Applied Analytical Chemistry (AAC)

Annual Report 2021

Applied Analytical Chemistry

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Applied Analytical Chemistry

The year 2021 was overshadowed by Corona. If Corona has taught us anything, it is that the general scientific education of large parts of the population and politicians is not sufficient to manage such a crisis sensibly. We need to find ways to help the population distinguish scientific facts from fake news. But this will be a lengthy process.

Despite all the adversities, I am pleased to present you another annual report.

The Applied Analytical Chemistry (AAC) is part of the Faculty of Chemistry at the University of Duisburg-Essen (UDE). The AAC 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 origin-of-life.

2021 was the ninth-year of the Applied Analytical Chemistry research group at the University of Duisburg-Essen and – despite all the problems – a very successful one.

The most important topic in 2021 was that we have further focused our research topics on Metabolomics/Lipidomics. In this regard, we are very pleased about further cooperations with the University Hospital Essen (Prof. Alpaslan Tasdogan) and the University Hospital Düsseldorf (Prof. Miriam Cortese-Krott), to name but two. This field is supported by our work in ion source development and multidimensional chromatography.

In addition, there were two other drastic experiences for the AAC. Dr. Martin Sulkowski, who rendered outstanding services in the organisation of the new laboratories, and Ms. Maria Madani, our good soul in the department with far-reaching contacts to all departments at the university, took their well-deserved retirement this year and had to be replaced. This will not be an easy task.

And in order to transfer the now digitally provided lessons from the winter semester 2021 back into a face-to-face lesson without losing the benefit of the 2020 experience, the year 2021 was very teaching-intensive.

Despite all these adversities, we managed to publish eight scientific papers in peer-reviewed journals, additional seven manuscripts are in the review process and two in preparation. Besides this one book chapter in the Liquid Chromatography: Fundamentals and Instrumentation (Third Edition) was written, which will be published next year, three posters were presented, and five lectures at national and international conferences (digital) were given.

In addition, seven doctoral theses, five master's, and four bachelor's theses were completed in 2021 in AAC and several projects, funded by BMBF, DFG, VW, and industry were started or continued, e.g. development of new ion sources (LC- and GC-LTP, GC-APPI and GC-APLI), a new LCxLC-MS platform for complex samples, and investigation of the metabolome/lipidome of various cells, bacteria, and archaea.

Last year, because of the inability to hold presentations in attendance at the UDE and the lack of conferences, we have decided to create a TRC forum together with Agilent, where internationally renowned analysts give a digital presentation on 10-12 dates per year. The TRC-Forum started in the winter semester 2020 and is still continuing.

I want to take this opportunity to thank the entire AAC team and all co-workers for their excellent work with all the hygiene regulations in the lab in this difficult year 2021 as well as 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 2022.

Essen, December 18, 2021

Applied Analytical Chemistry – Staff

Regular Staff

Prof. Dr. Oliver J. Schmitz	Head
Dr. Martin Sulkowski (now retired)	Senior Researcher
Dr. Sven Meckelmann	Senior Researcher
Dr. Florian Uteschil	Senior Researcher
Maria Madani (now retired)	Technician / Lab
Birgit Wöstefeld	Secretary
Constanze Dietrich	Secretary

Post-Docs

Dr. Juan Ayala Cabrera, Dr. Lidia Montero

Ph.D. Students

University Duisburg-Essen	External
Maha Alhasbani	Susanne Brüggen
Janosch Barthelmes	Tingting Li
Dominik Brecht	Wiebke Mehwald
Amela Bronja	Niklas Danne-Rasche
Yildiz Großmann	Dominic Mähler
Paul Görs	Ruzanna Mnatsakanyan
Claudia Hellmann	Chi Nguyen
Julia Klein	Simon Schastok
Timo Köhler	
Claudia Lenzen	
Christian Lipok	
Martin Meyer	
Alexandra Pape	
Kristina Rentmeister	

M.Sc. Students

Fariha Firoz Bristy, Yulia Dietle, Jasmin Heine, Hyerin Kim, Juang Luo, Pia Wittenhofer, Simon Schastok, Peter Stahlkopf

B.Sc. Students

Marc Hauke, Lars Klocke, Laila Orell, Christoph Schakau, Jessica Zierke

Guest Scientists

Věra Dosedělová (Erasmus student) and Assoc. Prof. Dr. Abul Khayer Mallik (AvH-fellower)

Apprentices

Lina Lantermann, Enola Sobel

Major News 2021

Teaching and Research Center for Separation

Agilent is developing a global network of world-class Centers of Excellence that can be linked together to broaden scientific collaborations. The University of Duisburg-Essen is world-wide the fifth university to join this network.

In 2018 as part of the collaboration, Agilent has supported the AAC with a broad range of instruments to equip a new Agilent-sponsored Teaching and Research Center for Separation (TRC). And this year we had the opportunity to exchange these fantastic instruments for the latest versions. In addition to research, the focus of the center is on teaching students and industry employees – from technicians to managers, graduates to postdocs – about separation science and training them in the use of modern analytical equipment.

"old" equipment   "new" equipment

Despite the corona situation, we could hold four digital courses in 2021 (1D- and 2D-gas chromatography, basic course LC, advanced course LC and LC-MS).

The next teaching courses will be given on:

06. – 08.04.2022	Basic Course Liquid Chromatography (Theory and HPLC)
18. – 20.05.2022	1D- and 2D-GC
01. – 03.06.2022	GC-MS
17. – 19.08.2022	Advanced Course Liquid Chromatography (2D-LC, LCxLC, SFC)
07. – 09.09.2022	LC-MS and Ion Mobility-MS
19. – 21.10.2022	ICP-OES, ICP-MS, and CE

For more information visit our website www.trc-separation.com

HPLC 2023 in Düsseldorf, Germany

Now the time has finally come. The 51th HPLC (Chairmen: Prof. Michael Lämmerhofer and Prof. Oliver J. Schmitz), postponed by Corona, will finally take place in June 2023. The Congress Centre in Düsseldorf (Germany) has been chosen as the venue, guaranteeing a professional environment for the conference and exhibition.

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The HPLC symposium series is known as the world-leading conference on liquid phase separations and related technologies. Its program covers all aspects of separation sciences in liquid and supercritical fluid phases as well as hyphenation with advanced detection technologies in particular mass spectrometry. The program will span from fundamentals and theory of chromatographic separations and detection principles, over methodological and technological advances including separation materials, column technologies, and instruments, to applications in various fields and quality assurance aspects. The symposium will feature workshops and tutorials, plenary and keynote lectures from the leading scientists in the field. Yet, the majority of lectures will be selected from submitted abstracts to make sure that participants can share and discuss their newest results with the audience. Besides, HPLC 2023 will have a big exhibition and vendor seminars in which attendees can see the latest innovations from the leading vendors in the field.

The conference topics will cover the advances in LC technologies in terms of fundamentals, hyphenation, and application.

Fundamentals

New column technologies, stationary phases and materials, Separation modes, Sample preparation, new instrumentation, mass spectrometric detection methods, Supercritical fluid chromatography, Capillary electrophoresis, and miniaturized formats, Preparative and process chromatography, Biochromatography, Green technologies, future challenges, and trends.

Hyphenated technologies

LC-MS, SFC-MS, CE-MS, microscale LC-MS, nanoLC-MS Ion-mobility spectrometry, Multidimensional separations, Untargeted and targeted analysis technologies Data processing for omics analysis technologies

Applications

Food analysis, environmental analysis, Pharmaceutical analysis, Biopharmaceuticals, biosimilars, monoclonal antibodies, and protein analysis Drug discovery, pharmacokinetics, natural products analysis, Omics technologies and biomarker analysis, Clinical and forensics analysis, doping control

Mark your calendar! We look forward to your participation.

For more information visit our website www.hplc2023-duesseldorf.com

Hero of the Year 2021

Dr. Juan Francisco Ayala Cabrera

He is now for about 2 years in the AAC working group and enriches it immensely.

Besides his excellent publications Juan is very successful in working on projects funded by industry and DFG about ion source development (GC-APPI, GC-LTP, LC-LTP) and in the supervision of theses in the research area of origin-of-life.

His above-average commitment in the laboratory is impressive and his friendly nature is a great enrichment for the Applied Analytical Chemistry.

List of Projects 2021
(Abstracts of these projects within the next pages)

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Origin of Life – Aliphatic aldehydes in the calcite of the drill core
Yildiz Großmann

Tube plasma ionization as a new ion source for GC-MS coupling
Jasmin Turkowski, Florian Uteschil, Juan F. Ayala Cabrera

Further development of an inverse low temperature plasma ion source for liquid chromatography mass spectrometry
Alexandra B. U. Pape, Juan F. Ayala Cabrera, Florian Uteschil

Comparison of ionization techniques for the gas chromatographic analysis of free fatty acids in plasma, serum, and cells
Paul E. Görs, Sven W. Meckelmann

A novel target approach to characterize the biosynthesis of cholesterol in cancer cells
Pia Wittenhofer, Juan F. Ayala Cabrera**,** Sven W. Meckelmann

Targeted and nontargeted analysis of lipids by means of LC-IM-qTOF-MS
Kristina Tötsch, Sven W. Meckelmann

TG-APPI-Orbitrap to analyze some vital fluids in the human body
Maha Alhasbani

Differentiation of industrial hemp strains by their cannabinoid and phenolic compounds using LC×LC-HRMS

Lidia Montero, Sven W. Meckelmann, Juan F. Ayala-Cabrera

Industrial hemp involves different varieties of cannabis that contain low concentration on psychoactive cannabinoid Δ⁹-Tetrahydrocannabinol (THC), but high concentration of non-psychoactive cannabinoids. These varieties are gaining more and more interest in the food, pharmaceutical, and cosmetic industries. Cannabinoids and phenolic compounds are two important groups of metabolites well known for their potential bioactive effects in these cannabis varieties. Besides, it is important to take in mind that one of the factors that highly affect the hemp composition is the genetic origin of the plant. Considering all these issues, we have developed a powerful separation method consisted of a comprehensive two-dimensional liquid chromatography coupled to high resolution mass spectrometry (LC×LC-HRMS) for the analysis of two industrial hemp strains, called cookie and gelato. A PFP and a C18 column were installed in the ^{1}D and ^{2}D, respectively. Although correlated separation mechanisms were used in both dimensions a high orthogonally was achieved using ^{2}D shifting gradients (63%). Thus, with the optimized method, a high separation capacity of the industrial hemp metabolite profile was achieved.

Then, a new demodulation process was used for the first time to transform 2D in 1D data. With this demodulation tool, a chemometric analysis of the 2D data acquired from the two industrial hemp was possible. We focus the objective of this work on the analysis and differentiation of the cannabinoid and phenolic profiles present in the two hemp strains. To do that, a suspected analysis of these two metabolite groups was carried out.

The figure shows the cluster analysis of the cannabinoids and phenolic compounds found in both samples. As can be observed, it was possible to differentiate the samples by their content in these metabolites. The cookie strain presented an important number of characteristic cannabinoids. Some of them were not previously reported, showing the complexity of the sample and the high separation power of the developed method. On the other hand, in the cookie sample, several phenolic compounds were detected as potential markers. The difference in the hemp strains composition could be valuable to target the use of each hemp strain to a specific pharmacological, food, or cosmetic destiny.
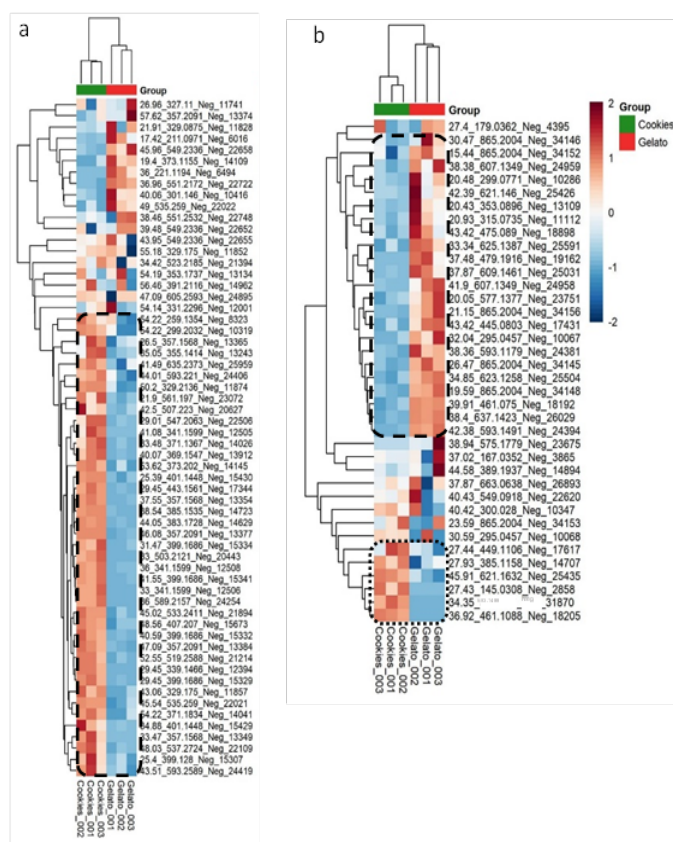

Figure: Heat maps of suspected cannabinoids (a) and phenolic compounds (b)

Non-target analysis of *Gynostemma Pentaphyllum* (Thunb.) Makino, a traditional Vietnamese medicine, by preparative HPLC, HPLC-IT-MS/MS and HPLC-Orbitrap-MS/MS

Peter Stahlkopf and Lidia Montero

Traditional medicine (TM) is very common in eastern countries and it is bases on ancient knowledge about folk and traditional plants and herbs that are used as formulated medical remedies for the treatment of common local diseases. In Vietnam the use of TM is very common, in particular to treat liver-related diseases.

From the chemical point of view, these herbal plants are very complex. They are composed of various bioactive ingredients with different physicochemical properties, which makes their analysis a real challenge. Therefore, there is a luck of information about the chemical composition of these traditional herbs, and consequently, the compounds that produce the therapeutic effect are unknown. The aim of this work was to evaluate the complete metabolite profile of a typical Vietnamese plant (*Gynostemma Pentaphyllum* (Thunb.) Makino) used for liver treatments to identify the potential compounds that promote the potential bioactivity.

To identify the ingredients of the TM plant, firstly a fractionation of the sample was achieved by using preparative liquid chromatography (Prep-LC) resulting in the collection of ten fractions (Figure). The individual collection of these fraction could be used to be individually tested in bioassays to determine if the effect of the plant is due to a synergic effect of all the compounds present in the sample or, on the contrary, to a specific compound or family of compounds.
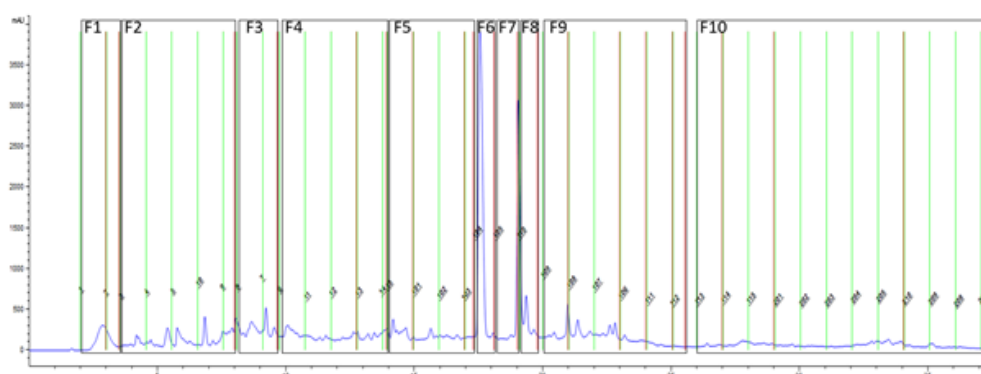

Figure: Fraction selection with the prep-LC

The gathered fractions were analyzed using a multiplatform analytical technique, i.e. an LC-ion trap mass spectrometry (LC-IT-MS) and LC-Orbitrap high resolution MS. This provided a complete information of the sample. On one hand, with the LC-IT MS, relevant structural information of the analytes was obtained, and on the other hand, with the LC-Orbitrap MS better mass resolution and mass accuracy and, therefore, higher degree of detection and identification of the compounds of the plant were achieved. Besides, as the secondary metabolite profile of herbs is very complex, involving compounds with different polarities and structures, the ionization was performed using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in positive and negative ionization mode. Phenolic compounds represented the largest group of compounds found in ten different fractions, but other types of analytes such as triterpenoids, fatty acids, lysophospholipids or lactones could also be detected.

Effect of the growing media on the metabolome of archaea *Sulfolobus acidocaldarius*

Lidia Montero

Sulfulobus acidocaldarius (*Saci*) is an extremophilic archaea able to thrive in hostile media of temperature and low pH. This type of organisms represent a valuable resource for new bioprocess for example for the utilization of glycerol and CO_2, two important industrial by- and waste-products. Therefore, the use of *Saci* with this purpose could have a positive impact in the environment due to the reduction of these waste products.

The objective of this work was the evaluation of the metabolome of *Saci*, focused in the central metabolic pathway, at different growing conditions. The variable in this study was the carbon source used for the cell growing, and three different culture media were tested: glycerol 20 mM, glycerol 40 mM and xylose 0.2%. The metabolomic study was focused on the phosphorylated hexoses pathways, amino acid metabolism, the glycerol degradation pathway, and citric acid cycle. For the analysis of these metabolites an LC-QTOF MS method was applied using HILIC as separation mode.

Regarding the phosphorylated sugars, there are two main metabolomics pathways in *Saci*, the Entner-Doudoroff (ED) and the Embden-Meyerhof-Parnas (EMP) pathways. The main phosphorylated sugars involved in both routes are glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-biphosphate (F16BP), glyceraldehyde 3-phosphate (GAP), dihydroxyacetone phosphate (DHAP), 2-phosphoglycerate (2PG), 3-phosphoglycerate (3PG), 2-keto-3-deoxy-6-phosgluconate (KDPG). Among the three carbon sources, with glycerol 20 mM the highest concentration of almost all the phosphorylated sugars were found. There were some exceptions, for example, GAP which was found in higher concentration in the xylose 0.2% growing media (Figure). Similar results were shown for the glycerol degradation pathway, where glycerol is transformed to glycerol 3-phosphate (G3P) and then to DHAP.
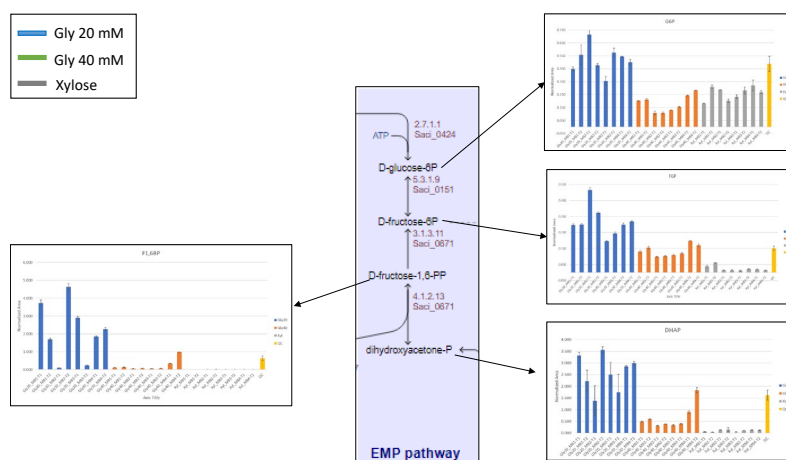

Figure: Phosphorylated sugars involved in the EMP pathway

The results showed that although all the amino acids had the same trend in each growing conditions, the overexpression of the individual amino acids varies between the three conditions. For example, there were some amino acids, like, asparagine, aspartic acid, threonine, an alanine isomer, phenylalanine, tyrosine, serine, glycine, lysine, and asparagine that were detected in higher concentration in the glycerol 20 mM conditions, however, methionine, valine, two alanine isomers, tryptophan, glutamic acid, proline, amino butyric acid, and creatine were found in higher concentration in the xylose 0.2% condition.

Finally in the citric acid cycle, xylose 0.2% was the condition that showed an upregulation of the metabolites involved in this metabolomics pathway with the exception of oxoglutaric acid that presented more intensity in the glycerol 40 mM condition.

Composition and size of exopolysaccharides in archaeal biofilms

Martin Meyer

As for bacteria, biofilms are the preferred way of life for archaea and protect the microorganisms against environmental influences, from antimicrobial agents, but also ensure the nutritional supply and the hydration of the archaeal communities. A major component of biofilms are exopolysaccharides, which have been studied predominantly for bacteria with clinical relevance, but are still poorly identified for archaeal biofilms. Our aim was to determine the monomeric composition of polysaccharides from biofilms of *Sulfolobus acidocaldarius* and additionally gain knowledge about the size or molecular weight of the polysaccharides and whether there are different polysaccharide fractions in the biofilm or the size is rather narrowly defined. To assess the composition, the extracellular polymeric substances were extracted from the biofilms, hydrolysed to cleave the polysaccharides into its individual monomeric units, and then analysed by RP-LC-MS after derivatisation. The results showed that the polysaccharide from *S. aci.* biofilms consisted of mannose, glucose, ribose, glucosamine, rhamnose, galactosamine and N-acetylglucosamine in the ratio 42:35:31:6:4:1:1. The size of this polysaccharide after extraction and enzymatic treatment to remove all non-polysaccharide macromolecular interferences was analysed by size exclusion chromatography coupled to evaporative light scattering detection. The analysis resulted in a single fraction with a very narrow molecular weight distribution for polysaccharides of microbiological origin, between 39 and 50 kDa, with the major mean mass at 44 kDa, which corresponds to a chain length, simplified from a linear polysaccharide, of 287 monosaccharide units.

Thus, we could give a first identification of monosaccharide moieties in polysaccharides and additionally gain insight into the size of the apparently narrowly defined, single polysaccharide secreted by this strain of archea as an extracellular polymeric substance. Hence, we fill a gap of knowledge in biofilm research of archea, a scientific field which provides a lot more potential for investigation.
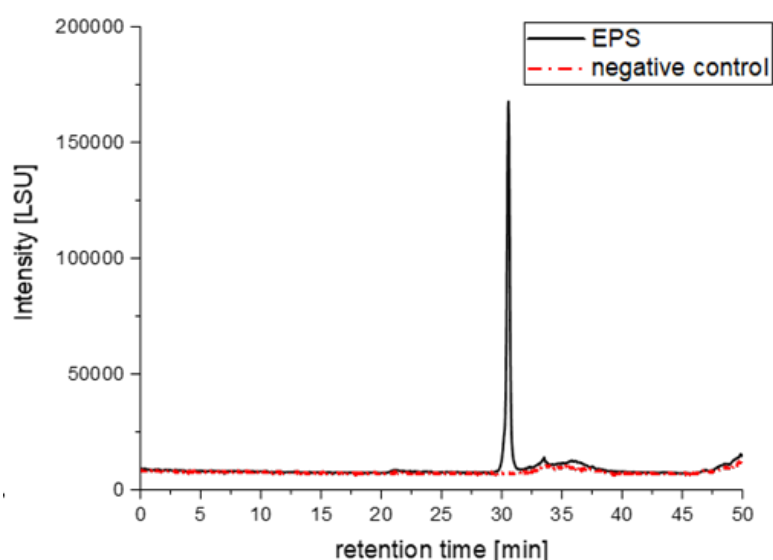

Figure: SEC chromatogram of *S. aci.* biofilm extract

Screening of sugar profiles in biological samples

Martin Meyer

Determining the composition of biological samples in terms of their sugar profile is a challenge but also desirable task from a scientific and medical point of view. Different sugars can lead to different biological, but also physiological activity of individual components. To identify the sugar profiles of different biological samples, a comparative study was initially carried out to investigate the separation performance and sensitivity of different chromatographic methods, namely supercritical fluid chromatography (SFC), hydrophilic interaction liquid chromatography (HILIC), reversed phase liquid chromatography (RP-LC) and gas chromatography (GC), each coupled to triple quadrupole mass spectrometry (QqQ-MS). It was found that RP-LC-QqQ-MS in multiple reaction monitoring mode (MRM) after derivatisation of the monosaccharides with 1-phenyl-3-methyl-5-pyrazolone (PMP) showed both the highest separation efficiency and the best sensitivity, with respect to the lowest limits of detection and quantification (LOD and LOQ). This method was finally used for the analysis of the sugar profiles of different biological/food samples, namely eight herbal liquors, two fruit pectins and one human glycoprotein. The herbal liquors were initially fractionated by solid phase extraction (SPE) into two separate fractions, one being the free sugars and the other being the glycosidically bound sugars. Finally, after sample preparation and analysis, the sugar profiles of the different samples could be determined both qualitatively and quantitatively. It was found that numerous herbal liqueurs in both fractions have a very similar sugar profile, differing only in sugar concentration. Only one sample was noticeably differing from the others, so that it can be assumed that a somewhat different manufacturing procedure is used here. The pectins from citrus peel and apples also showed strong similarities in terms of the monosaccharides present, but here differences in quantity were also found, which can be explained by the different degrees of esterification. The sugar residues of the human glycoprotein ORM, which plays a crucial role in the immune response of the human immune system, could also be determined and are shown in the adjacent figure. Overall, the method is thus suitable for determining the sugar profiles in various biological samples and the study helps to reduce the difficulties in sugar analysis.
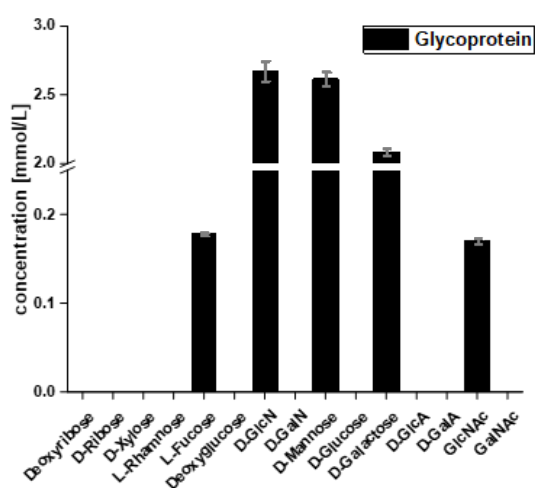

Figure: Sugar profile of human glycoprotein ORM

Monitoring of defective silicone-gel breast implants by determination of cyclic volatile methylsiloxanes in human whole blood

Janosch Barthelmes

The so-called PIP scandal in the early 2010s in which manufacturers of silicone-gel breast implants (SBI) used industrial-grade silicone for the production of their medical devices in hundreds of thousands of cases, has shed a light on the necessity of developing a sensitive, specific, and applicable method for the monitoring of the SBIs functional condition in-situ.

Over a decade later, the routine diagnostic tool is still mama sonography (SONO), which gives a sensitivity of 25-75%, depending on the operator's experience, and a specificity of regularly less than 50%. The gold standard in diagnosing defective SBI today is magnetic resonance imaging (MRI) to a sensitivity and specificity of >95%, which is nonetheless prevented from routine applicability by high cost and the need for specialized equipment.

Our group was able to define cyclic volatile methylsiloxane (vCMS) blood levels as significant threshold values for the diagnosis of ruptured SBI with a sensitivity of 77% and a specificity of 92%. We now aim for the establishment of a robust method that could be implemented in clinical routine as a more significant and only minimally more invasive alternative for the established SONO that would be more cost-effective than the MRI. Using static headspace analysis GC/MS with splitless injection and cryotrapping, we could significantly reduce the risk of contamination. Handling in laboratory environments holds a high risk of contamination of the sample with vCMS due to silicones' ubiquitous uses, for example as a sealant, flame retardant, or pump oil. Simultaneously, the complex matrix needs to be separated in the course of sample preparation to prevent contamination of the

Figure: SONO stepladder sign of defective Silicone-gel Breast Implant provided by DR. P. RUSCH of Essen University Hospital.

analytical system. The necessity to trade-off between these obstacles on applicability could be circumvented by our headspace method. To further improve sensitivity and meet the analytical criteria defined earlier in the project we proceed by implementing a cryotrap of linear design, in order to employ multiple headspace extraction as an enrichment technique.
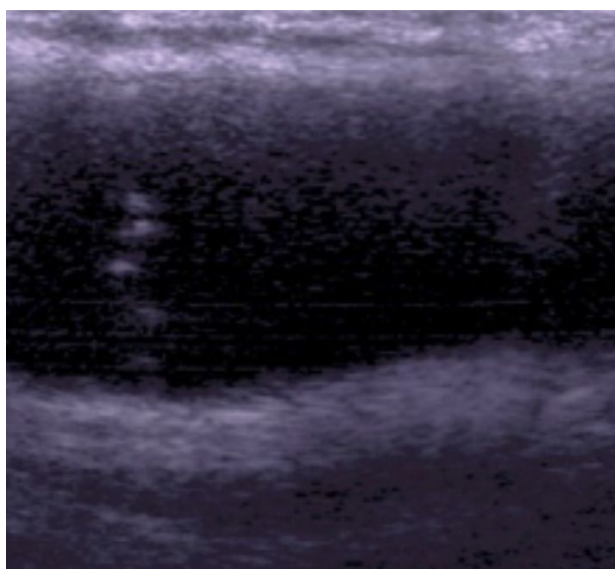
Development of a UHPLC-HRMS method to determine CLR01 in biological samples

Juan F. Ayala Cabrera

In the last years, scientific community is paying more attention on CLR01 as a possible drug against proteinopathies such as Alzheimer's and Parkinson's diseases. This molecular tweezer acts as a broad-spectrum inhibitor of abnormal protein self-assembly by binding to Lys residues (Fig. 1a). However, a powerful analytical technique is required to detect CLR01 at the common dosages in mice. Thereby, the main goal of this project lies on the development of a selective and sensitive ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) method to determine CLR01 in biological samples.

Previous studies showed that CLR01 can be efficiently ionized by non-conventional ESI conditions. The developed UHPLC-HRMS method using a C_{18} stationary phase achieved instrumental limit of detection (iLOD) of 0.15 nM but the extremely high carryover led to a low throughput analysis. Thus, different reversed-phase and hydrophilic interaction liquid chromatography (HILIC) columns were tested to reduce the interaction of CLR01 with the stationary phase. Among them, the ZIC-HILIC (150 x 2.1 mm, 3.5 µm) with PEEK coating provided the lowest retention, thus reducing the carryover effect and allowing a iLOD up to 0.01 nM. However, the carryover effect was increasing during the measurements due to the interaction of the analyte with the stainless-steel tubing of the system. This could be due to the strong interaction that hard Lewis acids (such as Fe, Ni or Cr) can have with hard Lewis bases such as phosphate groups. To minimize these effects, a daily cleaning step with CH_3CN/H_2O (4:1 *v/v*) + 0.1% H_3PO_4 was proposed. Once the determination was optimized, an extraction protocol was developed to extract CLR01 from serum samples. The low solubility of CLR01 in organic solvents as well as its interaction with proteins make the extraction challenging. The proposed protocol consisted of a protein precipitation followed by two liquid-liquid extrac-tions with hexane to get rid of less polar interferences and a final evaporation until dryness before resuspending at the optimal mobile phase conditions. Thus, CLR01 could be determined in serum samples down to 0.1 nM.
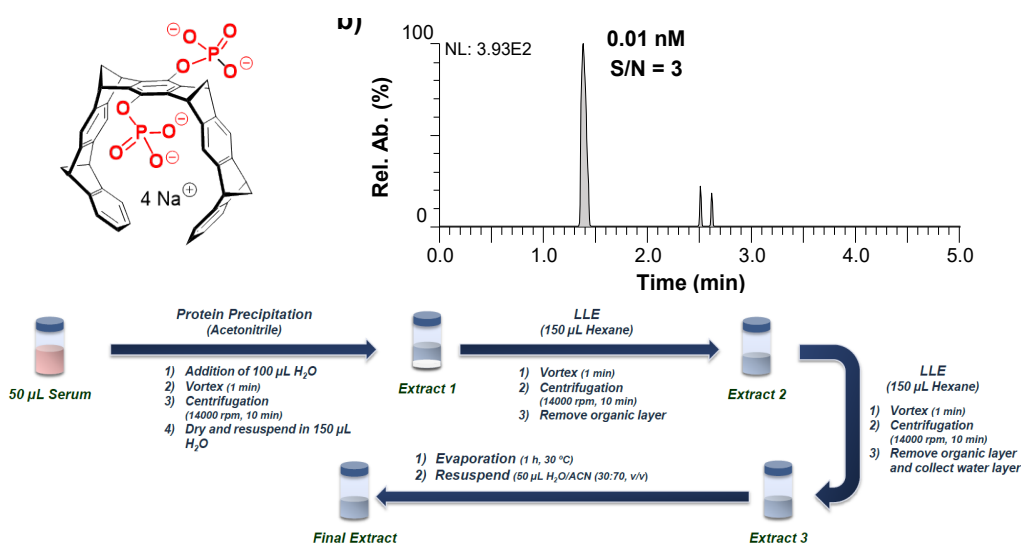

Figure 1: CLR01 a) chemical structure, b) extracted ion chromatogram (EIC) at 0.01 nM and c) extrac-tion procedure from serum samples

Non-targeted analysis by UHPLC-HRMS of Volcanic Eifel ancient samples

Jessica Zierke, Lidia Montero, Juan F. Ayala Cabrera

The origin of life still remains as an unsolved question for science. Along the history, different theories have been proposed although they showed weak points such as experiments where the early Earth conditions were not completely achieved or compatible for the formation of organic molecules as well as very low concentrations or fast degradation of these molecules at the conditions suggested. Recently, Schreiber *et al.* proposed that life could be formed in the deep-reaching tectonic fault zones. There, gases like N_2 or CO_2 at the high pressures and temperatures achieved at 340-740 m depth can get supercritical. Under these conditions, CO_2 behaves as a non-polar solvent which may help to dissolve non-polar compounds. Thus, CO_2 and water build a two-phase system with changing temperature and pressure where reactions between polar and non-polar compounds can happen to form organic molecules. This bubble-like inclusions could emerge to the Earth surface initiating the life. To demonstrate this theory, a drill core sample from the surrounding areas of Laacher See in the Volcanic Eifel area were analyzed. This sample (Fig. 1a) is assigned to the Devonian period and shows high porosity and fisures that offer a natural pathway for hydrothermal solutions. In the fissures calcite crystals, which may contain these bubble-like inclusions, are deposited. Previous studies have reported the presence of non-polar compounds such as aldehydes in this sample. Thus, the main goal of this project was to identify compounds (polar fraction) related to the origin of life.

To do this, a non-targeted analysis by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) was carried out. The analyses were done in both reversed phase (RP) and hydrophilic interaction liquid chromatography (HILIC) to obtain orthogonal information. Additionally, a Q-Exactive Orbitrap mass analyzer was employed to guarantee a high sensitivity and resolution (R_s: 140,000 FWHM). Preliminary results allowed us to tentatively identify interesting compounds in the sample such as pyroglutamic acid (confidence level: 2b), an uncommon amino acid cyclized derivate of L-glutamic acid. As can be seen in Fig. 1b and c, the tandem mass spectra of the $[M+H]^+$ ion led to two characteristic products ions that perfectly matched with the MS/HRMS library spectra from mzCloud. These identifications could help to support the theory of Schreiber *et al.* and finally answer how the life was originated.
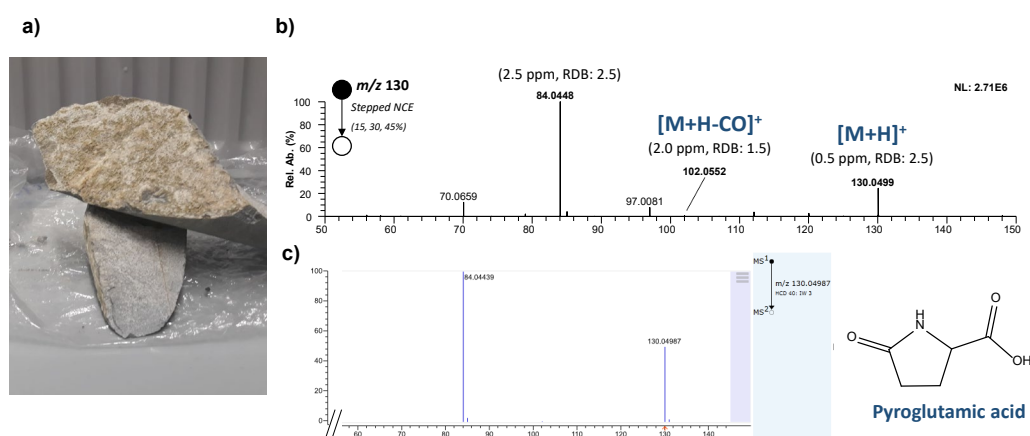

Figure 1: a) calcite sample. b) experimental and c) library tandem mass spectra (MS/HRMS) of pyroglutamic acid

Non-targeted analysis by UHPLC-HRMS of quartz liquid inclusions from Australia

Laila Orell, Isabelle Ardic, Yildiz Großmann, Lidia Montero, Juan F. Ayala Cabrera

Quartz crystals have grown in hydrothermal environment of tectonic fault zones in the upper continental crust, where the first prebiotic chemistry and the formation of protocells might have started. Under these conditions, sensitive organic molecules are well protected agains UV radiation and could undergo complex reactions in a two-phase system formed by hot water and supercritical CO_2. Thereby, these quartz crystals should have entrapped small amount of the fluid phase containing the formed organic compounds that may represent the original composition of the prebiotic stage of the planet.

To verify this hyphotheses, postulated by Schreiber *et al.*, these fluid inclusions in the archean quartz crystals have been analyzed to look for polar compounds that could be related to the origin of life. The sample (Fig. 1a) was taken from the Jack Hills (western Australia) which belongs to the Yilgarn craton and consists of greenstones, granites and granitic gneisses ranging from 3730 to 2550 Ma in age, when the major episode of plate tectonic activity on Earth happened. The sample was washed with sulphuric acid, cryo-milled and extracted with methanol/water (30:70 *v/v*) in the ultrasonic bath. After that it was centrifuged and concentrated up to 100 µL. Blanks of all the glassware material and all the extraction steps were done to avoid false positive results. The non-targeted analyses were carried out by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) using a Q-Exactive Orbitrap mass analyzer. The workflow developed for the data treatment (Fig. 1b) started with the identification of unique features coming from the sample with MSDial followed by the confirmation with the raw data. Then, a molecular formula was proposed for each exact mass of interest ensuring the identification by the mass error (>5 ppm), the isotopic cluster score (>80%) and a match in the ring double bond (RDB) equivalent. After that, the tandem mass spectra was considered to suggest a structure and tentative candidates were proposed by comparing with library spectra. Using these workflow, preliminary results have tentatively proposed interesting compounds such as leucine and guanidine derivatives which could be related to the origin of life.
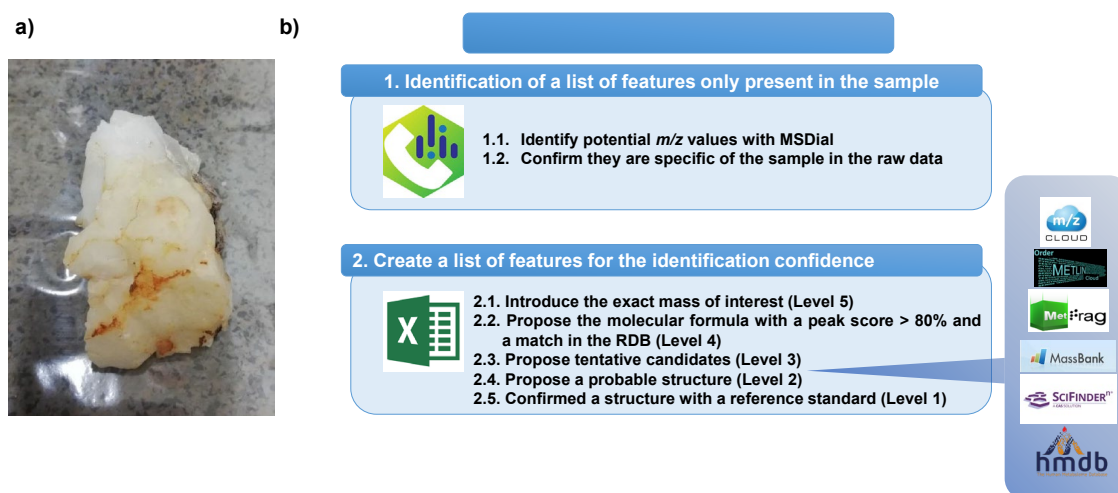

Figure 1: a) quartz crystal sample and b) proposed non-targeted workflow for the identification of interesting polar organic compounds.

Origin of Life – Aliphatic aldehydes in the calcite of the drill core

Yildiz Großmann

The origin of life is a question that has not yet been solved in the natural sciences. Some promising interpretative approaches are related to hydrothermal activities. Hydrothermal environments contain all necessary elements for the development of precursor molecules. There are possibly catalytically active surfaces and wide ranges of pressure and temperature conditions. The chemical composition of hydrothermal fluids together with periodically fluctuating physical conditions should open up multiple pathways towards prebiotic molecules. Already in 2017, our group detected prebiotic organic substances, including a homologous series of aldehydes in more than 3 billion years old Archean quartz crystals from western Australia. In order to approach the question if the transformation of inorganic into organic substances is an ongoing process, we investigated a drill core from the geologically young Wehr caldera in Germany at a depth of 1000 m. Here we show the existence of a similar homologous series of aldehydes (C_8 to C_{16}) in the fluid inclusions of the drill core calcites, a finding that supports the thesis that hydrothermal environments could possibly be the material source for the origin of life.

All measurements were carried out three times to verify the results. The analyses were done with an Accurate Mass Q-TOF GC/MS (Agilent 7890B GC with Rxi-5MS column and Agilent 7250 MS in full scan mode). Ionization was performed by electron impact (70 eV). Data Acquisition was performed and processed by MassHunter software version B.08.00 from Agilent and NIST Library.

Figure: Drilling in the Wehr caldera

Table: Concentration of aldehydes in the fluid inclusions of the calcite sample.

Aldehyde	Area	c [µg/kg]	RSD [%]
Octanal	15220	109	12.0
Nonanal	203139	582	2.1
Decanal	35050	142	2.6
Undecanal	5851	23	11.2
Dodecanal	111912	362	14.2
Tridecanal	6554	31	16.7
Tetradecanal	3420	18	1.9
Pentadecanal	6169	53	3.1
Hexadecanal	5813	36	13.3

A homologous series of long-chain aldehydes were identified in a concentration between approximately 10 and 600 µg/kg.

Returning to the question of whether the hydrothermal conversion of inorganic (CO, H_2, H_2O) to organic compounds, such as aldehydes, is an ongoing process that continues to occur today, we believe that our results demonstrate that this is in fact the case.

Tube plasma ionization as a new ion source for GC-MS coupling

Jasmin Turkowski, Florian Uteschil, Juan F. Ayala Cabrera

The interest about atmospheric pressure ionization (API) sources for gas chromatography-mass spectrometry (GC-MS) coupling is growing since they provide a soft ionization which largely preserve $[M]^{+\bullet}$ and/or $[M+H]^+$ ions. Thus, there is no longer a compromise between detection capability and selectivity usually required with high vacuum ionization techniques such as electron ionization. Among API sources, plasma-based ionization techniques are gaining more attention due to the renewing ionization region which decrease memory effects, their high stability and the posibility to easily modify the ionization. Here we present the tube plasma ionization (TPI). This source consists of a half dielectric barrier discharge based on an inverse low temperature plasma configuration where the high-voltage is applied to a stainless-steel pin electrode and the reference ground potential is the aluminum housing itself (Fig. 1a). The source design includes a connection to the GC transfer line, an external plasma gas supply controlled by a mass flow controller and a height manipulator for the TP probe to control the geometric parameters. The TP probe was connected to a HV pulse power supply through a shielded HV cable, allowing the ignition of the plasma gas (Fig. 1b).

The ionization by TPI was evaluated by means of an EPA mix of contaminants including different groups of compounds (PAHs, esters, ethers, nitro-compounds etc.). Among them, 18% of the analytes only led to the molecular ion, which mainly consisted of halogenated compounds with more than one halogen atom in the structure. In contrast, 14% of the compounds, mainly esters, ethers and nitro-compounds, only yielded $[M+H]^+$ ions. However, most of the analytes (64%) could undergo both charge-exchange and proton transfer reactions leading to both, $[M]^{+\bullet}$ and $[M+H]^+$ ions. Among them, it was observed that proton transfer reactions were promoted in the TPI source since the $[M+H]^+/[M]^{+\bullet}$ ratio was always higher than 1. It must be pointed out that the absence of functional groups as well as the presence of halogens generates an increased competition between both ionization mechanisms as happened for PAHs (ratios: 1.9-5.9) and halogenated compounds (ratios: 1.3-4.0). On the other hand, the presence of hydroxy or ester groups as well as the absence of aromatic rings increased the yield of the $[M+H]^+$ ion as observed for esters (ratios: 118-594). Different plasma gases were tested (He and Ar) achieving an increased ionization efficiency with argon compared to helium. Moreover, the use of an auxiliary gas (N_2) flowing around the GC column is required to guide the molecules through the ionization region towards to the MS inlet. Finally, the TPI was compared with other API sources used in GC-MS, showing the posibility to ionize a wider range of compounds which could led to a more universal ionization (Fig. 1 c).
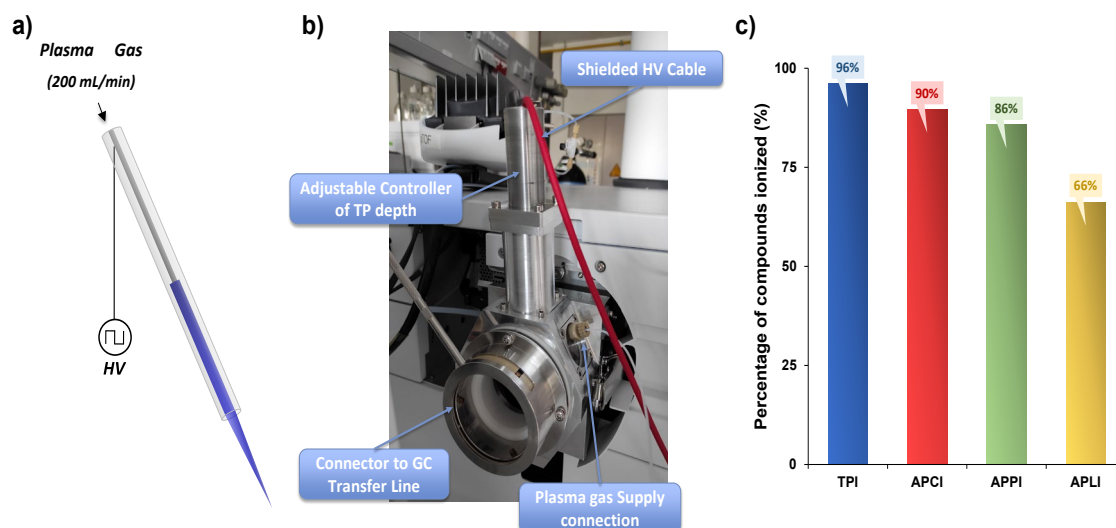

Figure 1: Tube plasma ionization a) configuration b) source design and c) comparison with other API sources for GC-MS

Further development of an inverse low temperature plasma ion source for liquid chromatography mass spectrometry

Alexandra B. U. Pape, Juan F. Ayala Cabrera, Florian Uteschil

A plasma consists of ions, free electrons, neutral atoms and molecules, and usually reaches temperatures of up to several thousand degrees. On the other hand, in a low temperature plasma (LTP) the electrons have much higher energies than the other particles in the plasma. Therefore, it is also called "non-equilibrium plasma". Such an LTP can be created by applying voltage to an electric circuit where either one or both electrodes are covered by an insulating material, also called "dielectric barrier" (cf. Fig. 1). When the voltage applied to the electric circuit is high enough, the electrons "breach" the insulator and travel from cathode to anode. In between the two insulators, the carrier gas, preferably helium or argon, is ignited, meaning that the highly energetic electrons excite the abundant gas molecules and atoms.
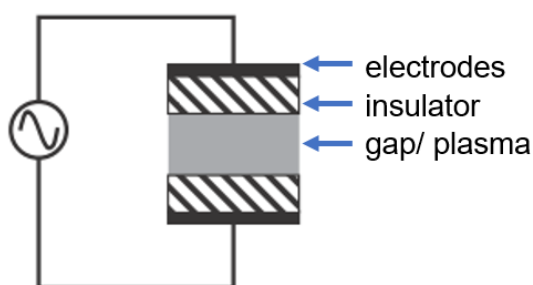

Fig. 1: Electric circuit diagram for a dielectric barrier discharge (adapted from Bruggeman *et al.* 2017)

This feature is made use of in a novel kind of ion source for mass spectrometry: the excited carrier gas species have the ability to ionize molecules through a series of reactions involving atmospheric nitrogen and water. Therefore, this type of ion source falls into the category of atmospheric pressure ion sources which also count atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI), among others. LTP-type ion sources can be coupled to both liquid chromatography (LC) and gas chromatography (GC), and have already proven to be capable of ionizing both unpolar and polar compounds, thus creating the possibility to analyze a wider range of compounds within one sample.

The here developed inverse LTP (iLTP) ion source has a slightly different design than introduced in Fig. 1. The ring-shaped ground electrode surrounds the needle-shaped high voltage inner electrode, separated by the insulator in form of a quartz tube and the gap through which the carrier gas argon is supplied (Fig. 2).

The aim of this project is it to enhance the previously developed iLTP ion source by optimizing parameters such as source geometry and dimensions. Further specifications include for example diameter of the quartz tube, size and material of the electrodes, as well as angle and distance of the iLTP towards the MS inlet. Coupled to Hitachi UHPLCs, the iLTP ion source provides an efficient and versatile ion source capable of analyzing a large variety of compounds in a wide field of applications.
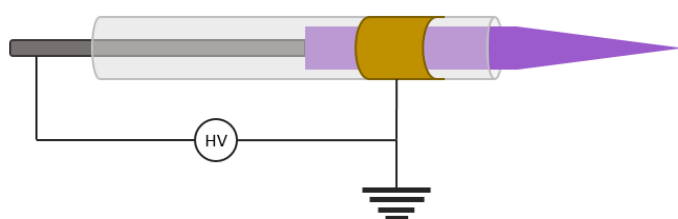

Fig. 2: Schematic set-up of an iLTP

Comparison of ionization techniques for the gas chromatographic analysis of free fatty acids in plasma, serum, and cells

Paul E. Görs, Sven W. Meckelmann

Fatty acids are essential for biological systems because of their role in energy storage and metabolism, as well as building blocks of complex lipids. Moreover, free fatty acids (FFA) can act as signaling molecules. Considering the low concentration in the nanomolar to the micromolar range together with the complex matrix, the analysis of free fatty acids is still a challenge and requires high sensitivity, a wide linear range, and the ability to detect a large number of saturated as well as unsaturated fatty acids.

A derivatization using penta-fluorobenzyl-bromide (PFB) offers a method for the selective and sensitive analysis of FFA. We compared three different GC-MS methods that were using either atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization ionization (APPI), and negative ion chemical ionization (NICI) for the analysis of FFA in negative ionization mode. The same GC parameters were chosen for all ion sources to allow comparison between the different measurements.
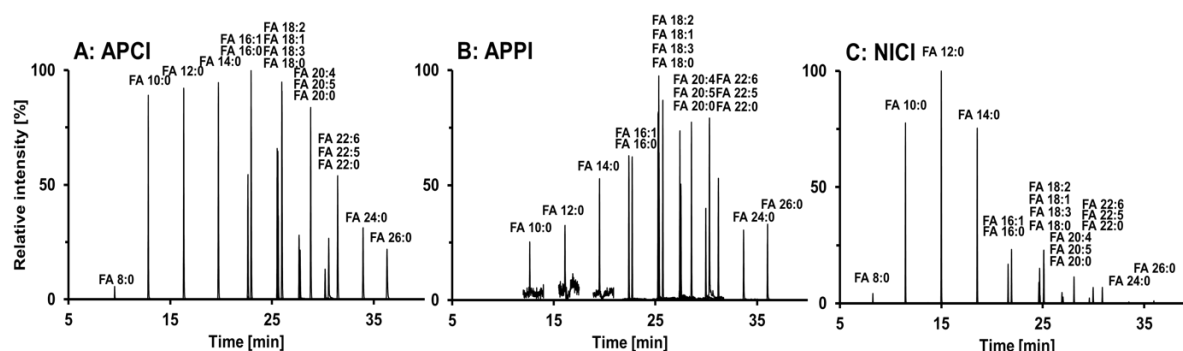

Figure: Analysis of a 30 µM free fatty acid standard using APCI (A), APPI (B) or NICI (C) source. All measurements were performed with the same GC parameters and optimized MS parameters in each case.

After validation of all methods according to the EMA-guidelines, the APCI-method was the most sensitive method with LODs from 3 to 30 fmol on-column. This enables trace analysis of free fatty acids in small amounts of sample because the method does not require the preconcentration of FFAs for example by SPE. Accordingly, concentrations of FFAs in plasma, serum, and cells were determined to demonstrate the applicability of the method to analyze clinically and biologically relevant samples. The samples were extracted according to Matyash et al. (J. Lipid Res. 2008; 49:1137-1146) and derivatized with PFB. The results obtained by APCI were comparable to values found in the literature, demonstrating the performance of the method for the analysis of FFAs in different biological matrices.

A novel target approach to characterize the biosynthesis of cholesterol in cancer cells

Pia Wittenhofer, Juan F. Ayala Cabrera, Sven W. Meckelmann

There are still many open questions in the research of carcinogenesis and further cancer development because often the necessary methods to analyze and understand pathways, changes in the actual biosynthesis of important biomolecules, and developments of the altered biosynthesis are missing. Currently, it is getting more and more clear that cholesterol homeostasis plays an important role in tumor initiation and progression offering several new therapeutic targets for cancer treatment. However, how and why the cholesterol homeostasis and, therefore, the biosynthetic pathways are altered remains unknown to a certain extent due to many difficulties in the analysis of cholesterol and the corresponding precursors.

Due to the structural similarity, the different concentration levels (factor 1000-10,000 between cholesterol and the biological precursors), the poor reproducibility, and the interferences between the sterols and the matrix, the simultaneous analysis of the whole biosynthesis is challenging.

To achieve a selective and sensitive quantitative detection, an improvement of the separation performance is needed. For this purpose, we started to develop a multidimensional liquid chromatographic heart-cut method (LC-LC) that allows separating cholesterol and biosynthetic precursors in one chromatographic analysis. Moreover, to ionize these compounds with high efficacy we will build an inverse low-temperature plasma (iLTP) ion source that is less prone to matrix interferences compared to other ionization techniques such as ESI and is well suited for the ionization of middle- to non-polar compounds. In the final stage, we will apply this method to study metastasis in pancreatic cancer (cooperation with AK Grüner) and tumor progression in melanoma (cooperation with AK Paschen).
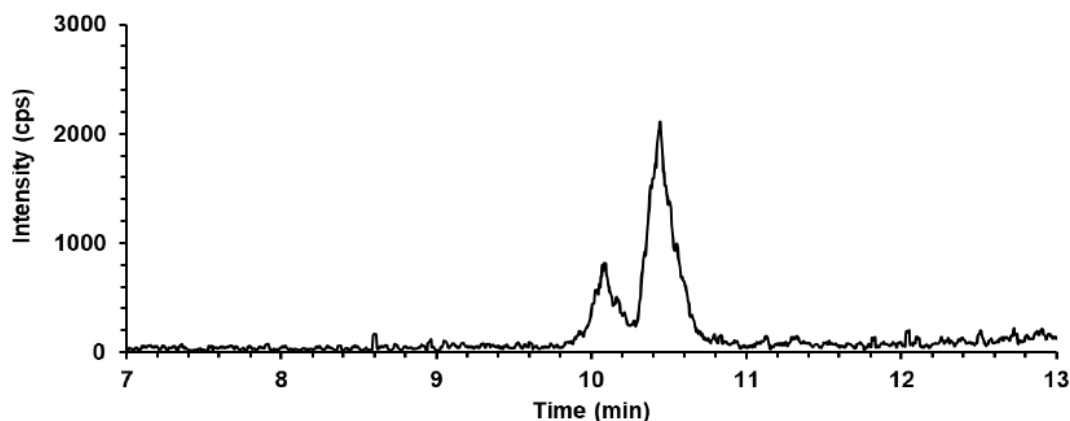

Figure: LC-iLTP-QqQ-MS analysis of cholesterol and lathosterol. Displayed is the transition of the m/z 369 to 161. Chromatographic separation was carried out using a Kinetex PFP-column (1.7 µm, 150 mm x 2.1 mm) as a stationary phase and a linear gradient of A: 10% ACN + 90% H_2O and B: 90% ACN + 10% H_2O both acidified with 0.1% acetic acid and 5 mM ammonium acetate, with a flow rate of 0.2 mL/min and an injection volume of 10 µL with a concentration of 250 nM of both.

Targeted and nontargeted analysis of lipids by means of LC-IM-qTOF-MS

Kristina Tötsch, Sven W. Meckelmann

Ion mobility as an additional separation dimension helps to resolve and annotate metabolite and lipid biomarkers and provides important information about the components in a sample. The identification of relevant information in the resulting data is challenging due to the complexity of the data and data evaluation strategies for both targeted and non-targeted workflows.

Feature analysis is often used as a first step to search for differences between samples in discovery workflows. However, follow-up experimentation often leads to more targeted data extraction methods (e.g. target lists in Skyline). In both cases, optimizing data sets for data extraction can make an important contribution to the overall results.

In the current study, the effect of experimental conditions including acquisition sampling rate and data pretreatment on lipid standards and lipid extracts as examples of complex biological samples analyzed by liquid chromatography coupled to drift time ion mobility quadrupole time-of-flight mass spectrometry was evaluated. The results show that by optimising the sampling rate, both peak variation and background noise can be reduced. The use of data pretreatment including data smoothing, intensity thresholding, and spike removal also play an important role in leading to improved detection and the identification of analytes from complex biological samples whereas, non-optimal data sampling rates and preprocessing can lead to adverse effects including the loss or alternation of small, or closely eluting low-abundant peaks. The results were published in Tötsch et al. J Am Soc Mass Spec 2021 32 (10), 2592-260.
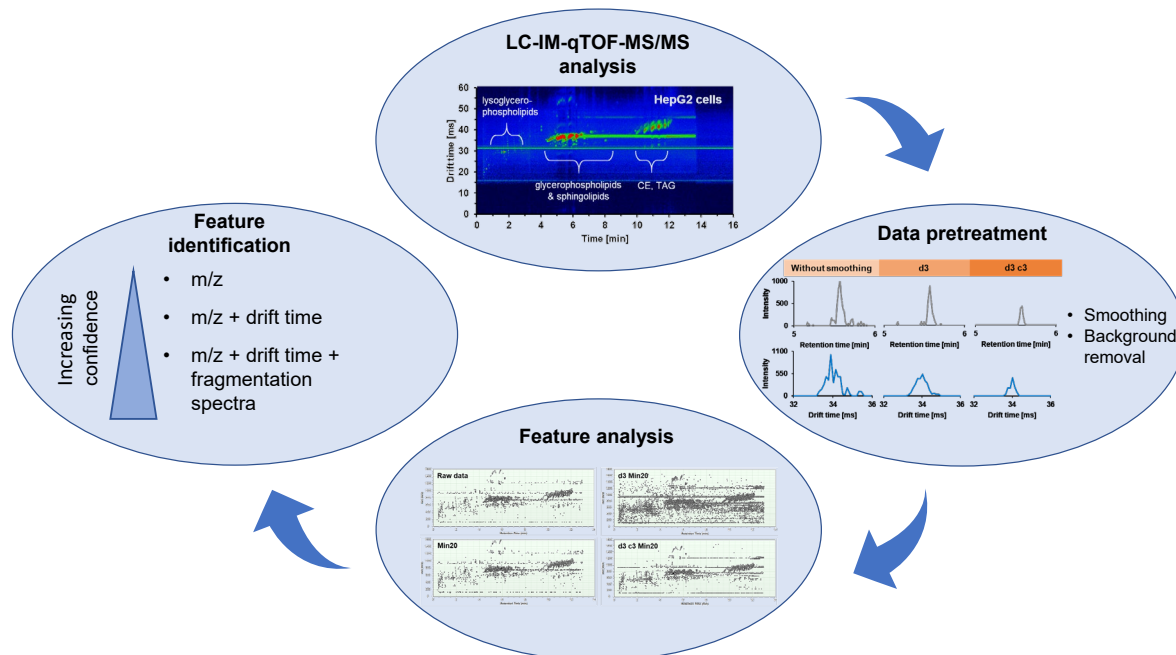

Figure: Optimized LC-IM-qTOF-MS workflow for the analysis of lipids in various biological matrices

TG-APPI-Orbitrap to analyze some vital fluids in the human body

Maha Alhasbani

Thermogravimetry coupled to high-resolution mass spectrometry (TG - Orbitrap) is a new technique to analyze the vital fluids in the human body, such as urine and blood. In urine, it was able to detect more than 65 metabolites depending on the exact mass and the extract ion current. One of the metabolites found as a biomarker of urine was creatinine with the highest signal that can be seen in figure 1. The creatinine concentration was successfully calculated by three different methods (external and internal calibration (by using Creatinine – D3) and standard addition procedure.

For blood analysis, the Human Metabolome Database (HMDB) was used for data evaluation and search of metabolites. In the data evaluation so far, 70 metabolites from various groups such as vitamins, amino acids, fatty acids, steroids and other organic compounds could be detected (figure 2). After analysing the standard for each hormone, it was obvious that a matrix effect prevents quantification. In order to ensure a reliable quantification of the hormones, two methods are to be tested. The first option is to extract all fragments for each hormone and make a comparison with the blood sample by establishing an ion ratio

Figure 1: The TIC of Urine Analysis and the Creatinine signal]M+H|$^+$

between the ion molecule and the in-source fragment in the standard and in the blood or an ion ratio between two fragments. The second option is an extraction procedure, to extract e.g. the lipids from the plasma.

The TG-MS will be very promising in quality control laboratories for qualitative and quantitative determination.

Figure 2: The EIC for the steroid hormones

Doctoral Theses accomplished 2021

Dr. Ruzanna Mnatsakanyan (external Ph.D. student)

Development and Application of Quantitative Proteomics Method for S-Nitrosylation Analysis

Cysteine reversible post-translational modifications are emerging as important players in cellular signaling and redox homeostasis. Among them S-nitrosylation has been shown to function as a redox-switch in several (patho)physiological events. However, investigation of S-nitrosylation particularly on pro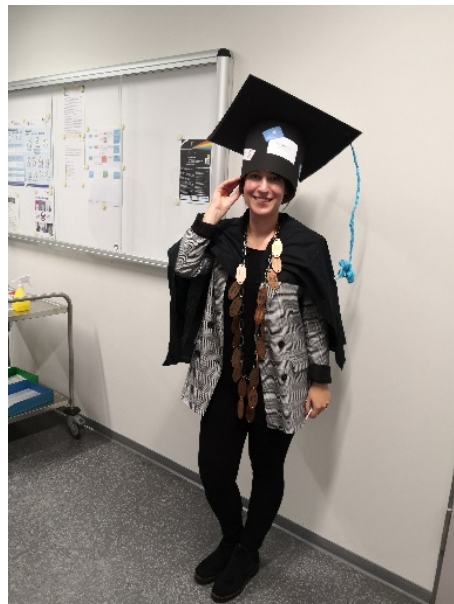teome-wide level and in the native cellular environment remains challenging, due to the lack of the highly efficient analytical methods. In this work, a novel chemical proteomics strategy for quantitative analysis of reversibly modified cysteines using biorthogonal cleavable-linker and switch technique (Cys-BOOST) was developed. Direct comparison of Cys-BOOST with iodoTMT for analysis of total Cys from HeLa cell extracts demonstrated threefold higher sensitivity, considerably higher specificity and technical reproducibility for Cys-BOOST. The application of Cys-BOOST for the analysis of S-nitrosylation in S-nitrosoglutathione-treated and non-treated HeLa cell extracts identified unprecedented number of S-nitrosylated proteins (3,632) and unique S-nitrosylation sites (8,304), covering a wide dynamic range of the HeLa proteome. Based on the quantitative data S-nitrosylation consensus motifs were deduced for S-nitrosylation sites with differential reactivity to S-nitrosoglutathione. These motifs prove the relevance of local hydrophobicity and acid-base catalysis for S-nitrosylation on proteome-wide scale and shed light on the exquisite target-selectivity of S-nitrosylation. *In-vivo* S-nitrosylation analysis in SH-SY5Y neuroblastoma cells revealed 2,158 unique S-nitrosylation sites on 1,443 proteins in basal condition and significantly changed SNO levels in proteins involved in neuro(axono)genesis, glutamatergic synaptic transmission, cadherin binding, NADH metabolic process, protein folding, translation, and DNA replication as response to early nitrosative stress. Collectively, these findings establish Cys-BOOST as an efficient tool for proteome-wide quantitative S-nitrosylation analysis and suggest S-nitrosylation as a global regulator of protein function akin to phosphorylation and ubiquitination.
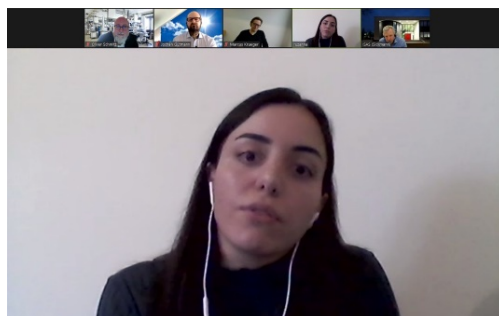
Dr. Susanne Brüggen (external Ph.D. student)

Development of a flexible and modern screening for water monitoring using LC-HRMS

The State Agency for Nature, Environment and Consumer Protection (LANUV) monitors a large number of inorganic and organic substances in water samples at over 2000 measuring points for water protection. Among them are many organic trace substances, such as herbicides, biocides, drugs and sweeteners, which are analyzed using classic target LC-MS/MS methods. In this work, a flexible and modern screening method using LC-HRMS was developed, which is able to identify not only known substances but also previously unknown compounds. For this purpose in the first step, blank value studies were performed. These are essential, especially for high-resolution systems, because the non-selective screening approach can result in a signal for each possible contaminants from chemicals and materials. Therefore different materials like eluents, pipettes, vials and analytical columns were tested. A surprising result was that although only high-purity vials were selected for the analysis, the purity of the different vials differed from one another, and a constant quality could only be achieved by heating the vials at 400°C. Additionally the large differences in the purity of the analytical columns were surprising, too. Some columns were not suitable for coupling to a highresolution mass spectrometer due to their blank value. Subsequent the gradient was optimized based on a selection of internal standards (ISTDs). In addition, investigations on the optimal sample pretreatment and carry-over test were carried out. The evaluations of these tests showed that the addition of a combination of buffer and acid to adjust the pH value is necessary to obtain good peak shapes and stable retention times in real water samples. The carry-over tests showed that there was no carry-over from one sample to the other by the autosampler. In the second part of the thesis, the developed concept consisting of target screening, suspected target screening and non target screening was applied to real samples. A simplified quantification concept with a calibration point of 0.1 µg/L (general precautionary value) and zero was established for the target screening. A comparison of 211 analytes ocer three matrices (surface water, groundwater and cleaned wastewater), which were evaluated with the classical quantification concept using a multi-point calibration and the simplified quantification concept, showed a mean deviation of 20.27%. No significant difference could be found in the mean deviations of the different matrices (surface water 22.64%, groundwater 20.02% and cleaned wastewater 18.14%), thus matrix effects could be excluded. In addition, the simplified quantification concept was applied to round robin tests, where the results of both quantification concepts were comparable and the deviations were significantly smaller. Thus, the simplified quantification concept provides comparable results to the classical method. Additionally to the quantitative results, the suspected target screening enables qualitative results on the occurrence of further substances in water samples. The *Gewässersteckbriefe* developed in this work, contain qualitative information on the occurrence and frequency of e.g. organic trace substances, which have not yet been analysed

in a LANUV routine method so far. By comparing different surface waters with each other, it is possible to identify substances that are ubiquitously distributed or occur only in specific water bodies. In contrast to common target analyses it is possible to get more information about a water bodies. The concept of suspected target screening also shows very good results analyzing special samples, where it is often not clear which target analysis method suits the best for the given analytical question. Due to the approach of non target screening, it was possible to identify two compounds, which were not known in groundwater so far. The substances were perfluoropentanesulfonic acid (PFPS) and perfluoropropanesulfonic acid (PFPrS) with a maximum concentration of 2.09 µg/L for PFPS and 1.51.

Dr. Julia Klein

Electrophoretic and chromatographic separation methods coupled with drift-time ion mobility spectrometry – powerful analytical platforms for complex samples

Drift tube ion mobility spectrometry (IMS) provides great separation power in combination with liquid chromatography (LC) and high resolution mass spectrometry (HRMS). Besides, it allows the determination of collision cross sections (CCS) as an intrinsic molecular descriptor and additional parameter to identify compounds in complex mixtures. A drift tube ion mobility quadrupole time-of-flight mass spectrometry (DTIM-QTOF-MS) system, which has been commercially available since 2014, was investigated with respect to its CCS determination properties and coupled to different analytical separation methods in liquid phase for the analysis of different complex samples. For this purpose, a comparison of CCS values of small molecules determined by drift tube ion mobility mass spectrometry (DTIM-MS) and traveling wave ion mobility mass spectrometry (TWIM-MS) was carried out to check the possibility of a common database. A good correlation of both devices were observed with a deviation < 1% for most of the investigated components. However, deviations of up to 6.2% indicate that CCS databases cannot be used mutually. Even if the comparability between the two types of instruments was not fully ensured, the CCS values can be used reciprocally to exclude clearly incorrect allocations when identifying unknown substances. In conclusion, instrument type and CCS method should always be taken into account when setting up a CCS database and using it. Also, the influence of different ionization conditions on the reproducibility of CCS values was investigated. Therefore, different LC and ionization source parameters (composition and flow rate of the LC eluent, dry gas temperature and flow rate) were varied to determine whether changes in the drift spectra occur and whether these have an influence on the resulting CCS values. For the standards investigated, no significant influence of the different ionization conditions on CCS values was found. The maximum deviation of the average experimental CCS values from the database values was 0.4%, which is within the range of uncertainty for this instrument. In addition, different IM modes (standard and multiplexed IM mode) were compared to evaluate the influence of different ion trap times on signal intensity and mass accuracy. For all three investigated standards, significantly larger peak heights were achieved in the multiplexed IM mode for the same concentrations than in the standard IM mode. The use of the multiplexed IM mode resulted in shorter trapping times in the trapping funnel, causing reduced space charge and detector saturation effects. This increases the signal intensity and the sensitivity and improves the mass accuracy for the investigated standards. Besides, the limit of detection could be reduced for the smallest of the investigated molecules by using the multiplexed IM mode.

Furthermore, the first coupling of a capillary zone electrophoresis (CZE) to a DTIM-MS instrument was realized and applied for separation and characterization of native and APTS

labelled N-glycans showing the advantages of combining two powerful analytical techniques. The separation power of different one- and two-dimensional LC-techniques with subsequent IM-QTOF-MS detection was evaluated. A standard LC method with a 250 mm and a 1000 mm column (coupling of four 250 mm columns in series), respectively, were compared to a LC+LC and LCxLC method in terms of chromatographic resolution, ion suppression and peak capacity. Finally, an existing LC+LC method was successfully further developed by miniaturization in the first dimension and by selecting a suitable combination of stationary and mobile phases in order to significantly reduce peak tailing in the first dimension. Subsequently, the cLC+LC method was further optimized for the analysis of complex samples. The analysis of various herbal liqueur samples was used to illustrate the repeatability of the optimized cLC+LC method and the reduced tailing in the first dimension in real matrices. In addition, some phenolic sample components could be identified using reference standards.

Dr. Timo Köhler

Development and application of gas chromatographic methods to characterise the extracellular volatile metabolome of Pseudomonas aeruginosa in biofilm

Cystic fibrosis (CF) is an autosomal recessive hereditary disease that leads to the production of thickened mucus in the infected organ (e.g. lung). Conditions in the infected lung favour polymicrobial infections, such as chronic lung infections with *Pseudomonas aeruginosa*. *P. aeruginosa* is the major pathogenic bacterium that colonises CF-lungs at the end of the lifetime of CF-patients.

The aim of the present work is, on the one hand, the development of an *in vitro* system for the cultivation of biofilms of the bacterium *P. aeruginosa* and the development and characterisation of a sampling and analysis method for bacterial, extracellular volatile metabolites from the *in vitro* system. On the other hand, the model system will be optimised concerning an *in vitro* study of the extracellular volatile metabolome of *P. aeruginosa* strains in the context of cystic fibrosis. Finally, an *in vitro* characterisation of the extracellular volatile metabolome of *P. aeruginosa* will be performed. The *in vitro* biofilm model consists of a Petri dish into which a solid nutrient is placed and the biofilms are cultivated on this nutrient. A sampling of volatile extracellular metabolites was performed by thin-film microextraction (TFME) using polydimethylsiloxane films (PDMS). The analysis of the loaded films was carried out by gas chromatography coupled with a quadrupole mass spectrometer and thermal desorption (TD-GC-qMS).

After the development and characterisation of the *in vitro* biofilm model and the sampling and analysis methodology, which is presented in the first part of the thesis, an *in vitro* study on influencing parameters on the extracellular volatile metabolome of *P. aeruginosa* in the context of CF-disease was conducted in the second part of the thesis. These investigations revealed that under two different oxygen conditions (aerobic vs. anaerobic) as well as between different phenotypes (mucoid vs. non-mucoid) of the different strains, differences in the volatile metabolome could be observed. Thus, e.g. 1-undecene was released only under aerobic conditions whereas 2-undecanone only under anaerobic conditions. Volatile metabolites of the substance classes of ketones and alcohols seem to be particularly suitable to distinguish between mucoid and non-mucoid strains of *P. aeruginosa* since ketones such as 2-undecanone could only be detected with non-mucoid strains (here: *P. aeruginosa* ATCC 10145 and PAO1) and the alcohols only with mucoid strains (here: *P. aeruginosa* FRD1). Based on the knowledge that the nutrient medium influences the extracellular volatile metabolome, a nutrient medium was developed which simulates the nutrient conditions in the CF-lung. With the help of this nutrient medium, the *in vitro* characterisation of the extracellular volatile metabolome of *P. aeruginosa* under CF-like conditions, as presented in the third part of this thesis, was carried out. In these studies, a significant difference between the three strains used could be demonstrated with an PCA, as well as by comparing the peak intensities. When

comparing the clinical strains P. aeruginosa PAO1 and FRD1 (CF-relevant), 31 mVOCs were determined, showing a significant change in peak intensity.

The development and establishment of a cultivation method for biofilms under CF-like conditions as well as the sampling and analysis method for extracellular volatile metabolites of these biofilms, presented in this thesis, can be used in the future for *in vitro* studies of clinical *P. aeruginosa* strains and other CF-relevant bacterial pathogens. The aim of these investigations is to identify CF-relevant bacteria from CF sputum based on selective metabolites. Furthermore, these results could be used for the development and establishment of a clinical non-invasive "at-bedside" breath analysis method for CF-patients.

Dr. Junjie Li

Determination of microbial metabolomics by comprehensive two-dimensional gas chromatography-mass spectrometry and inkjet-MALDI-MS

The purpose of this thesis was to develop a method for microbial sample preparation, which could be applied for the MS-based metabolic analysis. A sample preparation protocol for the analysis of the intracellular metabolites of a gram-negative bacteria, *C. marina* as the study object, was carefully optimized in particular from the quenching step to the derivatization with the mixture of BSTFA and TMCS. As a result, it was possible to detect more than 170 intensive signals corresponding to specific metabolites representing a "snapshot" of intracellular metabolic state, which were identified according to the NIST library. These analytes included three main groups of amino acids, alkane, and fatty acids. Repeatability analysis revealed that most of the signals have RSDs less than 20%, which implies superiority compared to previous work. Furthermore, this protocol was applied for the evaluation of intracellular state variation under different stresses. Oxidative stress inducing by treatment with ozone leads to a decrease of metabolites belonging to different groups such as L-proline, adenine and an increase of oleic acid. Moreover, it has been revealed that intermediate metabolic products such as cis-9-hexdecenal and 9-octadecenal could not be reduced due to the blockage of the enzyme by the stress as determined via visualization by GasPedal. Furthermore, the metabolic change of *C. marina* under artificially nutritional starvation was evaluated. There were significant changes in the signals of some metabolites, mainly amino acids, which are involved in metabolic pathways such as the glutathione redox cycles, the glucose pathway, and the shikimate pathway. Thus, it has been demonstrated that the proposed protocol and evaluation procedure could be utilized as a "toolkit" for the fingerprint MS-based analysis of such gram-negative bacteria. With the GasPedal software, it was much easier to find significant differences between images instead of naked eyes.

In the second part of this thesis, the use of the inkjet technique for introducing samples in MALDI-MS was evaluated and compared to traditional pipetting. To accomplish this, the formation of the droplets by an inkjet device was optimized in current pulse and driving voltage to get the optimum condition. After optimization, a voltage of 30 V and a pulse of 15 µs were chosen for the loading procedure with the multi-layer introduction method. It has been demonstrated that the induction sequence of 1.5-µL sample followed by 1.5-µL matrix was superior as compared to the "sandwich" method, which indeed locates the sample in between the matrix. Due to the fast evaporation of relatively small volume, the multi-layer method enables the spots on the MALDI plate to be prepared about 120 times faster than manual preparation with satisfying homogeneity. It has shown that a minimum of approximately 1000 bacteria is necessary for analyzing some analytes with MALDI-MS. Spotting by using an inkjet device has its obvious advantages, including precise location, adjustable volume, and uniform operation. Moreover, the multi-printing method for SA matrix

by using the inkjet device could be applied to reduce the typical "coffee-ring" effect observed after the crystallization of different matrices by the CCD camera. It could be a potential way to reduce the influence of outward capillary flow.

In summary, the protocol for *C. marina* provides a routine procedure to search for the significance and the possibility for potential biomarkers in biological pathways. In case of limited microbial samples, the inkjet technique could be an alternative for the sample preparation before MS detection.

Dr. Dinh Lien Chi Nguyen (external Ph.D. student)

Characterizing the impact of the unfolded protein response in glioblastoma via proteomics approaches

Diffuse glioblastoma is one of the most common type of human brain tumors and among the deadliest of all human cancers. Recent studies reveal that the unfolded protein response (UPR) pathway plays an important role in tumor proliferation and therapeutic resistance. Due to this reason, the UPR network has become a potential target for the cancer treatment aiming to induce cancer cell death by activating the UPR apoptosis site. However, little is known about the mechanism how the UPR can affect the outcome of tumor progression as well as tumor resistance against therapeutic treatments. The aim of this study is to perform a comprehensive and system wide investigation of the UPR activation in glioblastoma utilizing state-of-the-art mass spectrometry based proteomics techniques. First, a sensitive and straightforward targeted mass spectrometry method was successfully developed allowing the identification and absolute quantification of low abundant UPR key proteins for the first time. Applying this method, the UPR protein expression level could be quantitatively monitored over the treatment time describing the activation level of UPR under activation. Seconds, together with the research group of Dr. Medenbach at the University of Regensburg, a multi-omics approach in glioblastoma cells describing the mechanism of action of the UPR over time and at multiple biological levels was performed. By combining the transcriptomics and proteomics data, we could detect a group of UPR responsive proteins. Further validation experiments showed alterations in mitochondrial one-carbon metabolism triggered by UPR activation, too. Moreover, resistance against anti-folate agents interfering directly the mitochondrial one-carbon metabolism in glioblastoma cells could be observed in UPR activated cells. This finding opens new therapeutic directions and targets in suppressing the development of resistance against cancer therapies. In the final chapter, together with the research group of Dr. Knobbe-Thomsen at the University of Düsseldorf, the impact of UPR induction in treatment with the chemotherapeutic agent bortezomib was investigated in six different glioblastoma cell lines. We could identify clusters of proteins responding specifically either to bortezomib treatment or to ER stress induction. More important, a cluster of proteins showing significant correlation to cell viability under treatment with bortezomib was detected. This indicates a relationship between expression levels of proteins in this cluster and the cellular response to treatment with bortezomib. These proteins and their involving processes could be used as potential targets in therapeutic treatments.

Dr. Dominik Brecht

Development of atmospheric pressure ion sources for mass spectrometry

High throughput LC-MS is an increasing topic in analytical chemistry. Especially the idle time of a mass spectrometer should be reduced to use these devices more efficiently and save costs. Therefore, the first section of this thesis demonstrated how a dual ion source could be constructed. This ion source was designed to operate the typical ionization modes of LC-MS analyses, ESI, and APCI simultaneously and individually. Direct infusion experiments of reserpine and caffeine indicated that it is possible to run the two ion sources simultaneously. Furthermore, it was shown that switching between the two ion sources does not lead to instabilities in the ion current of the mass spectrometer. A six-port valve was used to couple a HPLC to the dual ion source for the initial chromatographic studies. Switching experiments were performed with cortisol and b-estradiol, resulting in a minimum switching time between the two probes of 6 s. Repeatability was investigated to be 0.28% intraday and 1.5% interday for APCI and 2.2% intraday and 2.8% interday for ESI. The limit of detection for the standard substances testosterone (ESI), vitamin D3 (APCI), and 25-hydroxyvitamin D3 (ESI) was 30 ng L^{-1} for testosterone and vitamin D3 and one µg L^{-1} for 25-hydroxyvitamin D3. The dual ion source was coupled to two HPLCs, making it possible to shorten the idle time of the mass spectrometer and to use individual methods for the two HPLCs. With this technique, the LOD of vitamin D3 could be reduced to 300 ng L^{-1}. The final experiment for multiple switching between ESI and APCI and the two UHPLCs shows good repeatability of the peak areas with standard deviations of 1.5% for testosterone and 3.8% for vitamin D3. In five runs, 50 analyses could be performed, requiring 1.5 min run time for one sample. Therefore, the dual ion source is well suited for the coupling of multiple HPLCs, accompanied by a low analysis time and a low idle time of the mass spectrometer.

The second part of this thesis dealt with developing an argon inverse-low-temperature-plasma (iLTP) ion source for LC tandem MS analysis. With flow rates up to 1000 µL min^{-1}. The iLTP ion source consists of a modified APCI ion source in which the iLTP probe replaced the APCI needle. This iLTP probe consists of a dielectric quartz tube, a tapered needle-shaped inner electrode made of stainless steel, and a silver ground electrode attached to the outside of the quartz tube. Optical emission spectroscopy was performed using a CCD camera to gain insight into the reactive species produced by the argon iLTP. These species are mainly hydroxyl radicals and metastable argon species (corresponding energies of 11.53 eV and 11.72 eV) that could serve as precursors for an ionization pathway such as metastable helium in helium LTP or via Penning ionization. Infusion experiments indicate a gentle ionization by iLTP, e.g., caffeine, testosterone, reserpine, vitamin D3, and 25-hydroxyvitamin D3, which resulted in the corresponding protonated molecular cations. The concentration dependence of the argon iLTP was demonstrated. Coupling with a UHPLC shows satisfactory inter-day repeatability

(n=10) of the analytes with RSD ≤ 5%. The instrumental detection limit study showed promising results for caffeine, testosterone, reserpine vitamin D3, and 25-hydroxyvitamin D3 of 10 ng L^{-1}, 50 ng L^{-1}, 500 ng L^{-1}, five µg L^{-1}, and five µg L^{-1}, respectively. The Ar-iLTP ion source can be operated with different discharge gases such as helium, nitrogen, synthetic air, or oxygen and lead to specific ionization pathways enabled by different discharge gases. It was demonstrated that an argon iLTP could compete with the helium LTP preferred in the literature. Investigations of pesticide standards and analysis of a forensic toxicology standard with a quantitation limit up to 500 ng L^{-1} demonstrate specific fields for applications. The iLTP ion source has comparable LODs to the dual ion source and can compete with the established methods like ESI and APCI.

Master's Theses Accomplished 2021

Yuliya Dietle
Method development for the analysis of aliphatic polyamines in urine using liquid chromatography

Hyerin Kim
A non-target analysis of complex natural substances: Industrial hemp (Cannabis sativa L.) for food product using 2DLC-IM-MS

Jiang Luo
Method development for lipids analysis by using HILIC coupled with ESI-qTOF-MS

Pia Wittenhofer
Characterisation of the free fatty acid profile of serum, plasma and cells by GC-APCI-, GC-APPI- and GC-NCI-MS

Simon Jan Schastok
Method development and application of a novel low-temperature plasma ion source for the quantification of 13 drugs in urine

Bachelor's Theses Accomplished 2021

Marc Haucke
Multiplexing in LC-IM-QTOF MS to optimise separation and quantification of complex samples

Laila Orell
Origin-of-Life: Analysis of fluid inclusions in quartz samples with Orbitrap-MS

Christoph Schakau
Investigation into the use of low-temperature plasma ionisation in HPLC-MS

Jessica Zierke
Non-targeted analysis of geographical ancient samples by HPLC-HRMS related to the origin of life

Scientific Publications 2021

Original Paper / Peer-reviewed

M. Meyer, L. Montero, S. W. Meckelmann, O. J. Schmitz, **Comparative study for analysis of carbohydrates in biological samples**, accepted in Analytical and Bioanalytical Chemistry

K. Tötsch, J. Fjeldsted, S. Stow, O. J. Schmitz, S. Meckelmann, **Effect of Sampling Rate and Data Pretreatment for targeted and non-targeted Analysis by means of Liquid Chromatography coupled to Drift Time Ion Mobility Quadruple Time-Of-Flight Mass Spectrometry**, Journal of the American Society for Mass Spectrometry (2021) 32(10):2592-2603.

T. T. Bui, T. H. Nguyen, H. A. Duong, H. V. Pham, O. J. Schmitz, V. T. Nguyen, Q. A. Ngo, **Hepatoprotective effect of water extract formulation from Stixis suaveolens and Pandanus tonkinensis against liver injury induced by acetaminophen and in vitro CCl4-induced toxicity**, Vietnam Journal of Chemistry (2021) 59(6):836-845

D. Brecht, F. Uteschil, O. J. Schmitz, **Development of an inverse-low-temperature plasma ionization source for LC-MS**, Rapid Communication in Mass Spectrometry (2021) 34:e8845

F. E. O. Suliman, S. K. Al Burtomani, A. A. Elbashir, O. J. Schmitz, **Capillary electrophoresis and molecular modeling of the chiral separation of aromatic amino acids using a/b-cyclodextrin and 18-crown-6**, Electrophoresis (2021) 42(17-18):1800-1809

J. F. Ayala-Cabrera, C. Lipok, J. Li, E. Moyano, O. J. Schmitz, F. J. Santos, **Ionic Liquid Stationary Phase for Improving Comprehensive Two-dimensional Gas Chromatographic Separation of Polychlorinated Naphthalenes**, Journal of Chromatography A (2021) 1635:461732-461737

J. F. Ayala-Cabrera, C. Lipok, E. Moyano, O. J. Schmitz, F. J. Santos, **Atmospheric Pressure Ionization for Gas Chromatography-High Resolution Mass Spectrometry Determination of Polychlorinated Naphthalenes**, Chemosphere (2021) 263:127963-127973

P. Rusch, A. V. Hirner, O. Schmitz, R. Kimmig, O. Hoffmann, M. Diel, **Zinc distribution within breast cancer tissue of different intrinsic subtypes,** Archives of Gynecology and Obstetrics (2021) 303:195–205(2021)

Misc. Publications

O. J. Schmitz, **Origin-of-Life** (in German), The information platform of the German Chemical Society (GDCh) Fazination Chemie (https://www.faszinationchemie.de/wissen-und-fakten/news/ursprung-des-lebens/), September 2021

Poster Presentations

L. Montero, O. J. Schmitz, S. W. Meckelmann, **Evaluation and comparison of phenolic compounds in herbal liqueurs by multidimensional analytical platforms**, 49. Deutscher Lebensmittelchemikertag (49th German Food Chemists' Conference), August 2021 (digital)

A. Silva, R. Garrat, C. M. Rezende, S. W. Meckelmann, **Characterisation of lipids in green Arabica and Robusta coffee beans by LC-IM-qTOF-MS**, 49. Lebensmittelchemikertag (49th German Food Chemists' Conference), August 2021 (digital)

P. E. Görs, P. Wittenhofer, J. F. Ayala-Cabrera, S. W. Meckelmann, **Comparison of ionization techniques for the gas chromatographic analysis of free fatty acids in plasma, serum, and cells**, 1st International Lipidomics Society annual conference and 7th Lipidomics Forum, Regensburg, Germany, October 2021

Invited Lectures / Oral Presentations

Prof. Oliver J. Schmitz

APCI, APPI, APLI and DBD as powerful ionization methods for GC-MS
Gas Chromatography meets Non-Target Screening, afin-ts, February 2021 (digital)

APCI, APPI, APLI, and DBD: Uncommon ionisation methods for GC- MS
QuoVadis:LifeSciences, Opole, Poland, June 2021 (digital)

Dr. Lidia Montero

Differentiation of two industrial hemp varieties by comprehensive two-dimensional liquid chromatography (LCxLC)
XX Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA 2021), November 2021 (digital)

Dr. Juan F. Ayala-Cabrera

Insight of tube plasma as a new ion source for GC-MS coupling
XX Scientific Meeting of the Spanish Society of Chromatography and Related Techniques (SECyTA 2021), November 2021 (digital)

Martin Meyer

Carbohydrate analysis in biological samples: Which method is best?
31th Doctoral Seminar of the AK Separation Science of the Division Analytical Chemistry of the GDCh, Hohenroda, January 2021 (digital)

Miscellaneous

Editorial Tasks by Prof. Oliver J. Schmitz

- Editorial Board member of *Talanta open*

- Editorial Advisory Board member of *Trends in Analytical Chemistry (TrAC)*

- 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

Institute Colloquium
(in cooperation with the research group of Prof. Torsten Schmidt)

Dr. Joachim Weiss, ThermoFisher Scientific, Mixed-mode liquid chromatography – An exciting development in separation science, November 21, 2021

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, Ionmobility-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. In addition, a digital seminar, called TRC-Forum is organized. This year speakers from Belgium, Canada, Czech Republic, China, Singapore, UK and USA presented their newest results in the TRC-Forum.

Date [CET]	Title	Speaker
19th Jan 2021 5 pm	Droplet Microfluidics as a Tool for Chemical Sensing and High-throughput Screening	Robert Kennedy University of Michigan, USA
26th Jan 2021 5 pm	Adventures in multidimensional space	Tadeusz Gorecki University of Waterloo, Canada
2nd Feb 2021 5 pm	High-dimensional molecular phenomics in systems, synthetic, and chemical biology	John McLean Vanderbilt University (USA)
27th Apr 2021 5 pm	Utilizing Ion Mobility Spectrometry-Mass Spectrometry for rapid and confident exposomic analysis	Erin Baker North Carolina State University (USA)
11th May 2021 10 am	Lipidomics of Human Blood Plasma	Markus Wenk National University of Singapore
1st Jun 2021 10 am	Cell analysis on microfluidics combined with mass spectrometry	Jin-Ming Lin Tsinghua University (China)
8th June 2021 5 pm	In-depth characterization of biopharma-ceuticals using multi-dimensional liquid chromatography	Koen Sandra RIC (Belgium)
29th Jun 2021 10 am	Two-dimensional liquid chromatography for drug impurity analysis	Duxin Li Soochow University (China)
29th Jun 2021 10 am	Two-dimensional liquid chromatography for drug impurity analysis	Duxin Li Soochow University (China)
2nd Nov 2021 10 am	Multidimensional untargeted metabolomics and lipidomics empowered by ion mobility-mass spectrometry	Zheng-Jiang Zhu Shanghai Institute of Organic Chemistry (China)
9th Nov 2021 10 am	Multidimensional comprehensive GC in food analysis	Giorgia Purcaro University of Liège (Belgium)
30th Nov 2021 10 am	High-throughput lipidomics quantitation of biological samples based on mass spectrometry workflows	Michal Holčapek University of Pardubice (Czech Republic)

The TRC-Forum is organized via Zoom and I would like to invite you to have a look at the presentations and discuss them with us. If you are interested please send an email to my secretary (constanze.dietrich@uni-due.de). This can be done informally by simply stating your name. You will then receive a link to the respective lecture as soon as possible. Of course, the event is free of charge.

You can view the program on the following homepage:

https://www.trc-separation.com/trc-forum

Please note that the times given are German time, i.e. Central European Time (CET).

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. Meckelmann)

Lecture Analytical Chemistry II (in German, Prof. Dr. O. J. Schmitz)

Tutorial Analytical Chemistry II (in German, Dr. S. 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)

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. Meckelmann)

Lecture Analytical Chemistry II (in German, Prof. Dr. O. J. Schmitz)

Tutorial Analytical Chemistry II (in German, Dr. S. 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)

Exercise Environmental Chemistry: Soil and Waste (in English, Dr. M. Sulkowski)

Lecture Environmental Chemistry: Pollutants (in English, Dr. M. Sulkowski)

Tutorial Environmental Chemistry: Pollutants (in English, Dr. M. Sulkowski)

Magisterium

Lecture Environmental Chemistry: Soil (in German, Dr. M. Sulkowski)

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)

Practical Courses

Practical course analytical chemistry

Research practical courses

Teaching and Research Center for Separation

Course 1: Basci course HPLC (in German, Prof. Dr. O. J. Schmitz)

Course 2: One- and two-dimensional gas chromatography (in German, Prof. Dr. O. J. Schmitz)

Course 4: Advanced Course Liquid Chromatography (in German, Prof. Dr. O. J. Schmitz)

Course 5: LC-MS and Ion Mobility MS (in German, Prof. Dr. O. J. Schmitz)

TRC-Forum

(in Englisch, well-known scientists around the world will give a digital lecture about their research, open for everyone)

2021