conditions necessary for different columns such as the mobile phase (MP). It is therefore possible that the solvent from the first dimension leads to a breakthrough in the second column. The aim of this project is the development of an LC × LC method with high orthogonality using the identical stationary phase under different separation conditions in both dimensions. This approach is made possible by the use of a newly synthesized stationary phase (Sil-Lys-2C18). Unpolar C18 groups, polar functional and free amino groups are present in the chemical structure with the purpose of the employment of Sil-Lys-2C18 in RP and HILIC mode depending on the separation conditions (composition of the MP), which could be validated in previous 1DLC experiments. To investigate a Sil-Lys-2C18 (HILIC) × Sil-Lys-2C18 (RP) separation, herbal liqueur (“Underberg”) was used as a chemically highly complex standard sample.

In initial LC × LC experiments, orthogonal separation was demonstrated as shown in Fig. 1. A method development was carried out and led to an orthogonality that can keep up with established LC × LC methods. Qualitatively comparable results were also shown with another newly developed SP (Sil-Ala-C12). The next step planned is coupling with mass spectrometry to enable clear identification of the separated compounds.

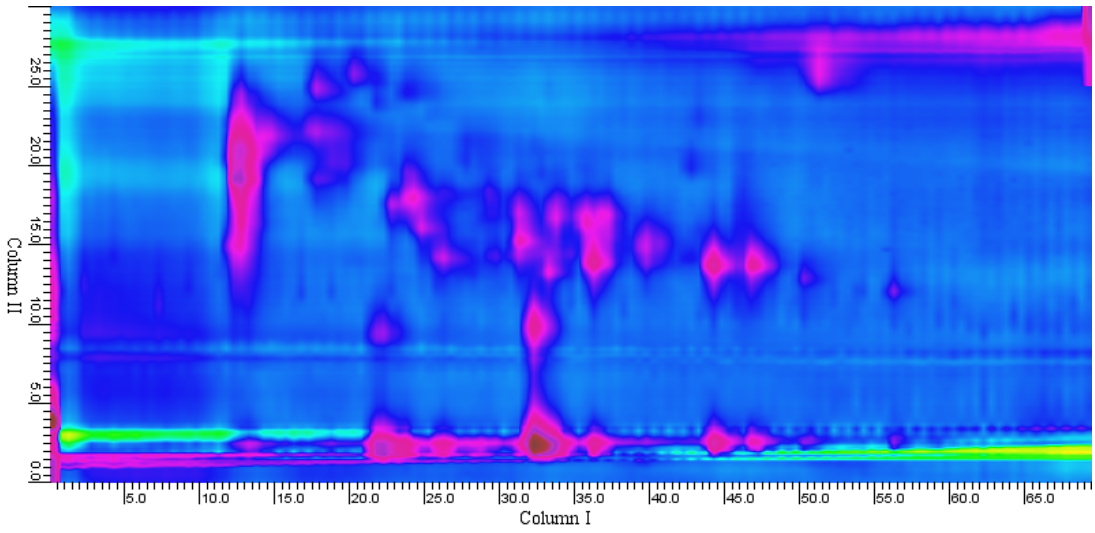


Fig. 1: 2D-LC plot of a Sil-Lys-2C18 × Sil-Lys-2C18 separation of herbal liqueur using HILIC-conditions in ^{1}D and RP-conditions in ^{2}D with a modulation time of 30 s and a wavelength of 254 nm.

Funded by: Alexander-von-Humboldt Foundation

Green automated lipid extraction for non-targeted Lipidomics

Pia Wittenhofer, Sven W. Meckelmann

Lipids play a vital role in organism function, substance transport, metabolic processes, and serve as key components in cell membranes. Imbalances in the lipidome are associated with numerous diseases and understanding the underlying biology is crucial for effective treatment. Therefore, accurate lipidomics analysis with a high degree of automatization is essential. In this context, sample preparation significantly impacts analytical accuracy. In addition, this step usually requires large amounts of organic solvents which are often harmful to the environment, toxic for the user, or from non-regenerative sources.

We developed an environmentally friendly alternative to the traditional solvent-based methods that are using MTBE, (methyl tert-butyl ether), chloroform, or hexane. Therefore, existing protocols were optimized and the solvents were replaced by ethanol and ethyl acetate as green alternatives. In addition, the protocol was optimized for the usage of an automatized sample preparation system. By comparing this protocol with extraction methods involving MTBE and hexane, we assessed their recovery and suitability for global lipidomics analysis. Results demonstrate that green solvents outperform traditional ones for different matrices, encompassing both internal standards and native lipids. Despite the higher polarity of ethyl acetate. Our findings offer insights into the good efficacy of environmentally friendly solvents in lipid extraction.

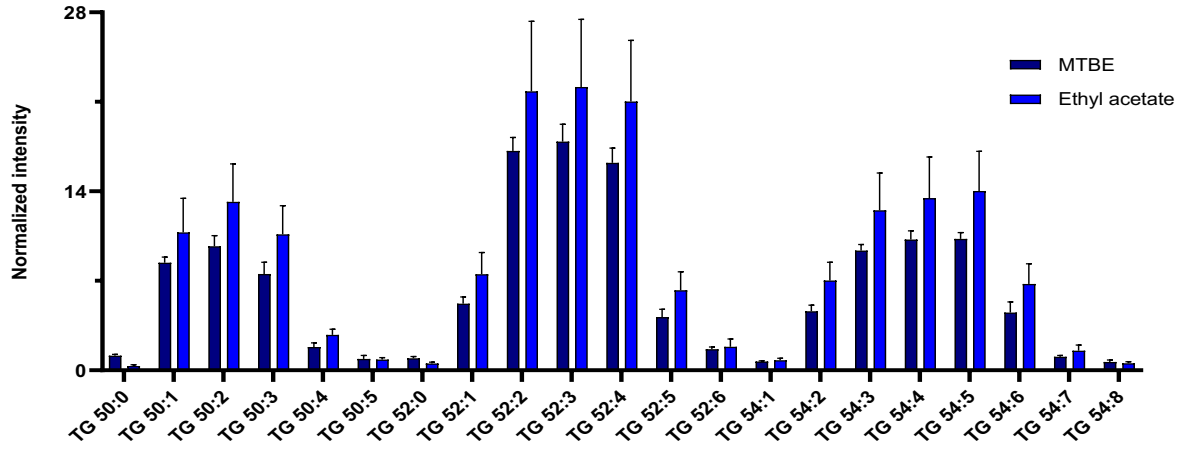


Fig. 2: Native lipids in human plasma, extracted with MTBE and ethyl acetate as a green alternative. Analysis was carried out by LC-ESI-IM-qTOF-MS using a Waters Acquity CSH C18 column (100 x 2.1mm, 1.7 µm).

Funded by: Deutsche Forschungsgemeinschaft (DFG) - ME 5800/1-1 and SCHM 1699/30-1

Using high-resolution demultiplexing (HRdm) to increase the separation power in ion mobility spectrometry (IMS)

Florian Stappert, Sven W. Meckelmann

The ion mobility is a ion-specific property which is defined as the proportionality factor between the drift velocity of an accelerated ion in an electric field and the electric field strength. A decisive factor is the number of collisions with neutral species, in which the accelerated ion loses kinetic energy, and can be described by the collision cross section (CCS). Nowadays, a wide range of analytical devices using the ion mobility for separation are available. A widely used method of ion mobility spectroscopy (IMS) is the drift tube IMS (DTIMS). In this project, a coupling of liquid chromatography, ion mobility and mass spectrometry (LC-IM-MS) is used, which allows multidimensional ion separation with high resolution and is therefore used for chemically highly complex samples. The non-targeted analysis of biological samples require maximum resolution, which is why a further increase is of great interest.

To increase the resolution power of the system, the resolution power of the IM part should be increased. Therefore, the DTIMS could be replaced by a high-resolution IMS devices (HRIMS), but this requires complex and costly modulation of the entire system. An alternativ approach to increase the resolution power of a classic DTIMS is the combination of multiplexing and high-resolution demultiplexing (HRdm). Multiplexing is a method to release an ion package into the drift tube by a sequence of several low-density pulses, while the classic single-pulse mode releases the ion package in a single puls. The total release time is identical in both cases. After data processing (demultiplexing), classic IM-specra can be obtained in multiplexing mode, which have a lower noise level and therefore lower LOD compared to single-pulse mode. Furthermore, the ion density per pulse is reduced, which increases the upper limit of the LOD and the linear range. The HRdm is a subsequent data processing using a high-resolution peak deconvolution to increase the resolution power.

We used the combination of both multiplexing and HRdm for the investigation of biological samples (for example human blood serum) and compared the resolution and the total number of detected species with the measurements in multiplexing mode only and in single-pulse mode. The resolution could be increased for selected signals from about 27 to about 202. Additionally, the total number of detected species could be increased for selected groups of lipid as shown in Fig. 1. To vary the qualitative effect of multiplexing and HRdm, further biological samples will be analyzed and compared.

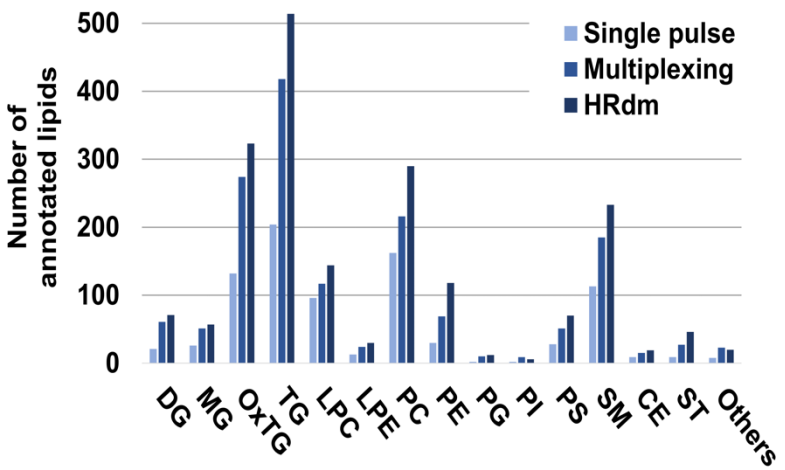


Fig. 1: Total number of detected lipids from human blood serum using LC-IM-MS in single pulse mode, in multiplexing mode, and after using HRdm.

Optimization of conventional and emergent extraction techniques for *Sambucus nigra*

Katharina Wetzel, Tatyana Tishakova, Lidia Montero

More and more antibiotic-resistant bacteria are appearing, and the existing drugs also show strong side effects or patient-dependent effects in many other diseases. Perhaps the ancient knowledge of monastic medicine can help to make further progress. For example, the treatment of liver diseases with European medicinal plants has a centuries-old tradition in monastic medicine. To isolate and preconcentrate biologically active compounds, extraction as a multi-component system has to be evaluated regarding crucial extraction parameters. Conventional and emergent extraction techniques (infusion, magnetic-, ultrasound- and microwave-assisted extraction) were optimized on different plant parts for *Sambucus nigra* via a design of experiment by MODDE. The parameters solvent-to-plant ratio, ethanol content in the extraction solvent, extraction time and temperature, and microwave power were varied and the total phenolic content, antioxidant and radical scavenging activity were determined. After optimization of the extraction parameters, the greenness of the respective technique was assessed according to the AGREE metrics (Fig. 1). Infusion as conventional technique has the significant drawback that water is used as extraction solvent. Aqueous ethanol is more favorable since a wider range of compounds can be extracted. For magnetic-assisted extraction, the lowest greenness value was observed due to low sample throughput and high energy consumption while it yields the highest total phenolic content. Ultrasound-assisted extraction has a low energy consumption and low sample throughput compared to high sample throughput and average energy consumption for microwave-assisted extraction. The bioactivities for all obtained extracts are not significantly different for conventional and emergent extraction techniques but the highest antioxidant and radical scavenging activity is observed for microwave-assisted extraction. Due to the significantly reduced extraction time, greenness and high bioactivity of the investigated extracts, microwave-assisted extraction is chosen as the most suitable technique and applied to other plants of interest such as *Agrimonia eupatoria*.

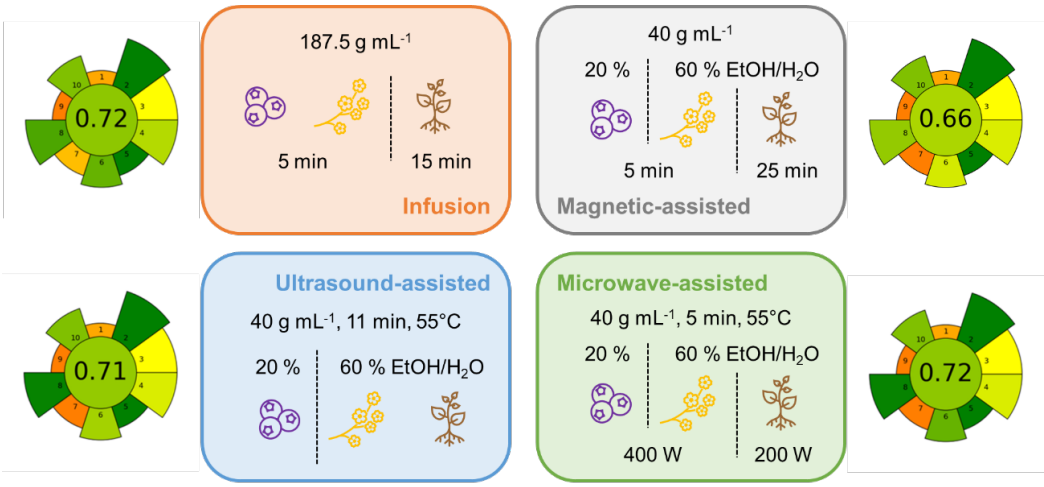


Fig. 1: Overview of optimized parameters and resulting AGREEprep metric values for the extraction techniques infusion, magnetic-assisted, ultrasound-assisted and microwave-assisted extraction.

Collaborative Project – Project Partner: Marvin Häßler (University Duisburg-Essen, Essen, Germany)
Funded by: Deutsche Forschungsgemeinschaft (DFG) - Projectnumber 504370143

Comprehensive chemical characterization of selected herbal remedies

Katharina Wetzel, Tatyana Tishakova, Leonardo Nuredin, Lidia Montero

The chemical characterization of traditional medicine remains a challenge for every analytical approach due to the complexity of metabolic profiles. In order to explore the full potential of herbal remedies, bioactive compounds have to be separated efficiently and identified for an implementation in our health systems.

Comprehensive two-dimensional liquid chromatography (LC x LC) is currently the most effective technique to enhance the separation power. As part of a bachelor thesis, a PFP x C18 method is under development to be capable of separating all plant extracts despite their individual compound compositions.

One of the most promising plant extract is derived from the leaves of *Agrimonia eupatoria* which has an equivalent radical scavenging activity as α-tocopherol according to DPPH assay. Beside this, plant parts of *Angelica archangelica*, *Sambucus ebulus* and *nigra* were analyzed and their bioactivity were determined using colorimetric essays such as Folin-Ciocalteu method (TPC) or for antioxidant activity ABTS and DPPH essay (Fig. 1).

For an identification of bioactive compounds, the LC x LC system will be coupled to high resolution mass spectrometry and evaluated as a non-targeted approach. In order to focus on the most bioactive compounds, prior to MS analysis the plant extracts will be fractionated via preparative LC to reduce data size before identification.

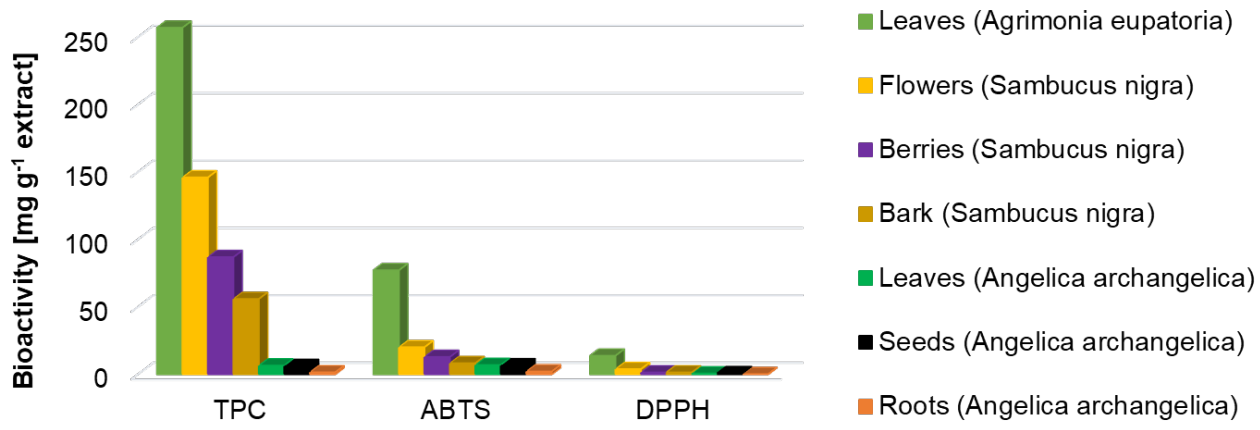


Fig. 1: Comparison of the bioactivity determined by Folin-Ciocalteu (TPC) and antioxidant activity by ABTS and DPPH assay of different plant parts from *Agrimonia eupatoria*, *Angelica archangelica*, *Sambucus ebulus* and *Sambucus nigra*.

Collaborative Project – Project Partner: MSc Marvin Häßler (University Duisburg-Essen, Essen, Germany)
Funded by: Deutsche Forschungsgemeinschaft (DFG) - Projectnumber 504370143

Fast metabolome analysis by HILIC-Orbitrap-MS

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

Non-targeted metabolomics has become a fundamental technique in life sciences as it enables the analysis of a wide range of metabolites in various samples. However, the quality of the final results depends directly on the identification level applied in the used method. Most of the published data for non-targeted metabolomics utilizes open source databases without a reference chromatographic method, and consequently without the alignment of retention times. This results in generally lower data quality compared to standard targeted approaches.

Furthermore most HILIC methods applied for polar metabolites have long analysis times and therefore consume instrument time and ressources, which makes them inappropriate for high throughput screening in large clinical studies.

For this purpose we have developed a fast HILIC seperation method using an Agilent AdvanceBio MS Spent Media coulumn (150 mm x 2.1 mm, 2.7 µm) followed by high-resolution mass spectrometry including data dependant MS2 acquisition and used this method to establish a database for over 500 polar metabolites including exact mass, retention time and fragmentation pattern.

This method is able to separate complex samples from the human metabolome within 8 minutes under high repeatablity. The dataset is yet including about 360 of the most important human metabolites and is therefore covering almost all important analytes for fundamental research in life sciences. The data was transferred into a libray file, which is compatible to open source software as MSDial, enabling a fast and robust data processing with the highest possible identification level in classical LC-MS based techniques.

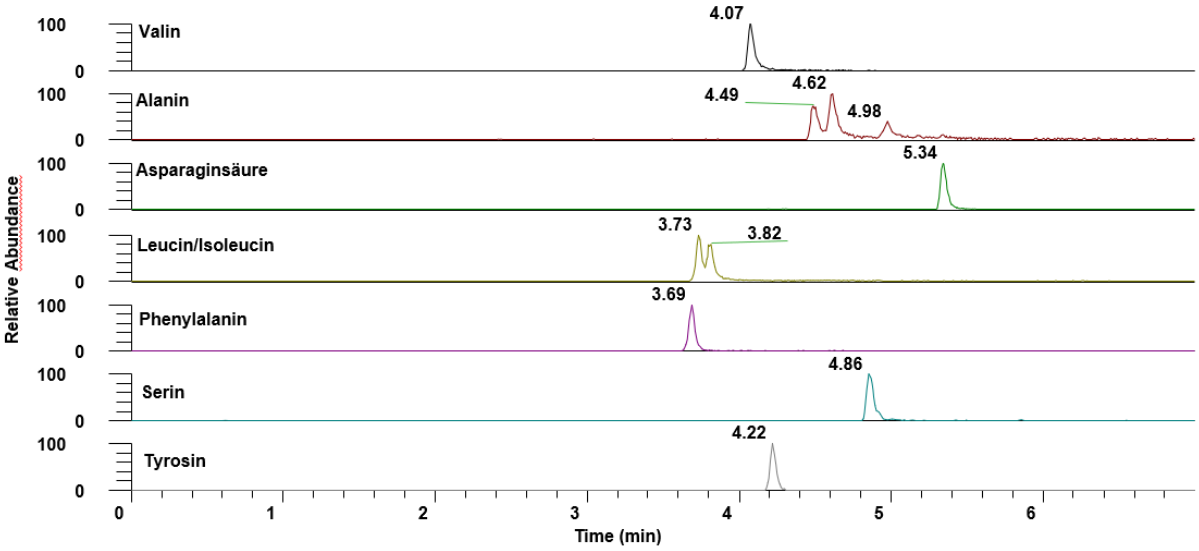


Fig. 1: Example for the separation of amino acids within less than 6 minutes.

Funding: 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)

Kinetic studies on polyamine metabolism by isotope tracing mass spectrometry

Jonas Rösler, Pia Wittenhofer, Gabriele Allies, Sven Meckelmann, Alpaslan Tasdogan

The disregulation of polyamines in cancer cells is known to correlate with cancer growth and proliferation, making the accurate determination of these compounds crucial for corresponding metabolic studies.

Hence, our group has recently developed a method for stable and simple quantification of polyamines using LC-QqQ-MS, which found feasible application in this field.

In this study, we report the improvement of this method by implementing the possibility of stable isotope tracing experiments, which adds a kinetic perspective studying the metabolism of polyamines.

By the use of LC-Orbitrap-MS it was possible to show the isotopical enrichment of the M+4 isotopologue of putrescine using isotope labeled ornithine or arginine as feedstocks for the cells as shown below.

The ratio between the labelled and unlabelled analyte over time thus provides information about the activity of this metabolic pathway. Using this information the method was applied in a pending study to elucidate the effect of nutrition on the kinetics of polyamine metabolism.

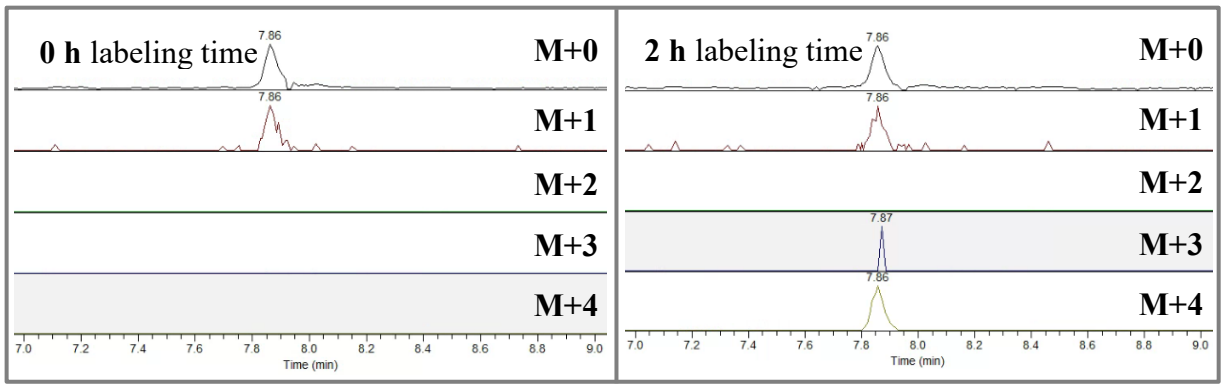


Fig. 1: Labeling of the polyamine putrescine after zero (left) and two hours (right) of exposition to 13C-labeled arginine. The characteristic M+4 isotopologue is clearly distinct from the natural isotope ratio.

In these experiments it was not just possible to visualize the dependence on different feedstocks, but moreover the data show changed metabolic behaviour induced by fasting, which is to be further investigated. The isotope labeling thereby enables a deeper understanding of mechanisms in the polyamine metabolism.

Collaborative Project – Project Partner: Prof. Omer H. Yilmaz, MIT, Cambridge, USA.

Funding: 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)

Characterisation of the metastatic liver organotropism

Jonas Rösler, Alpaslan Tasdogan, Sven W. Meckelmann

Organotropism in metastasis, the preferential spread of cancer cells to specific organs, is a crucial but poorly understood phenomenon. In this study, we focus on liver metastasis (LM) and its unique metabolic characteristics. LM occurse in several cancer and has a poor prognosis and resistance against therapy.

The loss of the Pip4k2c gene hypersensitizes cancer cells to insulin, leading to increased invasion into the liver, while sparing other organs. To understand the role of metabolic adaptions and organ-specific mechanisms in cancer metastasis we analyzed different liver and lung metastasis tissue from mice by non-targeted LC-MS based metabolics.

The analysis was carried out using an Agilent 1290 Infinity II Bio LC system with a AdvanceBio MS Spent Media column (150 x 2.1 mm, 2.7 µm). Mass spectrometric detection was performed by coupling the LC-system with a Thermo Orbitrap Q Exactive. The metabolic analyses revealed distinct fingerprints in liver metastases, with elevated TCA (tricarboxylic acid) cycle metabolites. The results are accepted for publication in Nature Cancer.

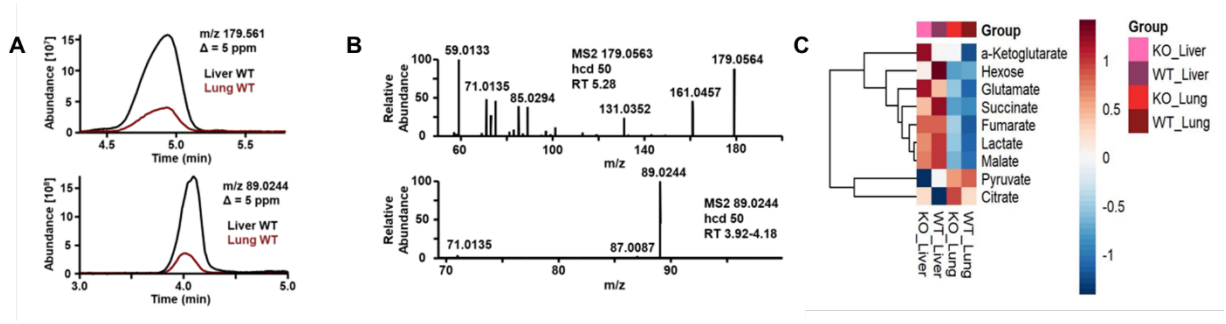


Fig. 1: Extracted ion chromatograms (**A**) and corresponding MS2 spectra of the putatively identified hexose (e.g. glucose) with an accurate mass for the $[M\text{-}H]^-$ ion of 179.5610 (**B** top) and of lactic acid with an accurate mass for the $[M\text{-}H]^-$ ion of 89.0244 (**B** bottom). Statistical analysis (**C**) revealed several TCA-related metabolites that differ significantly in both lung and liver metastases.

Collaborative Project – Project Partner: Prof. Benjamin Izar (Department of Medicine, Columbia University, New York, NY, USA)

Characterization of metabolites in A. Niger under reduced gravitation

Sven W. Meckelmann, Yassine Oulad El Majdoub, Jonas Rösler, Marvin Häßler

Filamentous fungi, like *A. Niger*, are ubiquitous sources of both infection and valuable resources in diverse materials and biological systems. While these fungi can pose health risks, they are also used for the production of antibiotics, vitamins, and food supplements. Understanding their adaptation, growth, and colonization within enclosed environments, such as the International Space Station (ISS), holds significant potential for monitoring and utilizing them in space exploration, including food production. In collaboration with the Aerospace Microbiology Research Group at the DLR, we started a project to explore the impact of gravity on the metabolic processes of *A. Niger*. Therefore, samples were cultivated under both normal gravitational conditions and supported microgravity environments. These samples were subjected to polar metabolite extraction and subsequent analysis via LC-MS. For HILIC separation, we employed an AdvanceBio MS Spent Media column (Agilent Technologies) with a rapid 7-minute gradient. Detection was carried out using an Orbitrap QExactive MS with data-dependent acquisition. Data analysis showed distinct metabolic signatures within the samples cultivated under supported microgravity conditions. Notably, these samples exhibited higher levels of amino acids, indicating an enhanced metabolic activity and growth rate under these conditions. This provides further insights into the adaptation of filamentous fungi in space environments, with promising implications for food production and other space-related applications.

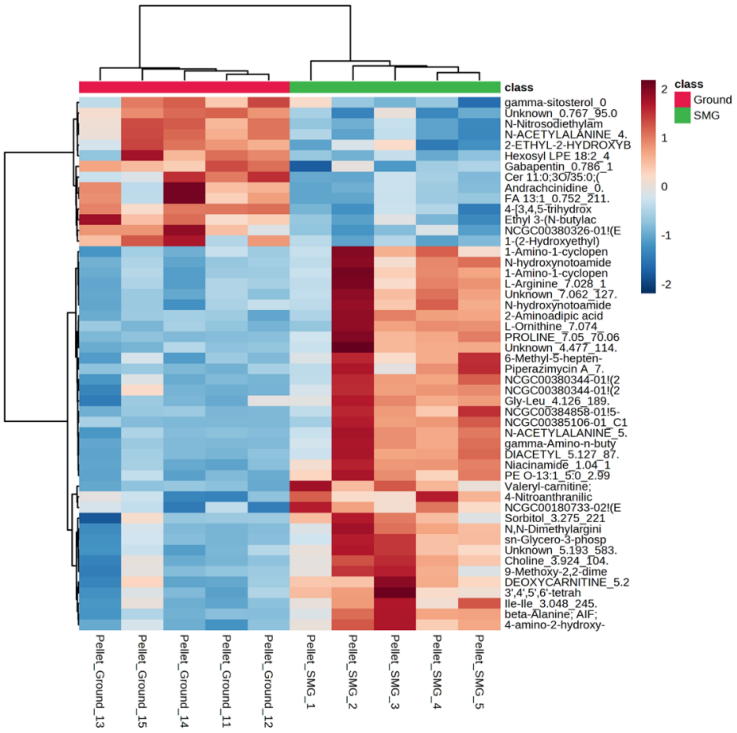


Fig. 1: Heatmap of the two different *A. Niger* samples grown under supported microgravity (SMG) and normal conditions (ground)

Collaborative Project – Project Partner: Prof. Dr. Ralf Möller and Dr. Marta Cortesao (Aerospace Microbiology Research Group, Institute of Aerospace Medicine, German Aerospace Center- DLR)

Application of Bioinfomatics to support data analysis in metabolomics and lipidomics

Jaqueline Leddin, Jonas Rösler, Constantin Krempe, Sven W. Meckelmann

Processing of data is often algorithmic, since different measurements are performed using just one experimental set-up, usually consisting of several, repetitive steps. The use of programs such as Excel has the advantage that the data can be easily processed. However, processing datasets with thousands of entries takes time (and can be tiresome), even when a basic familiarty with Excel is given. Therefore, programming languages are used to automate the processing of data within minutes instead of hours. There exist many open-source programming languages, e.g. *C, C++, Java, Python* or *R*. In our research group, mainly Python and R are used for data processing as well as statistical data analysis in different projects.

For example, in order to set up our own metabolomics library, the mass spectrometric data of fragments of hundreds of compounds was recorded unsing LC-Orbitrap-MS and preprocessed with Excel. The sheets contained the *m/z* value and relative intensity of all the fragments as well as the *m/z* value, retention time and adduct type of the corresponding precursor ions. For the construction of the library, the textfile of the values had to be brought into a predefined format, and missing items of the compound, like SMILES and InChIKey, had to be added. The developed python script reads out every sheet of the excel file and creates a textfile with the values for the database items. Regarding SMILES and InChIKey, the script automatically searches for the missing information in the database *ChemSpider* and adds them to the text file. Applying this python script, 67 Excel sheets were processed within a single minute. For postprocessing of MS/MS data from *MSDail*, functions were implemented in R. One function creates plots as an overview of the quality of the dataset so that the user can define thresholds for filtering out data with less quality. In the next step, the dataset can be processed for principal component analysis with R and the results are stored automatically in presentation slides. The whole process takes only several minutes per dataset which contained roughly twenty thousand signals before the filtering step.

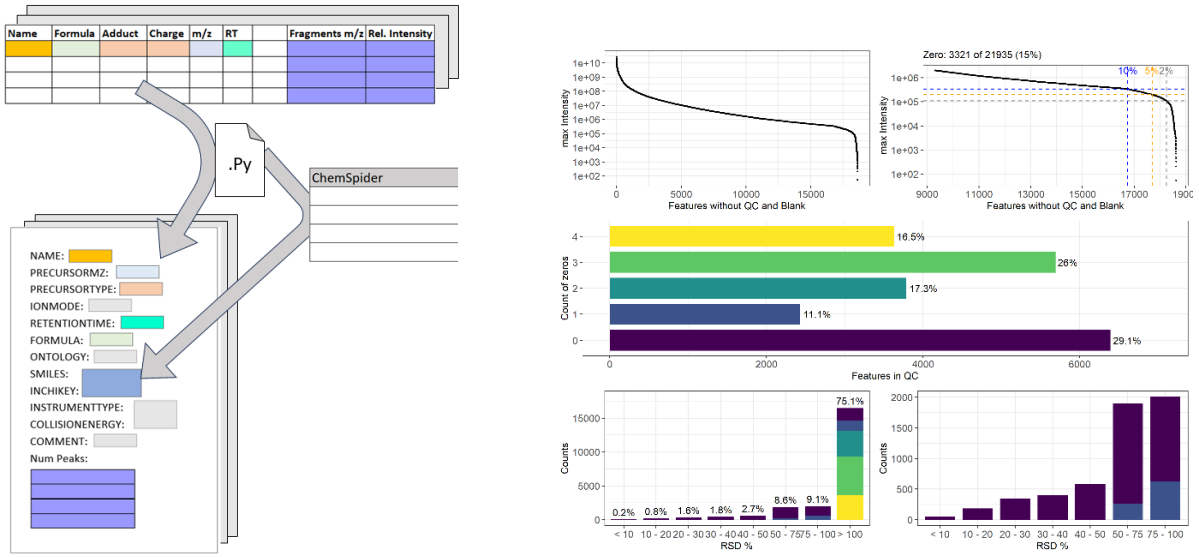


Fig. 1: Left: A Python script writes data from Excel sheets and an external database into a specific text file. Right: An overview of raw data to define thresholds for filtering out data with less quality created with R.

Inorganic examination of Mars similar deposit from Lüneburg Sole by ICP-OES for space research

Yassine Oulad El Majdoub, Leonie Nufer, Sven W. Meckelmann

In collaboration with the German Aerospace Center (DLR) which has brought back to life bacteria and archaea that had been preserved in the Lüneburg salt for 250 million years. Similar salt deposits had been found on Mars, which would indicate some possible life on such planet. Hence, the inorganic content of Lüneburg Sole is qualitatively and quantitativly characterized using ICP-OES.
The Lüneburg Sole sample was diluted with 18.5 MΩ deonized water containing 2% of Nitric acid and subjected to a qualitative screening to investigate the overall composition of the sample. The quantification of the detected inorganic elements was carried out using a multielement standard followed by a determination of the optimimal conditions in terms of suitable configuration of wavelength acquisition (radial, axial, synchronous vertical dual view), sample flow rate, etc.
Eight inorganic elements were detected, where only seven were quantified (Fig. 1). Sulfur, which is not quantified due to the unavailbility of the standard, and the following three components were considered as major elements present in high concentration, Na (80.32 g/L), followed by K (4.46 g/L) and Mg (3.32 g/L). Moreover, four minor elements were also detected in Lüneburg Sole sample with the following concentrations, Ca (660.83 mg/L), Cu (56.50 mg/L), B (24.40 mg/L), and Fe (0.27 mg/L).
The inorganic investigation would help to understand the optimal medium in which the bacteria and archaea was preserved for such long time in Lüneburg salt. This would also open up the possibility for investigation, in the future when it will be possible to obtain rock samples from Mars, whether similar microorganisms could survive on extreme conditions of that planet.

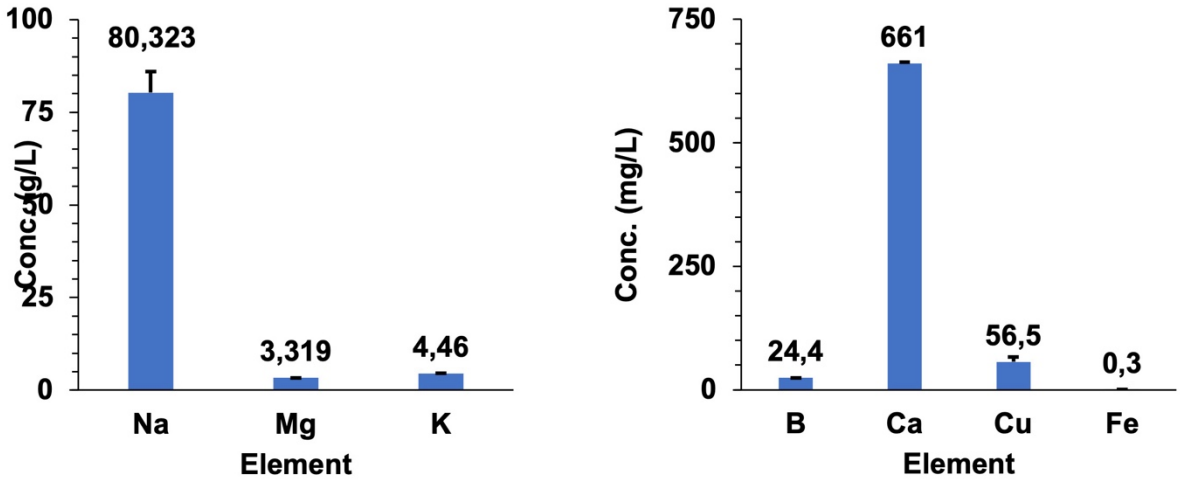


Fig. 1: Evaluation of inorganic content in Lüneburg Sole sample by ICP-OES

Collaborative Project – Project Partner: Prof. Dr. Ralf Möller and Dr. Marta Cortesao (Aerospace Microbiology Research Group, Institute of Aerospace Medicine, German Aerospace Center- DLR)

Semi-quantification of E&L found in polymeric materials of medical devices

Anneke Niehuus, Sarah Oßwald, Sascha Reinschmiedt, Denise Sievers, Sven W. Meckelmann

The determination of extractables and leachables (E&L) in polymeric materials has become imperative whenever a medical device is to be designed. Because of the potential adverse effects they can have on a patient's health, the presence of some E&L is restricted by various regulatories, such as the European Medical Device Regulation (MDR). Not only the identification is important but also a quantification is often demanded. The use of orthogonal analytical methods is indispensible though the focus during this project is on liquid chromatography coupled to a high resolution mass spectrometer (HPLC-HRMS). Direct quantification is often not feasible due to the huge variety of E&L and the lack of authentic reference standards since many standards - especially those which fall under the MDR restriction - are no longer produced or imported. Furthermore, E&L profiles consist of many unknowns, several of which are only tentatively identified and cannot be synthezised in the lab.

These obstacles can be partially overcome by performing a semi-quantification. During this project, different approaches of semi-quantifying analytes in pure solvents are compared to direct quantifications. The approach making use of a structurally similar internal standard yields errors of 0.42 x to 4.2 x. The accuracy can be improved by using multiple internal standards with varying response factors though the selection of such proved to be practically challenging thus far. Compared to that, the use of an experimental relative response factor (RRF) database leads to quantification errors of 0.52 x to 1.3 x. While the latter approach may lead to a more accurate quantification, it still requires reference standards to be measured upfront. A solution to this can be a regression model with which RRFs can be predicted if certain physicochemical properties of the analytes are known. So far, a model using multiple linear regression (MLR) has been established which takes into account the molecular mass, the octanol-water partition coefficient as well as the pK_a of the analytes. Preliminary results show deviations between the predicted and the experimental RRFs ranging from 0.14 x to 1.3 x. With further development, this model might have potential in the non-targeted analysis and the semi-quantification of E&L (see Fig. 1). In the next step, other regression models besides the MLR will be tested. Furthermore, the different semi-quantification approaches need to be extended to more subgroups of analytes and must be validated in commonly used polymer matrices and for real-life samples.

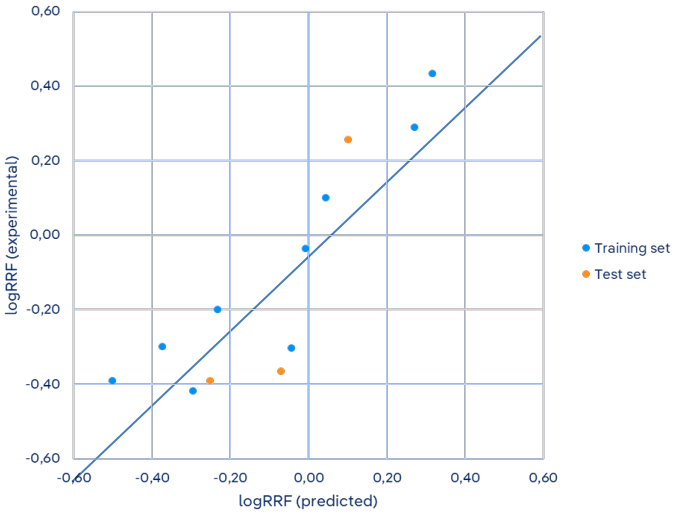


Fig. 1: The predicted RRF is plotted against the experimentally determined RRF; the training set (blue) was used to develop the model while the test set (orange) was used to validate it.

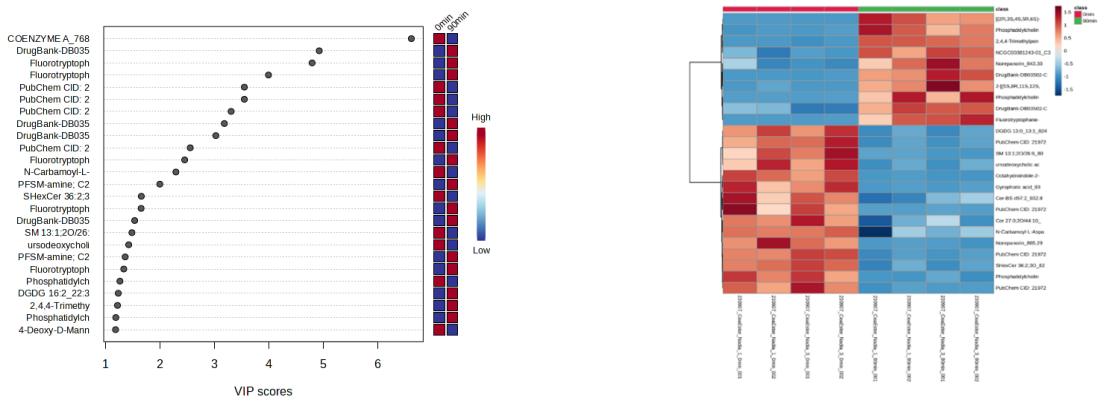
Collaborative Project – Project Partner: Drägerwerk AG & Co. KGaA, Lübeck

Anaerobic degradation of the three-ring polycyclic aromatic hydrocarbon phenanthrene, from lab to field

Nadia A. A. Samak, Marvin Häßler, Sven W. Meckelmann, Rainer U. Meckenstock

Polycyclic aromatic hydrocarbons (PAHs) threaten human lives, since they are extremely toxic and carcinogenic, and accumulate in the food chain. They reach the environment through oil spills, gasoline, domestic heaters, or any incomplete combustion processes. The European Environment Agency (EEA) identified PAHs as one of the priority pollutants in the Water Framework Directive (WFD), particularly in areas near industrial facilities and heavily trafficked roads. Moreover, it was found that PAH contamination in German groundwaters is prevalent, posing risks to both human health and the environment. Phenanthrene is a frequently found PAH and considered as priority pollutant by the World Health Organization. Due to their low bioavailability, PAHs are recalcitrant and accumulate especially in anoxic sediments where degradation rates are extremely slow. However, the hydrophobicity and strong adsorption to sediments makes it is difficult to assess the biodegradation of PAHs in the environment. Hence, an often-applied technology to assess biodegradation of PAHs is the detection of signature metabolites which requires a profound knowledge on the degradation pathways to identify unique metabolites. Signature metabolites can be extracted from ground-water or sediments and are used to qualitatively assess the presence of anaerobic PAH-degradation with LC-MS or GC-MS. Hence, the fundamental knowledge on the degradation pathway can be used as an assessment tool for engineers and consultants to evaluate the degradation processes on contaminated sites. However, since anaerobic PAH degradation has hardly been investigated, it is of general interest whether such important hazardous compounds can be degraded at all and under which environmental conditions. High-resolution mass spectrometry (HRMS) will help clarifying the degradation of various compounds. By comparing the samples at 0 and 90 minutes incubation time, as shown in Figure 1, the metabolites of the anaerobic degradation pathway of phenanthrene could be identified. The detection of metabolites, such as acetyl-CoA, confirms the degradation taking place. This discovery indicates that the phenanthrene degradation pathway is reflected in the biochemical principles of anaerobic naphthalene degradation.

Fig. 1: PLSDA and heatmap of the 25 most important features differences between 0 and 90 min



Collaborative Project – Project Partner: Prof. Rainer Meckenstock

Elucidating fatty acid double bond positions through GC-APCI-MS and in-source fragmentation patterns

Paul E. Görs, Sven W. Meckelmann

The positional arrangement of double bonds in unsaturated fatty acids plays a crucial role for their biological effects. Characterizing them analytically remains a challenge. The ionization of unsaturated fatty acids through gas chromatography-atmospheric pressure chemical ionization (GC-APCI) produces regiospecific in-source fragment ions, offering method to locate the double bond position. These fragment ions are primarily oxidized species that predominantly originate from the double bond nearest to the carboxylic acid group. This effect can be further enhanced by introducing benzaldehyde as a gas-phase reactant. Such a technique facilitates the identification of the Δ-notation of the fatty acid, and when combined with additional data such as mass-to-charge ratio (m/z) and retention time, it enables the accurate annotation of the corresponding fatty acid.

This method also streamlines the quantification of fatty acids in a single step, characterized by both high selectivity and sensitivity. Additionally, it allows the identification of rare fatty acids in suspected target analyses, often not available as standards. The final method and the application was published in J. Am. Soc. Mass Spectrom. 2023, 34, 11, 2538–2546.

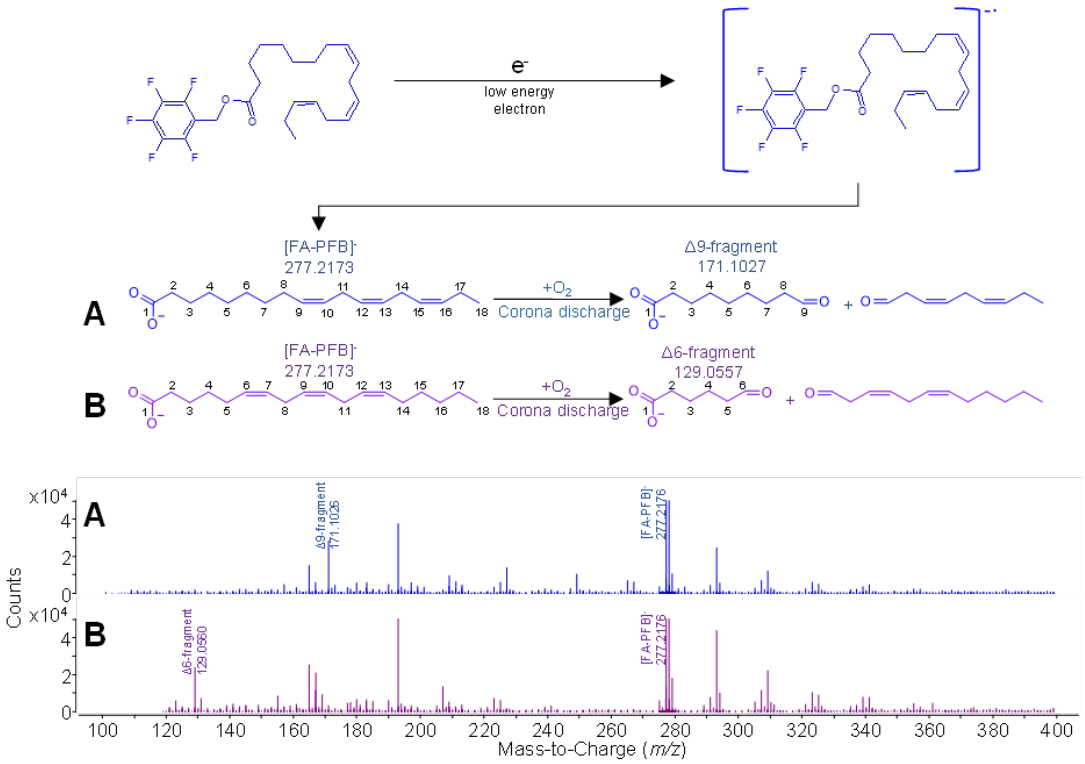


Fig. 1: Ionization of FA 18:3 after derivatization with PFB in alpha (ω3/Δ9) and gamma (ω6/Δ6) configuration. The mass spectra show the regiospecific in-source fragment ion m/z 171.1027 for alpha (A) and m/z 129.0557 for gamma (B), allowing to determine the position of the first double bond.

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

Metabolic labeling reveals fatty acid presence and biosynthesis in archaea

Paul E. Görs, Sven W. Meckelmann

Fatty acids (FAs) play vital roles in biological systems in eukaryotes and bacteria. However, in archaea, while the presence of FAs has been suggested, conclusive proof through metabolic labeling using ^{13}C-labeled substrates was not performed. Given the anticipated low concentrations of FAs in archaea, we developed a sensitive analytical method [1]. This GC-method involved the derivatization of FAs with pentafluorobenzyl bromide (PFB) followed by ionization using atmospheric pressure chemical ionization (APCI). Our project partners grew the Archaea on ^{13}C-labeled glycerol as the exclusive carbon source. The ^{13}C-labeling enabled a clear distinction between FAs produced by the archaea and potential contaminants, facilitating the sensitive analysis of archaeal fatty acids. In Figure 1, EICs of the fatty acids FA 8:0 (A), FA 10:0 (B), and FA 12:0 (C) following metabolic labeling are shown confirming the presence of these fatty acids.

Additionally, a knock-out experiment was conducted targeting a gene associated with the proposed FA biosynthesis pathway. The results indicate that the knock-out mutant contains a similar quantity of FAs compared to control samples but fewer FAs than the previously examined wild-type MW00G. This study provides new insights on the presence and biosynthesis of FAs in archaea, offering valuable informations regarding their metabolic processes.

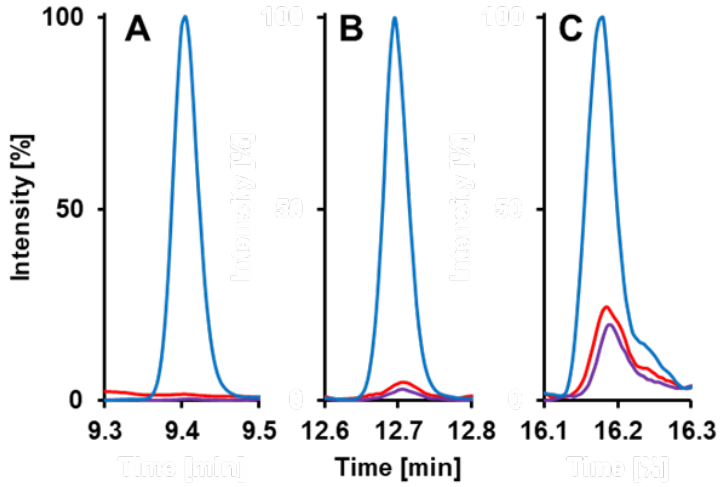


Fig. 1: Metabolic labeled ^{13}C fatty acids found in Archaea MW00G using ^{12}C-labeled substrate (red), ^{13}C-labeled substrate (blue) as well as in a knock-out mutant grown on ^{13}C-labeled substrate (violet). The cells were hydrolyzed, extractedwith MTBE], and measured by GC-APCI-QqQ-MS. The amount of ^{13}C fatty acids found in the wild type MW00G (A: FA 8:0, B: FA 10:0, C: FA 12:0) is increased compared to the control samples grown on a ^{12}C-labeled substrate, and the knock-out mutant grown on a ^{13}C-labeled substrate.

[1] P.E. Görs, et al., Anal. Bioanal. Chem. 414.22 (2022): 6621-6634.

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

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

Lipidomics analysis of YUMM cells reveals mechanism of ferroptosis

Jonas Rösler, Sven W. Meckelmann, Alpaslan Tasdogan

Lipidomics is besides metabolomics and proteomics a major analytical approach in life science research. The lipidome is not just crucial for membrane formation of cells, but also heavily involved into catabolic, anabolic and redox processes in the human metabolome.

As the other Omics technologies lipids require a specialized workflow for their analysis. Although the polarity of lipids is in general more unpolar compared to other metabolites covered with metabolomic approaches, a wide polarity range must still be covered in sample preparation and separation.

In this work a lipidomics workflow based on a former developed and published separation method by our group[1] was developed and optimized for a qExactive Orbitrap mass spectrometer. This setup was applied for the analysis of clinical samples and showed good sensitivity, repeatability, and coverage of the lipidome.

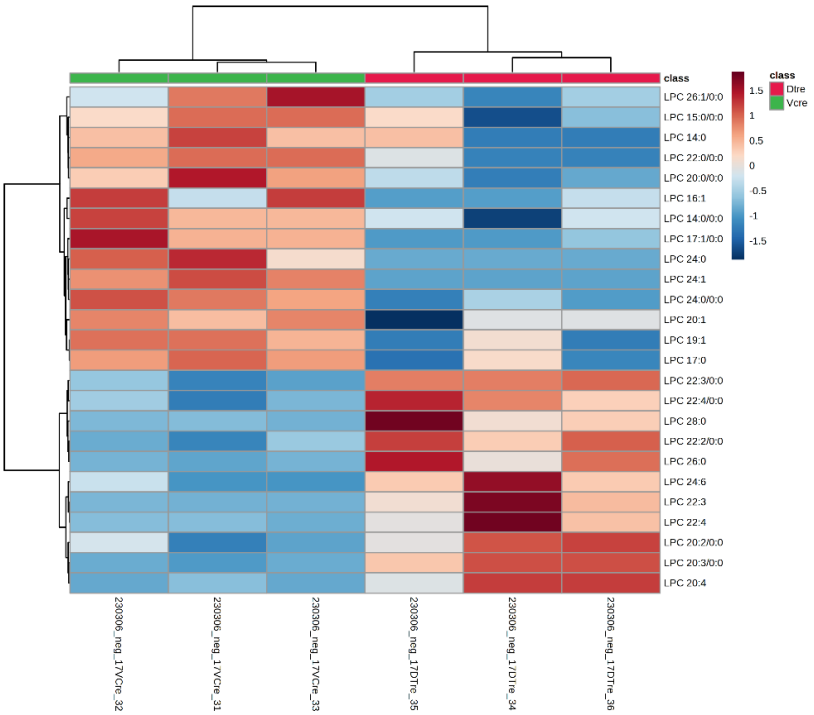


Fig. 1: Heatmap of the two different treatment strategies for LPCs, showing the upregulation of polyunsaturated lipids in the DT samples.

The data is clearly showing a strong influence of different treatment strategies on the lipidome and enables to evaluate the corresponding effects. Most importantly it was possible to show the accumulation of polyunsaturated lipids as depicted (Fig. 1) for the LPCs depending on the used treatment strategy. This data was the final indication for the correlation of the investigated phenotype to the process of ferroptosis, which could be approved in later experiments.

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[1]: K. Tötsch, J. C. Fjeldsted, S. M. Stow, O. J. Schmitz, S. W. Meckelmann, *J. Am. Mass Spectrom.* **2021**,*32*, 10.

Collaborative Project – Project Partner: Prof. Jonathan P. Sleeman (Medical Faculty, University of Heidelberg)

Funding: 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)

Use of different extraction methods coupled with thermal desorption-gas chromatography / Q-TOF mass spectrometry to analyse polycyclic aromatic hydrocarbons

Isil Gazioglu

Polycyclic aromatic hydrocarbons (PAHs) are a class of chemicals that occur naturally in coal, crude oil, and gasoline. They are also produced when coal, oil, gas, wood, garbage, and tobacco are burned. PAHs generated from these sources can bind to or form small particles in the air. This project provides the sensitive determination of PAHs.

For the determination of the PAHs, three different micro-extraction devices have been applied. These devices were a novel sol-gel phenyl/methyl/poly(dimethylsiloxane) sorbent coating which was created on polyester fabric substrate for fabric phase sorptive extraction (FPSE), DVB/ PDMS poly(divinylbenzene) film, and HLB (Hydrophilic-Lipophilic Balance) film. When the analytical performances of the devices evaluated, DVB microextraction film showed the highest adsorption capacity against to PAHs. DVB/PDMS applied as extraction device to monitor human exposure to selected polycyclic aromatic hydrocarbons (PAHs) including naphthalene, acenapthene, phenanthrene, anthrecene, flourenthene, benzo(a)anthracene, chrysene, benzo(k)flourenthene, and benzo[a]pyrene by using TD-GC-qTOF-MS (Fig.1). With this method, analysis of PAHs on DVB/PDMS was possible with direct desorption. Factors that influence the extraction efficiency, such as extraction temperature, extraction time, desorption temperature and time were optimized. Quantification methods of major PAHs were established, and a good linearity ($r > 0.998$) was obtained, with detection limits in the range of 2-56 ng/L. The new SPME fiber were successfully applied in the analysis of mussel samples. Compared to other devices DVB/PDMS films, the new material has higher extraction efficacy and higher precision. Hence, it is suitable for the determination of PAHs of various sources.

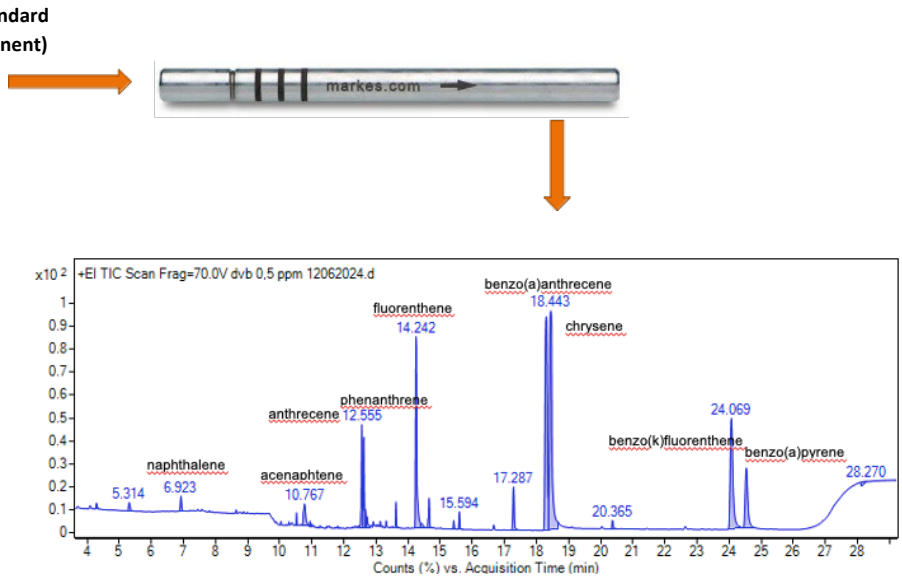

Fig.1: PAHs separation achieved after the optimization of thermal desorption by using GC-qTOF-MS.

Collaborative Project – Project Partner: Carlos Gil, Schauenburg Analytics and Prof. Janusz Pawliszyn (University of Waterloo, Canada)

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

Sarah Klaus, Florian Stappert, Sven W. Meckelmann

Sample preparation is an important step in the chemical analysis of metabolites, which requires a high level of precision and accuracy to achieve comparable and reliable results. To ensure the reproducibility, automatization is desirable to reduce inaccuracies and measurement errors to a minimum. The aim of this project is to develop such an automated sample preparation that enables analysis using gas chromatography - mass spectrometry (GC-MS). The analytes to be investigated are polar metabolites with a special focus on organic acids (for example pyruvic acid or aspartic acid), which are known as biomarkers for a wide range of physiological and pathological conditions.

Based on an established sample preparation, a derivatization with *N*,*O*-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was manually performed and optimized for a mixture of standard substances. Subsequently, the substance mixture was extended by further metabolites of interest, which were to be made accessible for GC-MS experiments by adding an additional methoximation step. The developed method enables the analysis of a wide range of polar metabolites by GC-MS, as shown in Fig. 1. In addition, the effect of varying the measurement conditions and the various sample preparation parameters could be determined.

In the next steps, the automatization of the sample preparation with a MultiPurposeSampler (MPS) will be developed using the findings and experiences of the preliminary investigations. This includes recovery experiments to test and demonstrate the robustness and precision of the developed method. The finished method should be able to be applied to a large number of different organic samples with different matrices, such as plasma, cells, or organ tissue samples. Thereby a wide range of application can be achieved, which will also demonstrated with real samples.

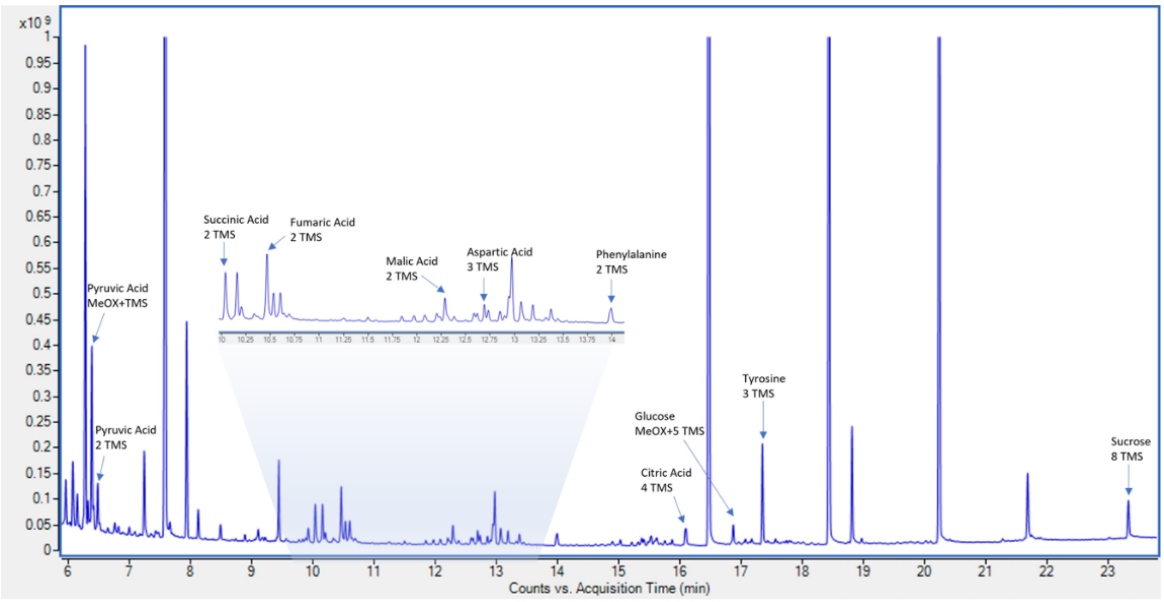

Fig. 1: Exemplary GC-MS spectrum of polar metabolites after manual sample preparation (derivatization)

Derivatization of non-aromatic compounds with ionization marker for GC-APLI-MS

Ling Tang, Florian Uteschil

Atmospheric pressure ionization (API) techniques mainly include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI), which are applied for the analysis of polar, moderately polar and non-polar compounds, respectively. In 2005, our group, together with Prof. Benter´s at University of Wuppertal, developed a novel ionization method, atmospheric-pressure laser ionization (APLI), which enables high selectivity and high sensitivity detection of non- to low-polarity aromatic compounds and is a powerful complement to existing atmospheric pressure (AP) ionization techniques.

The increasingly ambitious tasks faced in analytical chemistry placed high demands on instrument hardware, so the development of orthogonal techniques has attracted the keen attention of scientists. Mass spectrometers (MS) have become the most important group of detectors for the analysis of organic compounds in complex matrices. After the coupling of APLI and time-of-flight mass spectrometry (TOF MS) was first realized in 2005, the hyphenation of APLI with Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) and ion mobility mass spectrometry (IMS) have been successively developed. In order to simple sample preparation and reduce ion discrimination in complex mixtures, chromatographic separation techniques, high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) were coupled with APLI-MS.

Ion trap (IT) mass spectrometry is playing a significantly increasing role in modern chemical analysis. Its inherent high sensitivity and specificity facilitate many applications, including threat detection, investigation of chemical and biochemical systems, illicit drug identification, planetary exploration, and environmental monitoring. But there is currently no report on the coupling of APLI and ion traps. Here, the hyphenation of ion trap mass spectrometry and GC-APLI is reported at the first time and the schematic and photograph of this construction is shown in Fig. 1. To detect non-aromatic compounds, some ionization markers were used to address this problem. In future, this method will be applied for the identification of some real sample.

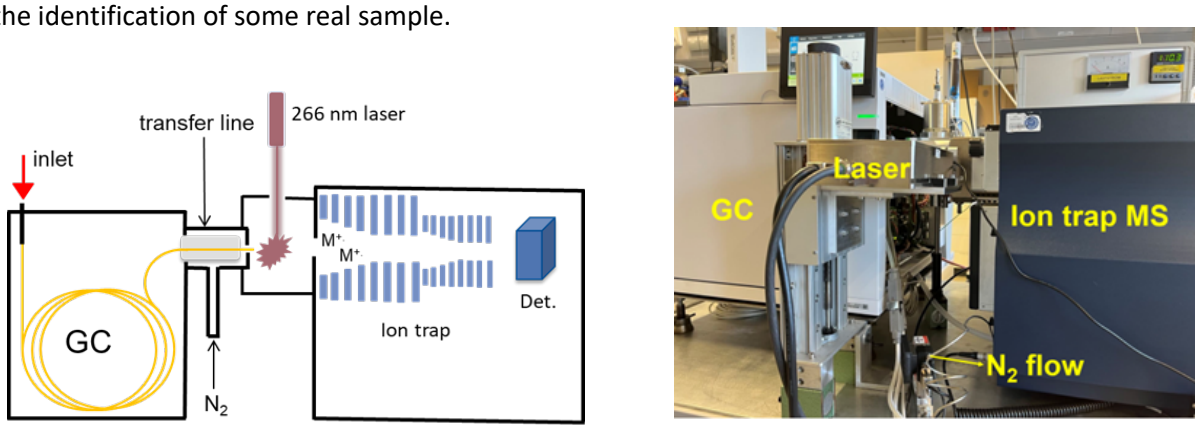

Fig. 1: Schematic of GC–APLI–(Ion trap) MS (left). Photograph of the entire GC–APLI–(Ion trap) MS setup (right).

Funded by: International cooperation training project for innovative talents" from China Scholarship Council (CSC)

Development in the novel design of the housing for a multi-ion-source

Marvin Häßler, Florian Uteschil, Juan Ayala Cabrera

The analysis of differently polar substances within complex plant metabolomes is the focus of various applications in plant research. The efficient analysis of these substances requires the ability to differentially ionize them. This requires the combination of different ionization sources, in particular the combination of electrospray ionization (ESI) with atmospheric pressure chemical ionization (APCI) or tube plasma ionization (TPI). Tube plasma ionization in mass spectrometry is crucial for analyzing less polar compounds due to its ability to ionize a wider range of compounds compared to APCI or APPI. This makes TPI a powerful tool for the analysis of less polar compounds, which are often difficult to ionize using other methods. In this Project a housing for a dual-ion source was conceptualized and fabricated. A housing was designed that allows the simultaneous use and evaluation of different ionization sources. This approach makes it possible to take advantage of different ionization methods and create optimal conditions for the analysis of different substances within the plant metabolome. The housing for the dual ion source has been successfully developed and the performance of the new housing is being evaluated with different standard solutions. The new housing will allow the combination of ESI with APCI, APPI or TPI in a flexible way.

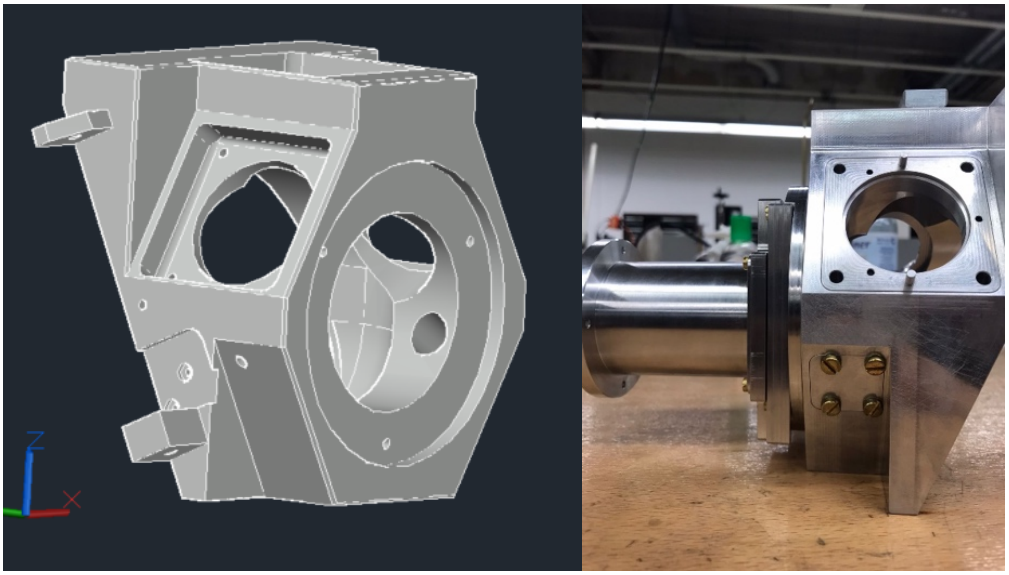

Fig. 1: 3D model of the finished Ion source created in AutoCAD and the finished product crated by the workshop

We plan to further optimize the housing and potentially make improvements to enhance the performance of the ionization sources. Additionally, further experiments will be conducted to assess the practical applicability of the housing in various analytical applications. The final goal is the coupling of the optimized dual-ion source to a multi-^{2}D LCxLC method.

Collaborative Project – Project Partner: Agilent Technologies (Santa Clara, USA), Katharina Wetzel (University Duisburg-Essen, Essen, Germany)

Funded by: Deutsche Forschungsgemeinschaft (DFG) – Projectnumber 504370143

Potential of tube plasma ionization for the determination of estrogenic compounds

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

Estrogens, along with androgens, progestogens and stilbestrols, are steroid compounds that can be used to promote growth in animals. This use of growth promoting substances in livestock is prohibited in the European Union by Regulation 96/22/EC due to the adverse influence of these steroid compounds interfering with the human hormonal system. Therefore, reliable and powerful analytical methodologies are necessary to determine growth promotors in various biological and food matrices. These methodologies usually involve chromatographic separation by either gas chromatography (GC) or liquid chromatography (LC) followed by tandem mass spectrometry (MS/MS) detection. However, these methods have drawbacks such as the necessary derivatisation and high in-source fragmentation in electron ionization (EI) for the GC-MS methodologies or the ion suppression and lower performance observed using electrospray ionization (ESI) in LC-MS approaches. Therefore, considering the analytical issues on the analysis of growth promoters, tube plasma ionization (TPI) could offer a novel approach to overcome these analytical challenges. In this project, plasma, source, and acquisition parameters were optimised for both LC-TPI-MS/MS and GC-TPI-MS/MS and quality parameters for these methodologies were evaluated. For LC determinations, the TPI approach was compared to electrospray ionization (ESI) as well as atmospheric pressure chemical ionization (APCI). TPI offered instrumental detection limits (iLOD) similar to APCI and significantly lower than those achieved by ESI in a 0.007 – 5.085 µg L^{-1} range. Additionally, TPI was capable of detecting more analytes that both APCI and ESI. Linearity was comparable with all ion sources whilst APCI offered better sensitivity than TPI. For precision and trueness, TPI and APCI achieved low relative standard deviations and relative errors and showed increased performance compared to ESI. Furthermore, the influence of matrix (bovine urine and meat) on the measurements of TPI and ESI was studied. These experiments showed a decreased matrix effect (40 % lower) in TPI compared to ESI. In addition to the LC-MS approach, a GC-MS approach was evaluated. This included the derivatisation with N-methyl-N-(trimethylsilyl)trifluoracetamid (MSTFA) and subsequent GC separation of the analytes. The GC-MS approach whilst having the downside of necessary derivatisation offers shorter analysis time than LC-MS methods and lower detection limits (0.009 – 3.460 µg L^{-1}). The evaluated quality parameters of this method show promising results with high linearity and sensitivity. Precision and trueness are acceptable but slightly higher than in LC-MS.

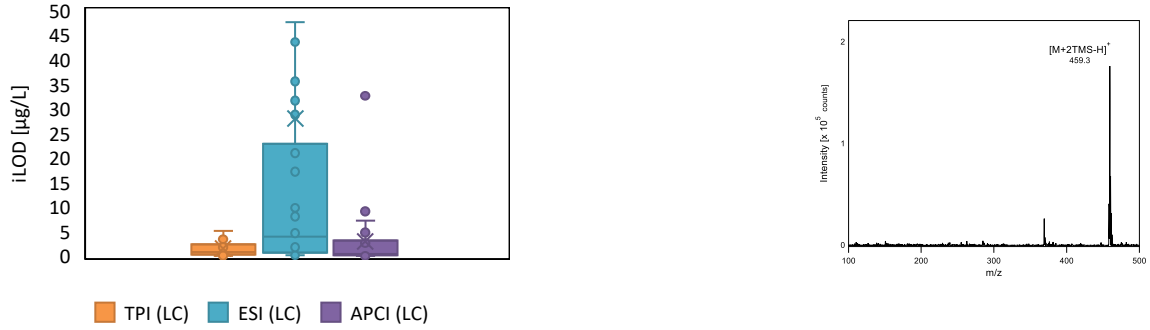

Fig. 1: Comparison of iLODs with different ion sources in LC-MS measurements (left). Mass spectrum of progesterone showcasing low in-source fragmentation of the derivatised analyte in GC-MS (right).

Collaborative Project – Project Partner: Marco Blokland und Ane Arrizabalaga-Larrañaga, Wageningen Food Safety Research, Wageningen, The Netherlands

Evaluation and characterization of LC-QqQ-MS using ESI, APCI and TPI as ion sources for the analysis of sterols

Pia Wittenhofer, Sven W. Meckelmann

Characterization and validation are an important final step in the development of analytical method, ensuring their reliability and applicability. A method should have stable retention times, minimal ion suppression, and consistent results, especially when confronted with high matrix loads. In this study, we employed liquid chromatography coupled with triple quadrupole mass spectrometry for the analysis of sterols in complex biological samples and compared its performance using electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and tube plasma ionization (TPI). To assess ion suppression, we introduced a T-piece after the separation column, enabling the addition of an internal standard via a syringe pump. Considering matrix effects and the consistency of results, APCI stands out as the best choice showing minimal signal suppression. The TPI source also demonstrates comparable performance, while ESI experiences significant signal degradation. For further comparison, a method characterization according to the ICH guidelines (International Conference on Harmonisation) was performed. The results support the previous data on ion suppression showing a good performance for APCI and TPI for sterol analysis while ESI provides much higher limits of detection/quantification ranging from 300 to 1000 nM. The low detection/quantification limits for the APCI and TPI sources in the lower nM-range emphasize the suitability of these techniques for sterol analysis under high matrix conditions.

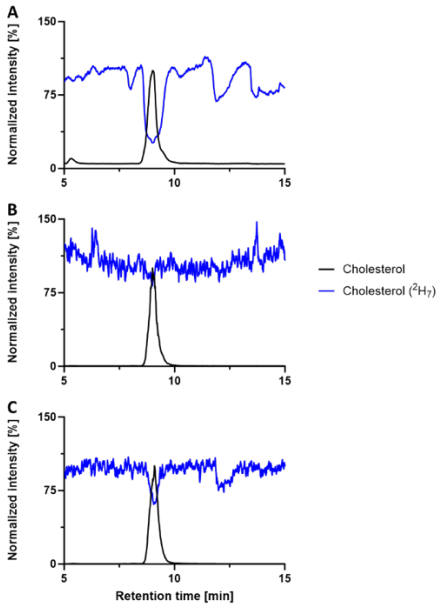

Table 1: LOD, LOQ and coefficient of regression for different sterols

		APCI	TPI	ESI
LOD [nM]	Zymosterol	10	30	1000
	Desmosterol	10	30	300
	Cholesterol	10	10	1000
LOQ [nM]	Zymosterol	30	100	1000
	Desmosterol	30	100	1000
	Cholesterol	30	30	3000
R^2	Zymosterol	0.997	0.9634	0.9455
	Desmosterol	0.9521	0.9612	0.9787
	Cholesterol	0.973	0.9645	0.9897

Fig. 1: LC-MS/MS analysis of human plasma samples. Chromatographic separation was carried out using a Kinetex PFP-column (1.7 µm, 30 mm x 2.1 mm, 1D). To monitor the ion suppression, deuterated cholesterol was spiked into the eluent stream before the ion source. The ESI (**A**) source is extremly susceptible to ion suppression which can be recognized by the large signal drops during the analysis. APCI (**B**) and TPI (**C**) are less susceptible to ion suppression effects and provide a relatively stabile signal throughout the analysis which is advantageous for quantification.

Collaborative Project – Project Partner: Prof. Barbara M. Grüner, (Cell Plasticity and Metastasis, University Hospital Essen, Germany); Prof. Annette Paschen (Molekulare Tumorimmunologie, University Hospital Essen, Germany)

Funded by: Deutsche Forschungsgemeinschaft (DFG) - ME 5800/1-1 and SCHM 1699/30-1

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

Jonas Rösler, Florian Uteschil, Sven W. Meckelmann, Alpaslan Tasdogan

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

This project is aiming to develop a novel ion source for single cell mass spectrometry, using ultrasonic nebulization for instant cell lysis and sample vaporization in combination with low temperature plasma ionization for efficient ionization prior to detection by high resolution mass spectrometry. With the current design it was possible to show extraordinary high sensitivity e.g. for forensic analytes even below one femtomol in source and furtheron we were able verify the function of the ultrasonic sampling system for cell lysis. Using this technology we were able to measure the mass of spermidine in living human cancer cells and to show a linear correlation with the expected cell counts down to even one single cell as depicted below. By experimental design it was also possible to proof, that these signals originate from the measured cells and that they are neither an artifact of the cell cultivation medium nor the used analytical materials.

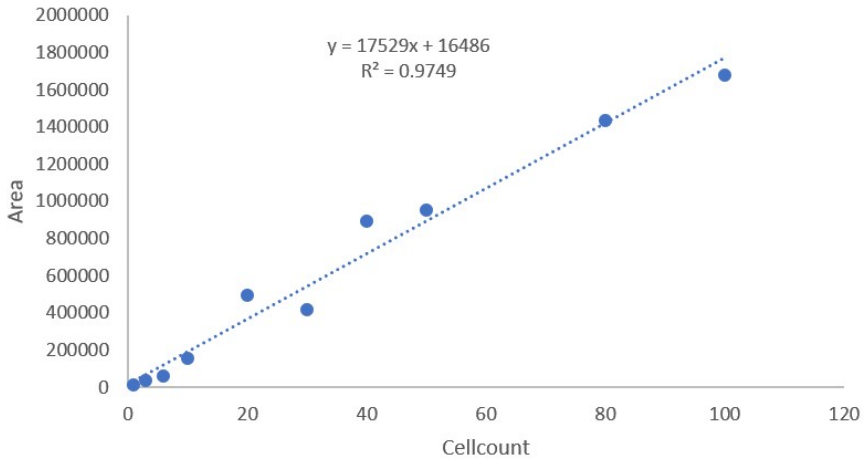

Fig. 1: Calibration curve of the measured signal of spermidine in living lung cancer cells correlated to the calculated cell count measured.

By demonstrating the sensitivity and ability to lyse cells, we have provided proof of principle for single cell analysis with our system. Right now the project focuses on the design and crafting of an improved prototype to further enhance the sensitivity and handling of the system. With this we are expecting our prototype to prove as an efficient and accurate device for single cell analysis.

Funding: 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)

Ultrasonic assisted low temperature plasma ionization for direct sweat analysis

Jonas Rösler, Christopher Jaeger

Direct analysis of samples is highly desired for applications in drug screening or routine controls, e.g., competitive sports or in security control. However, one of the major challenges in analytical chemistry is to carry out sample preparation, acquisition, data analysis and evaluation within a few minutes. Most available designs lack either selectivity or sensitivity in order to be used for economic application. Therefore this project aims to test and establish a self made prototype based on ultrasonic assisted sample injection combined with high resolution mass spectrometry for the direct analysis of liquid samples. Previous obtained results indicate an extraordinary high sensitivity for medium polar analytes as metamphetamine or caffeine and the total analysis time is about ten seconds per sample. As the device can work directly with organic solvents like methanol or ethanol it is going to be used for the analysis of human sweat, which can be easily extracted with ethanol water mixtures. For sweat extraction we will use “skin-wash”, an easily accessible device published by Malà et al. in 2021[1], which is capable to collect human sweat samples within two to five minutes.

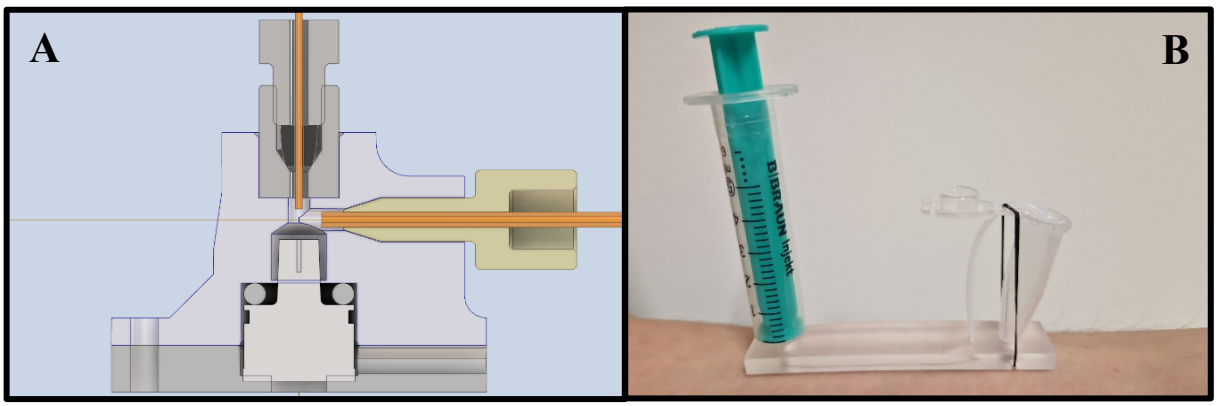

Fig. 1: Scheme of the ultrasonic nebulizer for liquid sampling in the DBDI ion source (A); sweat sampling device (B). The analysis is done using a Q Exactive Orbitrap mass spectrometer.

The combination of the ultrasonic assisted low temperature plasma ion source and the sweat extraction device is expected to offer simple handling, a total analysis time under five minutes and comparably low costs per measurement. Due to the good applicability of plasma ion sources for medium to non polar analytes, we expect high sensitivities for the volatile components in sweat as previously seen e.g. for caffeine.[2]

Currently, a variety of metabolic standards is being analyzed in order to evaluate the sensitivity of the setup. Further, the extraction of sweat sampling is being optimized. At last, the setup will be used to evaluate the influence of nutrition on the detectable sweat metabolites.

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[1]: M. Malà, P. Itterheimovà, L. Homola, J. Vinohradskà, P. Kubàn, *Separations* ***2021***, *8(12)*, 234.

[2]: J. Brunmair, M. Gotsmy, L. Niederstaetter, B. Neudischko, A. Bileck, A. Slany, M. L. Feuerstein, C. Langbauer, L. Janker, J. Zanghellini, S. M. Meier-Menches, C. Gerner, *Nature Communications* ***2021***, *12(1)*, 5993.

Collaborative Project – Project Partner: Prof. Petr Kubán (CEITEC Masaryk University, Czech Republic)

Improvement of the detection capabilities by inserting a repeller electrode into an electrospray ionization ion source housing

Alexandra Pape, Florian Uteschil, Florian Stappert

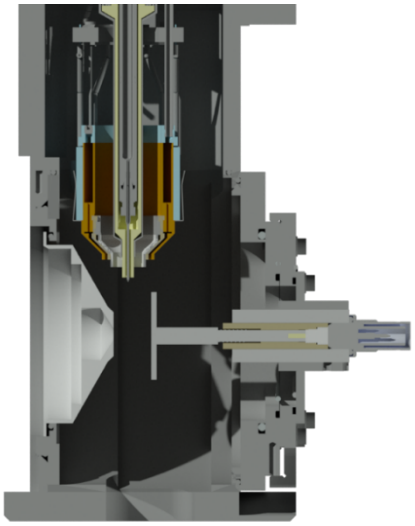

Fig. 1: Round, flat repeller inserted into the ion source housing.

In a cooperation project with Hitachi High-Tech, a method to improve the detection capabilities of an electrospray ionization (ESI) ion source was realized. The idea of positioning a repeller electrode, i.e. a piece of metal held at a defined electrical potential to create an electrical field which would push the analyte ions into the mass spectrometer inlet, was established (Fig.1). Initial experiments showed that the efficiency of the repeller depended on its shape, the applied voltage, its spatial position in the ion source housing, as well as the analyte solution flow rate and *m/z* of the analyte ions.

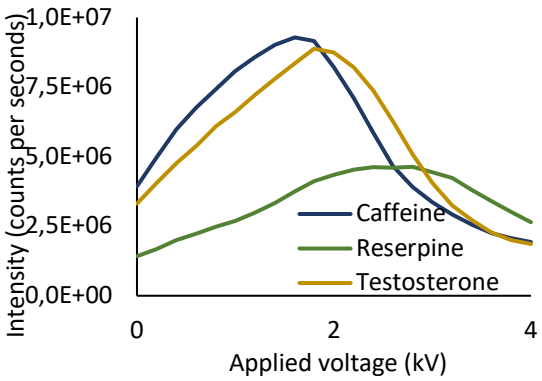

Fig. 2: Dependency of the averaged analyte peak height on the applied repeller voltage for a continuous injection of 35 µg/L caffeine and testosterone, and 25 µg/ L reserpine at 0.05 mL/ min. Measured with the rod-shaped repeller.

The best repellers were proven to be a rod of 4 mm diameter and a concave ball-shape of 12 mm diameter. In the first optimization experiments, analyte molecules were continuously sprayed and ionized via ESI, and spatial positioning and applied voltage were optimized to achieve increased signal heights of detected protonated molecule ions in the mass spectra (cf. Fig. 2). Since the applied voltage to the repeller and its spatial position were found to correlate with each other, a design of experiments was carried out to find the optimal instrumental parameters. With increasing analyte solution flow rate, the effects of the repeller lessened. The sensitvity gain for a real LC-MS application measuring the analyte molecules spiked into human plasma was limited. Nonetheless, two interesting discoveries were made regarding the implementation of the repeller: Analytes of higher *m/z* required higher voltages to reach their intensity maximum. Furthermore, applying no ionization voltage to the ESI capillary still created a potential difference between the capillary and the repeller, leading to a recordable mass spectrum and the detection of the analyte ions. However, even with only a nebulizer the repellers were capable of ionizing molecules on their own.

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

Pulsed electrospray ionization

Florian Uteschil

The demand on high throughput methods for LC-MS is a growing market in the analytical chemistry. Therefore, the our group is working on solutions to increase the method sensitivity of chromatographic analyses hyphenated with mass spectrometers. A special attention lies on the development of ion sources for the mass spectrometry. In this example an ion source should be created which is capable to ionize the analyte molecules with the presence of dynamic electromagnetic fields. This phenomenon is investigated on home-build micro(µ) and nano(n) electrospray ionization (µ/n-ESI) source setups. The challenge of this project is to create an ion source which is more sensitive comparable to conventional ESI ion sources, and works with sufficient sample throughput. However, the focus lies on the investigation of electrode configurations and its potential to increase the sensitivity. The electrical contact of the ESI emitter is investigated by means of liquid junction and induced ESI by applying the electrospray potential to an electrode which is placed as close as 2 mm to the ESI emitter tip (not shown).

Our first attempt to realize such a source is to pulse the ionization voltage of a home build ESI and verify, if the appearance of a *Taylor Cone* is visible at pulsed conditions. Therefore, a high voltage switching circuitry was realized in a setup that includes the electrospray emitter or an inducing electrode and a counter electrode, that simulates the front inlet of a mass spectrometer. Figure 1 presents pulsed ESI operations of pure methanol at ambient conditions. The electrospray was operated at 1.6 kV with pulse frequencies of 50 Hz at different pulse widths.

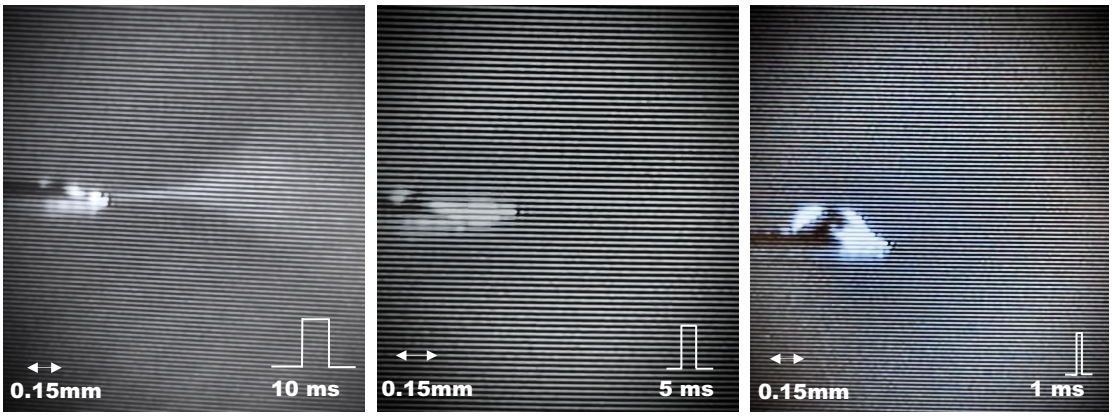

Fig. 1: Photographs of pulsed electrospray operations during infusion of 1 µL min^{-1} Methanol at 1.6 kV applied via liquid junction. The inserts show dimensions and applied pulse widths at 50 Hz pulse frequency.

The first experiments show working electrosprays when operated with pulsed ionization voltages. Especially the external control of applied voltage, frequency and pulse width contributes to important future applications such as single cell metabolome analysis.

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

Doctoral Thesis accomplished 2022

Dr. Claudia Lenzen

Development of a DIP-EESI-MS and the use of ambient ionization methods for the analysis of drugs

The present thesis is concerned with the development and optimisation of an ion source with a direct inlet system and based on electrospray ionization for the analysis of pharmaceuticals. Because the ionization mechanism of the new ion source is most likely based on the mechanism of extractive ESI, it is named DIP-EESI.

After optimisation of the source parameters, lower detection and quantification limits could be determined with the DIP-EESI-MS than with the DIP-APCI or DIP-APPI using pure substances that could be ionized well with the DIP-EESI. Only a low background level was generated by the ESI spray, resulting in a baseline level comparable to that observed with the DIP-APCI. The DIP-EESI can therefore be classified as having the same performance as the DIP-APCI. Comparable results were also obtained in comparison with related methods using the same ionization mechanism of the EESI described in the literature.

Based on the results obtained with the pure substances, the DIP-EESI could be applied for the analysis of pharmaceuticals.

In the analysis of pharmaceuticals, the DIP-EESI showed lower intensities of the active substances compared to the DIP-APCI due to the EESI mechanism. However, the DIP-EESI also shows a lower ionization of excipients, such as starch, stearate or povidone. This minimises interference from excipients and allows screening for unknown impurities and identification of known impurities.

Quantitative analysis of temperature-stable substances such as acetylsalicylic acid and screening for impurities is not possible with the DIP-EESI. However, the thermal decomposition products can be detected in situ and structurally elucidated.

With the DIP-EESI and the DIP-APCI, a rapid method could be developed to investigate the temperature-stable pharmaceuticals and to screen for unknown impurities. This method could be applied to all the pharmaceuticals examined without the need for time-consuming method development. However, the active ingredients of the tablets had to be dissolved because they were so well ionized that even with the smallest possible sample weight, the active ingredient could still be detected after the source had been heated up for several hours.

The DIP-EESI was used to examine tablets that had been halved and repackaged for patient-specific secondary blistering. These tablets were stored in their original or new packaging in a translucent plastic container in daylight without further precautions on the laboratory bench for up to 3 years.

Solutions of these tablets containing an amount of the active ingredient that could be analysed with DIP-EESI were compared with each other. Of nine substances investigated, a significant difference in

the concentration of active ingredient in the tablets was found for clonazepam and primidone between the original and repackaged tablets. The relative standard deviations for the assay of the active ingredients were on average 4.3 %.

In addition, screening for known impurities in the tablets was performed. The DIP-EESI is so sensitive that it can still detect these impurities in the commercially available tablets, even though their content is below the maximum permitted limit. An identification of the known impurities could be done via fragmentation reactions. The results obtained with DIP-EESI were compared with those from DIP-APCI and HPLC-ESI-MS investigations and partially confirmed.

Since the DIP-EESI is less susceptible to interference by auxiliary substances than the DIP-APCI, it was also possible to screen solutions with a high content of dissolved auxiliary substances for unknown impurities or conspicuous masses. Some of the unknown substances detected in the tablet solution could be separated via the temperature gradient of the DIP to such an extent that they could be fragmented individually from one another and examined. For example, with the DIP-EESI, five different substances could be detected in amlodipine tablets due to the separation, which would have been detected as one substance without temperature separation. A structure could be postulated for some of these substances. It was possible to develop a rapid procedure for the analysis of pharmaceuticals with the DIP-EESI and to demonstrate the potential of the method.

Dr. Dominic Mähler

Development of a capillary electrophoretic analysis method for the characterisation of cell-free enzymatic reactions in miniaturised production systems

This work describes the development of a miniaturized enzymatic reactor system that enables a simple *in vitro* biosynthesis of the building blocks MalCoA and AcCoA for the production of potentially antibiotically active substances within the natural product class of polyketides with time-resolved, automated and quantifying CE analytics in a proof-of-concept application. For this purpose, a CZE-UV/Vis analytical method was developed for the quantification of the nucleotides AMP, ADP and ATP and the coenzymes CoA, MalCoA and AcCoA in enzymatic reaction matrices, which requires minimal sample amounts without any sample preparation and enables time-efficient analyses. The measurement method was systematically optimized with respect to capillary pressure, capillary voltage, capillary temperature, type of buffer, buffer concentration and pH, so that the nucleotides, coenzymes as well as the internal standard cAMP were completely separated. Sequential multiple injections of enzymatic matrices were then performed, which caused a number of separation issues when using the developed analytical method. Adjustment of the capillary dimensions, injection parameters, and conditioning method restored the original separation efficiency. Subsequently, the method was validated based on the guidelines provided by the ICH for validation of analytical methods in terms of specificity, selectivity, accuracy, precision, linearity and sensitivity. The CZE-UV/Vis method was then used to evaluate the stabilities of the critical analytes CoA, MalCoA, AcCoA, and ATP. It was found that quantified dimerization of CoA in the enzymatic matrix could be avoided by the addition of TCEP. In addition, hydrolysis-induced instability of AcCoA and MalCoA was quantified. ATP, on the other hand, was proved to be stable under the same conditions. In the further course, the cell-free enzymes malonyl-CoA synthetase Y3K-deGFP MatB, malonyl-CoA decarboxylase Y3K-mRuby MatA, and citrate synthase Y3K-eBFP CitZ were evaluated with respect to specificity, kinetics, and applicability for the production of the CoA fatty acid esters CoA, MalCoA, and AcCoA. For this purpose, sequential multiple injections of single as well as multi-enzyme reactions were performed and quantified in a time-resolved manner. This demonstrated the functionality of the native enzymes individually as well as in combination, and no by-products were observed. Subsequently, the enzymes were immobilized on magnetic Ni-NTA beads and the functionality of the immobilized enzymes was demonstrated by repeating the previously performed reactions with native enzymes. Furthermore, the activities of native as well as immobilized enzymes were determined by a developed and validated method for inactivation of enzymes by pH change. Finally, a miniaturized flow-through membrane reactor that can be integrated into the CE was developed as part of a proof-of-concept application for a simple *in vitro* biosynthesis of citrate and the polyketide building blocks MalCoA and AcCoA with an integrated CoA recycling system. In addition to direct coupling of the reactor with the analytical system, upscaling compared to previous batch approaches and working in a flow-through system was enabled. The reactor can be produced cost-effectively, used in a resource-saving manner and operated under defined reaction conditions. The modularity of the reactor, demonstrated by the sequential use of enzyme-loaded filter units, and the potential reusability of the enzyme units proved advantageous. However, some unresolved issues arose re-

garding the stability of immobilization, as reduced activities were shown with multiple uses of immobilized enzymes. Pending, but part of current research, is the coupling of the reactor to a free-flow electrophoresis (FFE) unit for continuous separation of products from the reaction matrix and the integration of a cell-free polyketide synthase for the production of a potential drug in the developed reactor system.

Dr. Claudia Hellmann

Possibilities and limitations of HPLC-ICP-MS coupling for the analysis of monomethylmercury in sediments

The speciation of mercury in sediments is a topic that has occupied analysts since the environmental disaster in Minamata. Not least due to the threat of climate change, mercury is still of great importance, which makes speciation indispensable for the assessment of the state of the environment. Speciation can be divided into several sections, which are defined by sample storage, extraction, enrichment, measurement methodology and quality control. It could be shown that each step for the analysis of monomethylmercury in sediments has to be considered critically and can decide about the success of speciation. This thesis dealt with the possibilities and limitations of HPLC-ICP-MS as a coupling technique for the analysis of monomethylmercury in sediments. Due to the absence of the need for derivatization and the enormous variety of separation methods, HPLC has many advantages and, in combination with the special properties of ICP-MS, forms a powerful coupling technique. Thiol compounds, which include L-cysteine, 2-mercaptoethanol and APDC, are common substances that are used frequently in the speciation of mercury. It has been shown that thiol compounds have a special position in the analysis of monomethylmercury and their specific choice can make quantification easier or more difficult. The extraction of sediments is a particularly critical step in the analysis of mercury species, especially monomethylmercury. This is due to the fact that the methylmercury concentration is only 0.1 - 1 % of the total mercury content, so sediments must be treated with special care. In the present work, some methods used in the literature have been listed and critically discussed. For the extraction of the sediments mainly acidic, alkaline or distillative methods are used. However, acid extraction is the most common method and is used in different variations. Based on the methods collected, three were determined for comparison. The methods included selective and non-selective approaches, but it could be shown that selective extractions with the help of dichloromethane led to significantly better results. With these recoveries between 82 and 94 % could be achieved and contamination of the measuring system could be minimized. As already mentioned, the concentration of monomethylmercury in sediments is extremely low, which makes a suitable enrichment method after extraction necessary. Based on this, a preconcentration column based on thiol silica and thioureasilica was manufactured and initially connected offline to the measuring system. A review of the preconcentration method resulted in a quantification limit of 22 ng/L for monomethylmercury. In order to enable a measuring method that was as automated as possible, an online enrichment method was subsequently developed, optimized and checked with suitable reference material. For the analysis of the reference material ERM-CC580 by means of Online-SPE-HPLC-ICP-MS recoveries between 82 and 101 % could be achieved. Based on the results obtained, it could thus be shown that the developed online enrichment method in combination with HPLC-ICP-MS is suitable for the analysis of CH_3Hg^+ in sediments and leads to satisfactory recoveries. The analysis by HPLC-CV-AFS is another common technique for the speciation of mercury. Based on the analysis of suitable reference material, recoveries between 91 and 96 % could be determined. In view of the results, it can therefore be concluded that the coupling technique corresponds to a powerful alternative for the analysis of monomethyl mercury in sediments.

Dr. Yildiz Großmann

Untersuchungen von hydrothermal gebildeten Flüssigkeitseinschlüssen in Gesteinsproben sowie die Analyse von Simulationsexperimenten zur Peptid- und Nukleotidbildung mit einer Phasengleichgewichtshochdruck-Apparatur

In the context of the present work, investigations on the topic of "Origin of Life" took place using new analytical methods and modern analytical equipment.

In the first part of the work, various rock/mineral samples were analyzed to find possible precursor molecules for organic life. In detail, these were calcites from a drill core from the Wehrer Kessel (volcanic Eifel), crackling salts from the Werra area (Thuringia) and quartz from Western Australia. All samples are fluid inclusions within deposits of hydrothermal origin. In these deposits, precursor molecules for organic life could be obtained over the long time periods. It is also of particular importance that these are formations of a self-contained system that was not in contact with the atmosphere. Thus, inputs from the outside can be ruled out. These are purely chemically formed molecules. The investigations specifically targeted aldehydes and other polar organic compounds as possible precursor molecules for organic life.

In that a calcite vain containing drill core sample, long-chain aldehydes of C_8–C_{16} were successfully identified by comparison with analytical standards using GC-Q-TOF. The quantitative analysis showed aldehyde concentrations of 18–582 µg/kg calcite. The aldehyde nonanal (C_9) had the highest concentration with 582 µg/kg and tetradecanal (C_{14}) the lowest with 18 µg/kg. These concentrations could only be detected through the very intensive cleaning and sample preparation process and an optimized and finally established analytical method. In addition, a novel DIP-Q-TOF MS coupling was tested to analyze the drill core solid sample in regards of the presence of short-chain C_2–C_7 aldehydes. It was found that there was insufficient sensitivity to detect low concentrations of aldehydes. A further investigation via headspace analysis using GC-FID also did not lead to any further results.

In the binocular analysis of the crackling salt sample KS-2.1 fluid inclusions were clearly visible. The analysis was done by GC-Q-TOF and initially did not reveal any identifiable substances. Therefore, future investigations should be repeated with the method proven with the calcites from the drill core in order to be able to make a reliable statement about the occurrence of entrapped precursor molecules in crackling salts.

The non-target analysis and evaluation of the Western Australian quartz sample MU23.1 using the UHPLC – Q Exactive Plus Orbitrap MS showed a large number of different polar organic compounds. Some compounds have a high number of nitrogen atoms. These are some of the most important compounds of the early Earth. In addition, a high bromine content was detected in the sample, which could enhance various reactions with alkenes, alcohols and metals. These results expand the knowledge of prebiotic chemistry. Here, the molecular formula and probable molecular structures can be determined; a final confirmation using reference standards still has to be done.

In the second part, the focus was on peptide and nucleotide formations in the phase equilibrium high pressure apparatus (PGGHA). It is assumed that peptides and nucleotides could be formed preferentially in environments with periodic pressure variation and associated alternation of CO_2 between subcritical and supercritical state.

The on this way obtained peptides were analyzed by UHPLC - Q Exactive Plus Orbitrap MS and an octapeptide (KSPPAAFF) was found, the exact sequence still needs to be confirmed. Further analysis of the peptides using NanoHPLC coupled with the Orbitrap Fusion Lumos Tribrid MS and subsequent *De-Novo*-peptide-sequencing using *Peaks® Studio Xpro* found possible peptide sequences with a score of 69–97 %. These are penta- or hexapeptides (experimental series B: FVTLK, TTALT, TTKALK, TAATP and experimental series C: SSKVGK, TAATP, TTKALK and TLLPPP) out of two series of four experiments (A–D).

The nucleotides which were also generated with the PGGHA were analyzed by using the Q Exactive Plus Orbitrap MS via the direct inlet method. There was a tendency that a dinucleotide (ApA) can form via a mononucleotide (AMP).

Since only a small number of the crackling salt and quartz samples have been evaluated and many more samples are still pending, through further research, based on the results obtained here, both on the rock samples and the respective simulation experiments, the following can be expected: In case of a future optimization of the experimental parameters and methods in connection with further analyses as well as the complete evaluation of all data, an identification of further organic compounds may be successful. Thus, further statements about the formation of precursor molecules of life can be made.

Master's Theses Accomplished 2023

Kiana Mellinghaus
Chemoselective and mass spectrometric analysis of microbiome-derived amino-metabolites

Cedric Thom
Optimization of the ion transmission efficiency of an electrospray ion source for HPLC-MS

Bachelor's Theses Accomplished 2023

Anne Neugebauer
Characterisation and application of new stationary phases for liquid chromatography

Scientific Publications 2023

Original Paper / Peer-reviewed

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

L. Mondello, P. Dugo, P. Donato, M. Herrero, L. Montero, Oliver J. Schmitz, **Comprehensive two-dimensional liquid chromatography**, Nature Reviews Methods Primers (2023) 3:86, doi.org/10.1038/s43586-023-00269-0

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P. E. Görs, J. F. Ayala-Cabrera, S. W. Meckelmann, **Unraveling the Double Bond Position of Fatty Acids by GC-MS Using Electron Capture APCI and In-Source Fragmentation Patterns,** Journal of the American Society for Mass Spectrometry (2023) 34(11):2538–2546, doi: 10.1021/jasms.3c00257

Z. Tietel, S. Hammann, S. W. Meckelmann, C. Ziv, J. K. Pauling, M. Wölk, V. Würf, E. Alves, B. Neves, R. M. Domingues (2023). **An overview of food lipids toward food lipidomics**, Comprehensive Reviews in Food Science and Food Safety (2023) 22(6):4302-4354, doi: 10.1111/1541-4337.13225

J. F. Ayala-Cabrera, L. Montero, T. Sahlabji, O. J. Schmitz, **Comprehensive two-dimensional gas chromatography with flow modulator coupled via tube plasma ionization to an atmospheric pressure high-resolution mass spectrometer for the analysis of vermouth volatile profile**, Analytical and Bioanalytical Chemistry (2023) 415(13):2561-2573, doi.org/10.1007/s00216-023-04688-6

A. K. Mallik, L. Montero, S. W. Meckelmann, O. J. Schmitz, **Design of -Alanine-Derived Novel C18 Stationary Phase Containing Embedded Urea and Amide Groups for the Separation of Versatile Analytes**, Analytical Chemistry (2023) 95:6172–6181

J. Lu, X. Hiong, M. Ma, B. Chen, Y. Chen, O. J. Schmitz, **Enhancing the compatibility of normal-phase chromatography x reversed−phase chromatography by combination of low−temperature sensitive aqueous-phase compatible normal- phase chromatography and at−column dilution modulation**, Journal of Chromatography A (2023) 1691:463821

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J. F. Ayala-Cabrera, L. Montero, S. W. Meckelmann, F. Uteschil, O. J. Schmitz, **Review on atmospheric pressure ionization sources for gas chromatography-mass spectrometry.**
Part I: Current ion source developments and inmprovements in ionization strategies, Analytica Chimica Acta (2023) 1238:340353

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Part II: Current applications, Analytica Chimica Acta (2023) 1238:340379

Misc. Publications

O. J. Schmitz, S. W. Meckelmann in **Liquid Chromatography** (Third Edition), Fundamentals and Instumentation, Handbookd in Separation Science, Chapter 27 – Identification and quantitation in liquid chromatography–mass spectrometry (2023) 707-726, Elsevier, https://doi.org/10.1016/B978-0-323-99968-7.00008-4

Poster Presentation

J. Rösler, F. Uteschil, A. Tasdogan, O. J. Schmitz. **New Tools in Cancer Metabolomics – Ion Source Development for Single Cell Analysis**, BCEIA 2023, Beijing, China, September 2023.

S. W. Meckelmann, P. E. Görs, J. F. Ayala-Cabrera, **Positionsspezifische Analyse von Doppelbindungen in Fettsäuren mittels GC-APCI-MS**, 51. Deutsche Lebensmittelchemietage, Bonn, Germany, August 2023

P. Wittenhofer, O. J. Schmitz, S. W. Meckelmann, **Heart-cut 2D-LC-QqQ-MS as a novel tool for the Characterization of the Cholesterol Biosynthesis**, HPLC 2023, Düsseldorf, Germany, June 2023

M. Häßler, K. Wetzel, L. Montero, J. F. Ayala-Cabrera, **Herbal remedies as complex samples – Challenge or possibility for new analytical techniques?**, HPLC 2023, Düsseldorf, Germany, June 2023

M. Herrero, J. F. Ayala-Cabrera, O. J. Schmitz, L. Montero, **Comprehensive two-dimensional liquid chromatography; strategies to improve poor resolution and characterization of complex food samples**, HPLC 2023, Düsseldorf, Germany, June 2023

F. Stappert, A. K. Mallik, Y. Oulad El Majdoub, L. Montero, J. Rösler, S. W. Meckelmann, O. J. Schmitz, **2D LC separation of herbal liqueur using the identical stationary phase in both dimensions**, HPLC 2023, Düsseldorf, Germany, June 2023

S. W. Meckelmann, P. Wittenhofer L. Montero, **Lipidomics Analysis for the differentiation of Parmigiano Reggiano and Grana Padano by LC-IM-qTOF-MS**, HPLC 2023, Düsseldorf, Germany, June 2023

J. Rösler, F. Uteschil, L. Martins Nascentes Melo, G. Allies, S. W. Meckelmann, A. Tasdogan, Oliver J. Schmitz, **Novel Tools in Cancer Research – Dissecting Metabolic Differences in the Metastasizing of Cancer Cells by LC-MS and Ion Source Development for Single Cell Analysis**, HPLC 2023, Düsseldorf, Germany, June 2023.

F. Stappert, A. Pape, J. F. Ayala-Cabrera, F. Uteschil, O. J. Schmitz, **Design and investigation of homemade argon plasma ion sources – An overview of source development, application, and ionization mechanism**, ASMS 2023, Houston, USA, June 2023

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P. Wittenhofer, L. Kiesewetter, O. J. Schmitz, S. W. Meckelmann, **Characterization of Cholesterol Biosysnthesis by means of Heart-cut 2D-LC-QqQ-MS**, analytica Vietnam, Ho Chi Minh City, Vietnam, April 2023

J. Rösler, F. Uteschil, A. Tasdogan, O. J. Schmitz, **New Tools in Cancer Metabolomics - Ion Source Development for Single Cell Analysis,** analytica Vietnam, Ho Chi Minh City, Vietnam, April 2023

A. Pape, J. F. Ayala-Cabrera, F. Stappert, F. Uteschil, O. J. Schmitz, **Investigation of homemade Low Temperature Plasma (LTP) ion sources**, Anakon 2023, Vienna, Austria, April 2023

P. Wittenhofer, L. Kiesewetter, O. J. Schmitz, S. W. Meckelmann, **Characterization of Cholesterol Biosysnthesis by means of Heart-cut 2D-LC-QqQ-MS**, Anakon 2023, Vienna, Austria, April 2023

J. Rösler, F. Uteschil, A. Tasdogan, O. J. Schmitz, **New Tools in Cancer Metabolomics - Ion Source Development for Single Cell Analysis**, Anakon 2023, Vienna, Austria, April 2023

S. W. Meckelmann, L. Montero, O. J. Schmitz, **Increasing the Separation Power and Lipidome Coverage by LC-IM-MS in Combination with Multiplexing and Post-Processing**, Anakon 2023, Vienna, Austria, April 2023

Invited Lectures / Oral Presentations

Prof. Oliver J. Schmitz

Ultra-high-resolution MS and ever more powerful ion mobility mass spectrometers, and we are still talking about chromatography: why?
BASF, Ludwigshafen, Germany, December 2023

Various applications of GC-El-qTOF-MS in the field of metabolomics, lipidomics and environment
Agilent GC/Q-TOF Virtual User Meeting, online, October 2023

On the way to single-cell metabolome analysis
Dalian Institute of Chemical Physics, Dalian, China, September 2023

On the way to single-cell metabolome analysis
Tsinghua University, Beijing, China, September 2023

Development of a new ionization source for single cell metabolome
20th BCEIA, Beijing, China, Plenary talk, September 2023

How to solve problems in non-targeted analysis
20th BCEIA, Beijing, China, September 2023

Why GC-EI-MS when you can do better?
54th Annual Conference on the DGMS 2023, Dortmund, Germany, May 2023

How to solve problems in non-targeted analysis
analytica Vietnam, Ho Chi Minh City, Vietnam, April 2023

Dr. Florian Stappert

Multi-^{2}D comprehensive liquid chromatography for the separation of highly chemically complex samples
Anakon 2023, Vienna, Austria, April 2023

Awards

In April 2023, during the analytica Vietnam conference in Ho Chi Minh City, Vietnam and in September during the BCEIA in Beijing **Jonas Rösler** was awarded with a Best Poster Award for his poster titled: **New Tools in Cancer Metabolomics - Ion Source Development for Single Cell Analysis.**

Cedric Thom was awarded with the **3rd Price of the Feralco Water Award** for his master thesis and **Laura Kiesewetter** (Bachelor thesis 2022 in AAC) was awarded with the **Analytical Chemistry Study Award** of the Analytical Chemistry Division of the German Chemical Society (GDCh).

Miscellaneous

Conference Organization

In addition to organizing the 51st HPLC, the conference for the 8th analytica Vietnam was organized in 2023 together with my colleague Prof. Viet Pham (VNU University of Science, CETASD). The two-day conference was a complete success and was extremely well attended with 582 participants.

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

At analytica Munich, the world's leading trade fair for laboratory technology, analysis, biotechnology and analytica conference, which will take place in Munich from 9 to 12 April 2024, we will once again be organizing a session at the analytica conference on 9 April with the title **“A dream comes true: Fantastic news from analytical chemistry”**.

Program		
9:30 – 10:00	Eberhard-Gerstel-Price	
10:00 – 10:30	Dr. Marleen Vetter Bruker/TOFWerk, Switzerland	Improved compound identification for target, suspect- and non-target analysis using a GC-EI&CI-TOF-MS system
10:30 – 11:00	Dr. Juan Liang-Schenkelberg Agilent Technologies, Germany	Less Work, Better Peaks - solution to solvent effects challenge in LC & LC/MS analysis
11:00 – 11:30	Tim Causon BOKU, Vienna, Austria	SLIM-based ion mobility-HRMS for deeper characterization of challenging molecular and isomer systems
11:30 – 12:00	Frank Steiner ThermoFisher Scientific, Germany	Intelligent multi flow-path solutions for characterization of biopharmaceuticals: from sequential to parallel – how simultaneous can you get?
Coffee Break and Poster session		
12:30 – 13:00	Luigi Mondello University of Messina, Italy	The Fascinating World of Fast Separation Using Narrow-Bore Columns from Theory to Practice
13:00 – 13:30	Robert Plumb Imperial College London, UK	Evolution of high throughput LC-MS to support drug discovery studies
13:30 – 14:00	Sebastiaan Eeltink Vrije University of Brussel, Belgium	Unlocking Ultra-High Peak Capacities: The Spatial 3D-LC Revolution
14:00 – 14:30	Tobias Werres IUTA, Germany	The development of a modular lab-on-chip platform. How 3D printing serves as a catalyst for innovation in analytical chemistry.
Coffee Break		
15:00 – 15:30	Jin-Ming Lin Tsinghua University, China	Open microfluidic device for single cell analysis
15:30 – 16:00	Derek Stein, Brown University, Providence, USA	Toward single-molecule protein sequencing with a nanopore ion source
16:00 – 16:30	Takehiko Kitamori National Tsing Hua University, Taiwan	From femto-liter MS sample interface to ton/year chemical production by micro- and nanofluidic
16:30 – 17:00	Thomas Bocklitz, University of Jena, Germany	AI based methods facilitate the usage of spectroscopic and image data for analytics and diagnostics

Mark the event in your calendar!

analytica China will also take place in Shanghai from 18 to 20 November 2024. Together with Prof. Jin-Ming Lin from Tsinghua University in Beijing, I am organising the accompanying analytica conference there under the motto **Analytical Chemistry for a better life** with the following sessions:

- Single-Cell Analysis
- Food Analysis
- Microfluidics
- Chromatography
- Mass Spectrometry
- Environmental Analysis
- New developments in analytical chemistry
- Imaging
- Sample preparation

Mark the event in your calendar!

TRC-Forum

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

Editorial Tasks by Prof. Oliver J. Schmitz

- 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 DAAD selection committee (Foreigners from Asia and Oceania)
- Member of the DAAD selection committee (Project-related people exchange with India)
- 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. Dr. O. J. Schmitz)
- Tutorial Analytical Chemistry I (in German, Dr. S. W. Meckelmann)
- Lecture Analytical Chemistry II (in German, Prof. Dr. O. J. Schmitz)
- Tutorial Analytical Chemistry II (in German, Dr. S. W. Meckelmann)
- Lecture Modern analytical methods for systems medicine (in German, Prof. Dr. O. J. Schmitz)
- Seminar Modern analytical methods for systems medicine (in German, Prof. Dr. 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. Dr. O. J. Schmitz)
- Tutorial Analytical Chemistry I (in German, Dr. S. W. Meckelmann)
- Lecture Analytical Chemistry II (in German, Prof. Dr. O. J. Schmitz)
- Tutorial Analytical Chemistry II (in German, Dr. S. W. Meckelmann)
- Lecture Applied Analytical Chemistry (in English, Prof. Dr. O. J. Schmitz)
- Tutorial Applied Analytical Chemistry (in English, Prof. Dr. O. J. Schmitz)
- Lecture Modern analytical methods for systems medicine (in German, Prof. Dr. O. J. Schmitz)
- Seminar Modern analytical methods for systems medicine (in German, Prof. Dr. 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. Dr. O. J. Schmitz)

Seminar

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

Practical Courses

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

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

- Basic course LC-MS (digital), Provadis, February 2023
- Advanced LC-MS (digital), Provadis, February 2023
- Method school: HPLC for beginners (digital), Klinkner & Partner, June 2023
- Method school: HPLC for advanced users (digital), Klinkner & Partner, June 2023
- Basic course GC-MS, Haus der Technik, Essen, Germany, September 2023
- Basic course LC-MS (digital), Provadis, November 2023
- ICP-MS course (digital), Klinkner & Partner, November 2023
- Advanced LC-MS (digital), Provadis, November 2023
- Master course GC-MS, Haus der Technik, Essen, Germany, December 2023