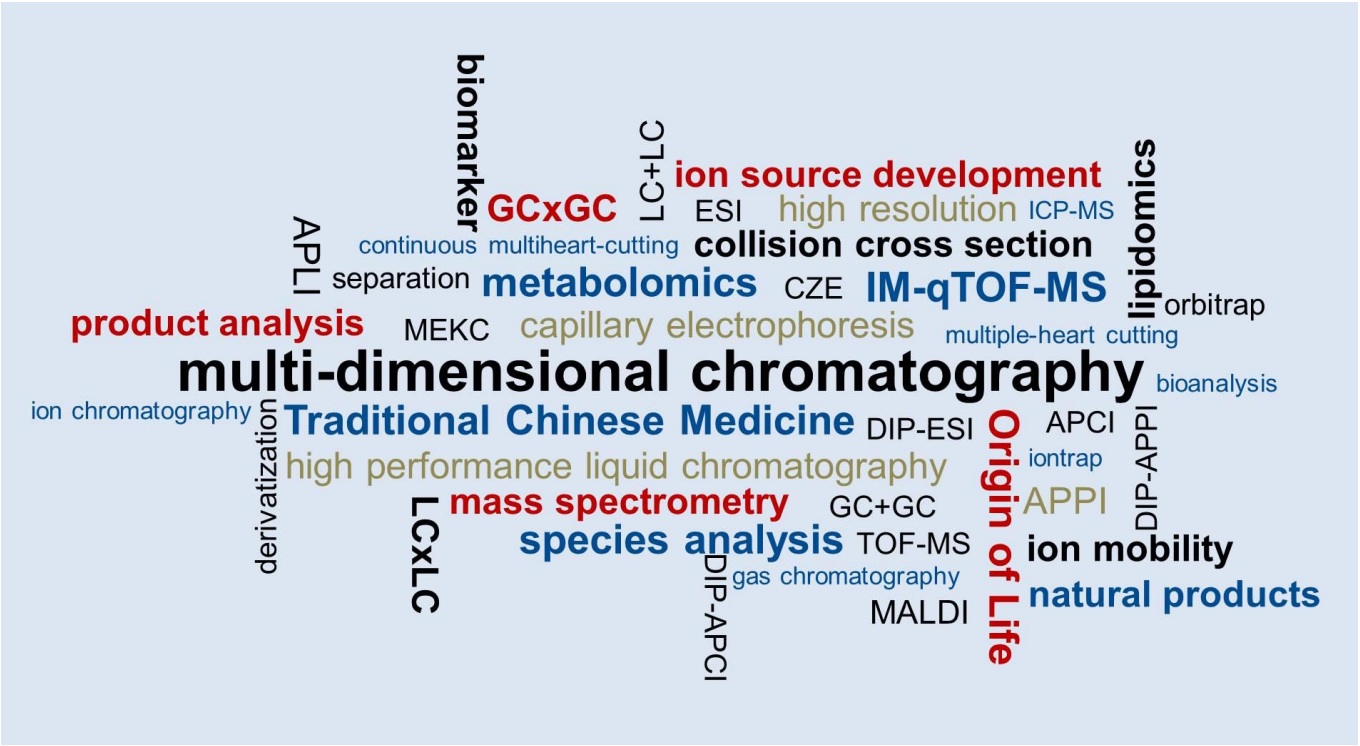
Applied Analytical Chemistry (AAC)

Annual Report 2019

applied analytical chemistry

Applied Analytical Chemistry

Annual Report 2019



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Faculty of Chemistry
Applied Analytical Chemistry
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Applied Analytical Chemistry

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 and the metal(oid) species analysis by ICP-MS in combination with gas chromatography (GC) or liquid chromatography (LC).

Prof. Dr. Oliver J. Schmitz
Head of the Research Group

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The most important topic in 2019 was that we have focused our research topics on Metabolomics/Lipidomics and Origin-of-Life. These fields will be supported by our further work in ion source development and multidimensional chromatography.

2019 was the seventh year of the Applied Analytical Chemistry research group at the University of Duisburg-Essen and a very successful one.

Nine scientific papers in peer-reviewed journals with a total impact factor of 41, two further manuscripts in review process and five in preparation, one book about Quality control of Chinese herbs (Springer) and 14 posters at national and international conferences with one poster award were published. Two doctoral theses, 3 master and 13 bachelor theses were successfully completed in 2019. In addition, several third-party funds were successfully raised and further national and international industrial cooperation were newly founded or extended. Many colleagues have contributed to an exciting year of research, teaching and last but not least to shouldering many other tasks.

During 2019 several new projects are started, e.g. development of three new ion sources (LC-LTP, GC-APPI and an ESI/APCI Dual source), LC+LC-MS with a new developed at-column dilution modulation for complex samples and investigation of the metabolome of various bacteria and archaen. Due to our outstanding equipment (many thanks to Agilent) we will not only deal with device and method development, but also work in the field of metabolomics and lipidomics. Therefore, in 2019 we started the cooperation with several working groups from the university hospital Essen and developed workflows in GC-MS and LC-MS for metabolome and lipidome analysis.

Based on our Origin-of-Life research – together with Prof. Ulrich Schreiber and Prof. Christian Mayer (both University of Duisburg-Essen), we are currently taking a 18 m long drill core (between 950 and 1000 m depth) near Laacher See in the Volcanic Eifel. This is a region in Germany, that is defined to a large extent by its volcanic geological history. Characteristic of this volcanic field are eg. volcanic tuffs, lava streams and volcanic craters like the Laacher See. The Volcanic Eifel is still volcanically active today. We hope that analyses of this core will confirm our theory regarding the formation of life in tectonic fault zones.

Nevertheless, in 2019 my group has, for the fourth time, organized the PhD seminar of the Working Group Separation Science of the Section for Analytical Chemistry of the GDCh in Hohenroda. Many thanks to Kristina Rentmeister and Timo Köhler for organizing this very successful and inspiring conference with 156 participants, 26 PhD lectures, 2 tutorials and 2 industrial talks.

As mentioned last year, it is also a pleasure and honor that the Permanent Scientific Committee of HPLC has commissioned us, Prof. Dr. Michael Lämmerhofer (University of Tübingen, Germany) and me, with the organization of the 51st International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2021), which will be held from June 20 to 24, 2021 in Düsseldorf, Germany. It will be the fourth time that the HPLC symposium series will come to Germany, after Baden-Baden in 1983, Hamburg in 1993, and Dresden in 2009. This year we have add several new information on the homepage (www.hplc2021.com) and continue the advertising to make HPLC 2021 successful. Mark your calendar! We look forward to your participation.

I want to take this opportunity to thank the entire AAC team and all co-workers for their excellent work in 2019 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 2020.

Essen, December 31, 2019

Applied Analytical Chemistry – Staff

Regular Staff

Prof. Dr. Oliver J. Schmitz	Head
Dr. Martin Sulkowski	Senior Researcher
Dr. Sven Meckelmann	Senior Researcher
Maria Madani	Technician / Lab
Birgit Wöstefeld	Secretary
Constanze Dietrich	Secretary

Post-Docs

Dr. Yingzhuang Chen, Dr. Lidia Montero, Dr. Florian Uteschil

Ph.D. Students

University Duisburg-Essen	External
Maha Alhasbani	Susanne Brüggen
Janosch Barthelmes	Annika Doell
Dominik Brecht	Tingting Li
Amela Bronja	Wiebke Mehwald
Yildiz Danisan	Niklas Danne-Rasche
Lin Gan	Dinh Lien Chi Nguyen
Simeon Horst	Bing Peng
Julia Klein	Dominic Mähler
Timo Köhler	Ruzanna Mnatsakanyan
Claudia Hellmann	
Claudia Lenzen	
Junjie Li	
Christian Lipok	
Martin Meyer	
Kristina Rentmeister	
Alexandra von Trotha	

M.Sc. Students

Alam Ashraful, Karim Chowdhory, Martin Dißner, Kendra Majewski (external)

B.Sc. Students

Imke Ackermann, Zaid Awad, Enise Bekar, Annika Funck, Jasmine Heine, Katharina Hellmann, Laurin Grabler, Jiang Luo, Simon Schastok, Hannes Schlottmann, Kevin Schulz, Philipp Swiderski, Kübra Temel, Pia Wittenhofer

Guest Scientists

Prof. Abdalla Elbashir (Khartoum University, Sudan) with a Alexander-von-Humboldt fellowship
Assistant-Prof. Taher Sahlabji (King Khalid University, Saudi Arabia)

Apprentices

Marcel Nolte, Christian Müller, Kim Verholen

Major News 2019

Teaching and Research Center for Separation

As mentioned last year, 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.

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). In addition to research, the focus of the center will be 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.

After a year of experience with this kind of courses, we have reduced the duration of the courses (1.5 day theory and 1.5 day practical course) despite the very good evaluation of the participants, in order to make the courses even more attractive for the industry.

The next teaching courses will be given on:

02. – 05.03.2020	1D- and 2D-GC
16. – 19.03.2020	GC-MS
07. – 09.09.2020	LC-MS and Ion Mobility-MS
21. – 23.09.2020	ICP-OES, ICP-MS and CE
19. – 21.10.2020	Basic Course Liquid Chromatography (Theory and HPLC)
07. – 09.12.2020	Advanced Course Liquid Chromatography (2D-LC, LCxLC, SFC)

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

6th International Ion Mobility Spectrometry (IMS) Meeting

In partnership with Agilent Technologies we will host the 6th International Ion Mobility Spectrometry (IMS) Meeting at the University of Duisburg-Essen (Campus Essen) in Germany on 18-19th February 2020. The scientific meeting provides an excellent forum for delegates to network with international guest speakers and Agilend R&D scientists about the newest fields in IM-MS.

Day 1 will consist of a full day of scientific IMS presentations delivered by guest speakers and Agilent R&D Scientists. The wide range of topics will be of interest to scientists that are interested in learning about the application of IMS for small and large molecule applications.

This first day is open to all scientists, regardless of their experience.

Day 2 comprises of in-depth training workshops covering different application areas using Agilent IMS and therefore the second day is designed to be primarily of interest to current users of the Agilent's 6560 IMS system.

Both days are free of charge and will provide an excellent forum for delegates to network with guest international speakers and Agilent R&D scientists. Complimentary lunch and coffee breaks are provided for delegates.

HPLC 2021 in Düsseldorf, Germany

It is a great pleasure to announce that the 51st International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2021) will be held at June 20-24, 2021 in Düsseldorf, Germany. Prof. Michael Lämmerhofer from the University of Tübingen and Prof. Oliver J. Schmitz from the University of Duisburg-Essen are the chairmen of this conference.

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 2021 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.hplc2021-duesseldorf.com

Obituary

PD Dr. Hans-Georg Schmarr

June 16, 1961 – August 20, 2019

Dr. Hans-Georg (Geo) Schmarr was an established food chemist, wine analyst and aroma researcher who worked for many years at the Service Centre Rural Area Rheinpfalz in Neustadt an der Weinstraße.

Through his habilitation, which he completed in 2015 in Analytical Chemistry at the University of Duisburg-Essen, he was closely associated with the Faculty of Chemistry at the University of Duisburg-Essen.

Geo Schmarr was an excellent scientist with an amazing detailed knowledge in the field of gas chromatography as well as of the main object of his research, wine, its aromas and its falsification. We will miss the hard fought table soccer games in Hohenroda with Geo very much. With him we are losing a colleague and a friend whom we will remember with pleasure.

Hero of the Year 2019

This time I would like to thank especially **Julia Klein**, who published 5 papers in peer-reviewed journals in her doctoral thesis (total IF 32) and got the 3rd International Poster Prize at the analytica conference Vietnam 2019. In this year she had unbelievable bad luck with defective devices but she never lost her courage and bravely repaired the systems.

List of Projects 2019

(Abstracts of these projects within the next pages)

Analysis of complex samples using multidimensional separation and detection techniques
Julia Klein, Sven Meckelmann

At column dilution (ACD) modulator developing for flexible and precise control dilution factors to ovcome mobile phase incompatibility in comprehensive two-dimensional liquid chromatography
Yingzhuang Chen, Junjie Li

Development of a method for quantification of pesticides in green tea using online SPE-HPLC-QqQ-MS
Simon Jan Schastok, Florian Uteschil

SFC- and HILIC-MS analysis of carbohydrates in archaeal biofilms
Martin Meyer

Characterization of HepG2 cell lipidome
Kristina Rentmeister, Sven Meckelmann

Lipidomic profiling of pancreatic cancer cells and corresponding liver metastases
Sven W. Meckelmann

Characterization of the metabolome from *Pseudomonas aeruginosa* in biofilm as lung infection model
I. Ackermann, T. Köhler

Development of a new GC-APCI ion source
Christian Lipok

Thermogravimetry coupled to an atmospheric pressure photo ionization quadrupole mass spectrometry
Dominik Brecht, Florian Uteschil

Thermogravitmetry coupled with mass spectrometry (TG-APPI-MS) for the analysis of honey
Maha Alhasbani, Florian Uteschil

Development of an ESI and APCI dual ionization source
Dominik Brecht, Florian Uteschil

Development of a LTP ion source for LC-MS
Dominik Brecht, Florian Uteschil

Origin of Life – Origin of Life in Deep-Reaching Tectonic Faults
Oliver J. Schmitz

Origin of Life – Molecular Evolution in a Peptide-Vesicle System
Yildiz Danisan, Martin Sulkowski

Ozone Stress Effect on the Intracellular Metabolites from Cobetia Marina by Two-dimensional Gas Chromatography with Mass Spectrometer
Junjie Li

LCxLC and LC+LC coupled to mass spectrometry for the analysis of chemical constituents in *Buddleja davidii* root
Jiang Luo, Enise Bekar, Lidia Montero, Yingzhuang Chen

A novel five-dimensional µLC+LC-IM-qTOF-MS/MS separation method for the analysis of complex proteomic samples
Annika Funck, Lidia Montero

Application of Comprehensive 2D-LC Coupled to MS using Mixed-Mode in the First Dimension and Reversed Mode in the Second Dimension for the Separation of Polar Compounds in Real Food Samples
Katharina Hellmann, Lidia Montero

Mass spec based immuno-capture assay enables full characterization of therapeutic antibodies after injection *in-vivo*
Annika Doell

Analysis of complex samples using multidimensional separation and detection techniques

Julia Klein, Sven W. Meckelmann

Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) is a powerful technique for the non-target analysis of complex samples. However, there are several challenges analysing very complex matrices. For example, in case of coelution of several compounds ion suppression takes place during the ionisation process. Besides, coelution of isobaric compounds is possible that cannot be separated by HRMS or by tandem-MS (MS/MS) in case of similar fragmentation.

One possible approach to improve the separation power is increasing the number of separation dimensions, e.g. by using comprehensive liquid chromatography (LCxLC) or by using liquid chromato-graphy based on a continuous multiple heart-cutting approach (LC+LC). The coupling with ion mobility spectrometry (IMS) offers the possibility of an additional separation dimension by separating compounds in terms of their shape-to-charge ratio. Furthermore, IMS enables the identification of substances according to their collision cross section (CCS). Coupling of two-dimensional liquid chromatography to ion mobility-quadrupole time-of-flight mass spectrometry (LCxLC- or LC+LC-IM-qTOF-MS) is a powerful four-dimensional separation and detection technique. To evaluate the separation power of different LC techniques, three complex matrices (plant extract of Traditional Chinese Medicine, waste water and biocoal) were analysed under comparable conditions using LC, LCxLC and LC+LC, each using IM-qTOF-MS as detector. In comparison with LC analyses, LCxLC and LC+LC showed significantly less ion suppression. Moreover, ion mobility enabled the identification of isobaric compounds after chromatographic separation according to their CCS, which would not be possible with HRMS and accurate masses only.

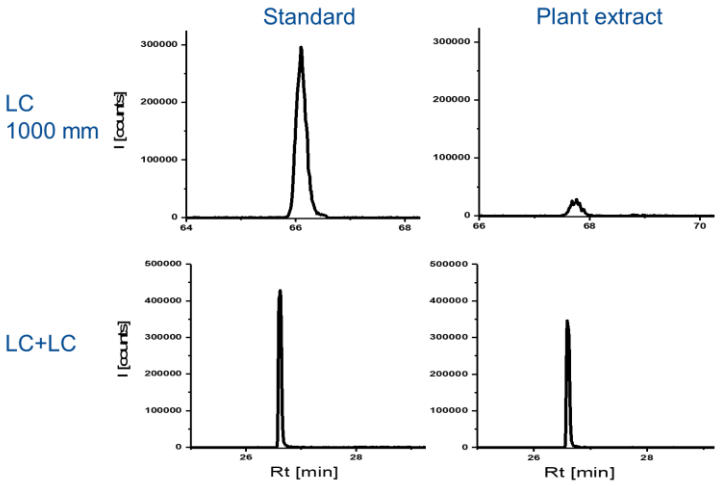


Figure: Extracted Ion Chromatograms (EICs) of 5-Methylbenzotriazole spiked in a blank (left) and plant extract sample (right), each analyzed with 1D LC (1000 mm) (top) and with LC+LC (bottom).

Collaborative Project – Project Partner: Dr. T. Teutenberg (IUTA, Duisburg, Germany) and Dr. J. Fjeldsted (Agilent Technologies (Santa Clara, USA)

Funded by: IGF Nr. N 03189/15

At column dilution (ACD) modulator developing for flexible and precise control dilution factors to ovcome mobile phase incompatibility in comprehensive two-dimensional liquid chromatography

Yingzhuang Chen, Junjie Li

With the combination of different mechanisms, two-dimensional liquid chromatography has brought revolutionary changes compared to the traditional one-dimensional separation, which dramatically improves the peak capacity in separation and meets the ever-increasing demand for the analysis of complex sample in different researching field, such as chemistry, medicine, etc. However, the incompatibilities between two columns due to the transport of the large sample volume and the solvent effect always limit the widely use of two-dimensional liquid chromatography. In order to resolve this problem, an at-column dilution (ACD) modulator, was established to overcome the solvent incompatibility in the orthogonal combination within the comprehensive two-dimensional liquid chromatography. This interface is modified from normal two-dimensional interfaces by an additional transfer pump, which realize the at-column dilution without a flow split during the transportation. Moreover, with the control of the transfer flow and the second-dimensional gradient flow, it is able to precisely regulate the at-column dilution factor and conveniently optimize the separation conditions in both dimensions.

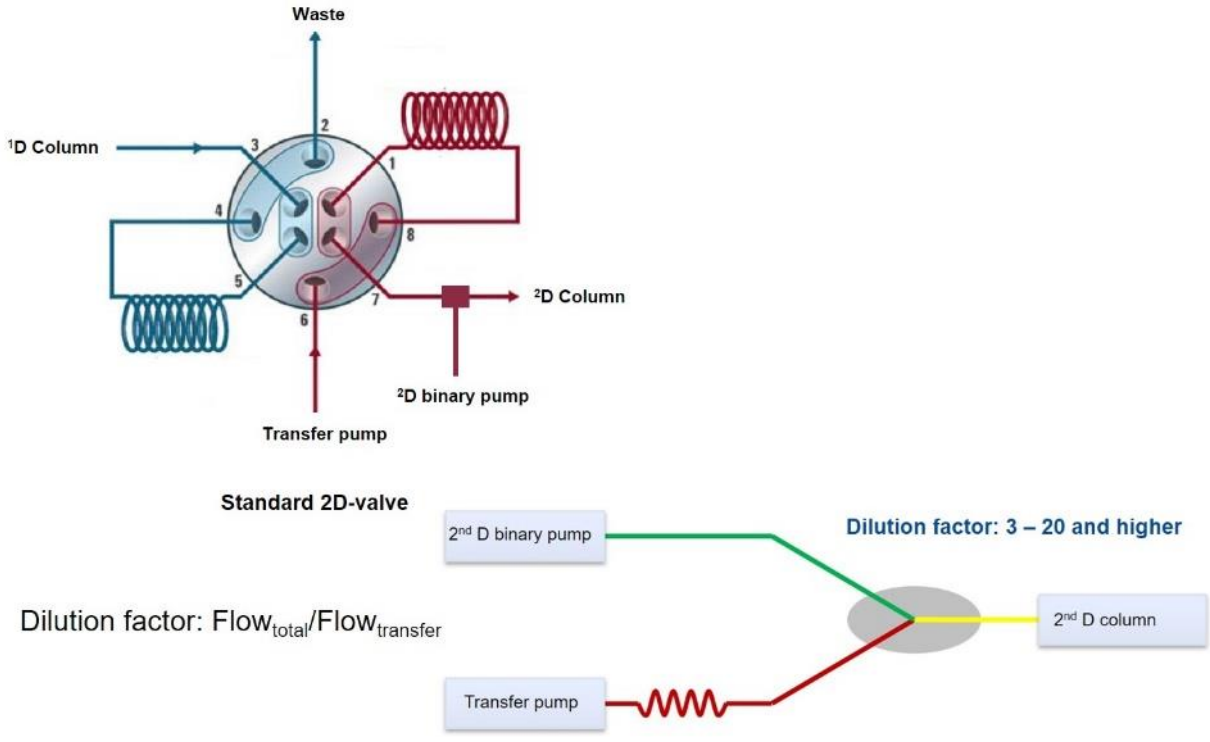


Figure: Scheme and principle of the ACD modulation

Funded by: China Scholarship council (CSC) and Agilent Technologies (Waldbronn, Germany)

Development of a method for quantification of pesticides in green tea using online SPE-HPLC-QqQ-MS

Simon Jan Schastok, Florian Uteschil

The methodology developed for more than 100 pesticides has proven to be practicable in the absence of real sample matrix for both on-column and online SPE injections despite negative influences on peak geometry in the form of tailing (online SPE). The conversion of the developed and optimized LC-MS methodology to an online SPE-LC-MS was carried out by extension of the necessary switching conditions and adaptation of the retention times. The enrichment and recovery of analytes were successful, with the exception of two analytes, acephate and methamidophos. An enrichment factor of 100 was sufficient to reduce the LOD and LOQ by 10. This can be considered promising with regard to possible optimization of the online SPE, due to the possibility of enriching even larger volumes than were used in the practical implementation of this work. In addition our new developed at-coloumn modulation can help us to reduce the peak width on the LC column, which could result in better sensitivity. The final quantification can be done by standard addition methods or optionally by isotope-labelled pesticide standards.

Figure: Online-SPE-LC-QqQ-MS

As a conclusion of the conducted comparative investigations it can be stated that the transfer of an on-column injection to online SPE injection is possible by adapting a small number of parameters in the range of LC and MS. However, the comparability of both injection procedures is less analogous. Online SPE peaks tend to tailing effects and as has been observed. Successful focussing at the LC column after elution form the online-SPE is strongly polarity-dependent, which makes the applicability to a broad spectra of analytes difficult in individual cases or even impossible by online SPE injection. Here, our at-column modulation should be a useful tool to minimize these problems.

Funded by: Agilent Technologies (Waldbronn, Germany)

SFC- and HILIC-MS analysis of carbohydrates in archaeal biofilms

Martin Meyer

Microorganisms, such as archaea, favour life in a biofilm rather than the planktonic form of life. A biofilm is defined as a community of microorganisms embedded in a self-produced matrix of hydrated extracellular polymeric substances, mainly polysaccharides (PS), proteins and DNA. The polysaccharides form a three-dimensional network, which provides stability of the biofilm and mediates the adhesion to surfaces.

Analysis of the monomeric composition of polysaccharides requires sample preparation by hydrolysis of the PS and subsequent chromatographic separation and identification by mass spectrometry (MS). The similar polarity and exact same masses of different monosaccharides from the same class of carbohydrates and the comparable polarity of the different carbohydrate classes themselves impede the analysis with classical reversed-phase chromatographic approaches without extensive sample praparation, such as derivatization. One resort towards this problem is supercritical fluid chromatography (SFC), which provides several advantages compared to classical reversed-phase chromatography, such as no need for derivatization, faster analysis due to higher flow rates and gentle ionization conditions. A SFC-MS method was developed, which allows screening and quantification of the main monosaccharide components of biofilms from *Sulfolobus acidocaldarius* with concentrations in the low µmol/L range. With this method, the main components in the PS of *S. aci.* biofilms were found to be Ribose, Mannose and Glucose. Additionally, a HILIC-MS method using a zwitterionic phase and high pH of the mobile phase was used for the analysis of the monosaccharide composition. Here, the limit of detection was even lower, resulting in analyzable concentrations up to the sub µmol/L range.

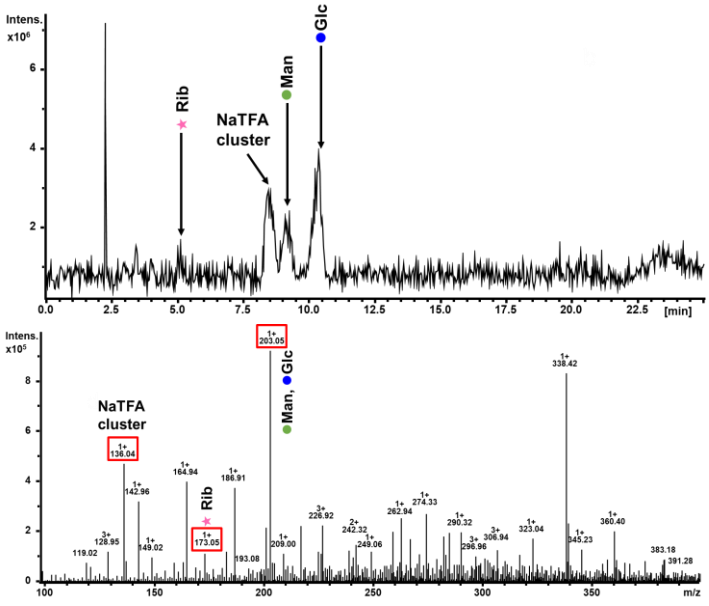


Figure: SFC-MS base peak chromatogram and corresponding mass spectrum of hydrolysated EPS extract from an archaeal biofilm.

Collaborative Project – Project Partner: Prof. Dr. B. Siebers, Dr. J. Wingender (University of Duisburg-Essen)
Funded by: DFG Project number: 393406057

Characterization of HepG2 cell lipidome

Kristina Rentmeister, Sven Meckelmann

Effects are mostly determined simply by cell viability or only the influence of a drug in a known metabolomic pathway is investigated. Only few studies concentrate on investigating the complete metabolome of HepG2 cells. Knowing the complete metabolome and lipidome is essential to study large sets of pathways simultaneously to understand the impact of growing factors (e.g. culturing conditions or supplementation of bioactive compounds) in biological studies more accurately.

Lipids are separated on a Waters ACQUITY UPLC CSH C18 column (2.1x100 cm, 1.7 µm) using a 16 min gradient with a mixture of water/acetonitrile and acetonitrile/isopropanol. Detection of the eluting lipids was performed by ion mobility time of flight mass spectrometry

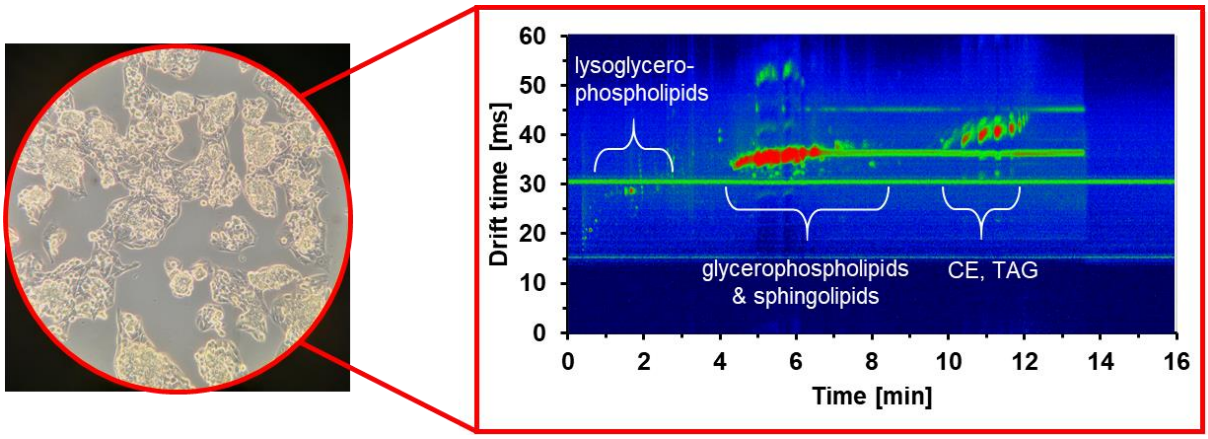


Figure: Lipid analysis with LC-IM-qTOF-MS/MS

(IM-qTOF-MS). This setup of chromatographic separation coupled with IM-qTOF-MS allows an identification of the detected lipids based on their retention time, drift time or collison cross section (CCS), accurate mass, as well as MS/MS spectra. Since fragmentation takes place after the drift tube, fragment ions have the same drift time as their precursor ions and can be easily assigned and due to the prior IM separation, the resulting MS/MS spectra are less prone to background noise.

The instrumental method as well as the bioinformatic data treatment was optimized using a set of 13 lipids, covering all major lipid classes and applied to HepG2 cells to characterize their lipidome.

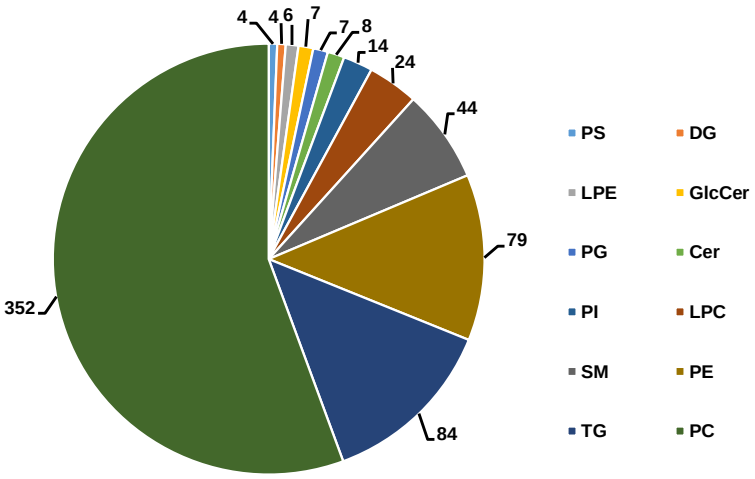
Lipidomic profiling of pancreatic cancer cells and corresponding liver metastases

Sven W. Meckelmann

People diagnosed with pancreatic cancer have a very poor prognosis as in early disease stages almost no symptoms occur. Only in later stages, symptoms are specific enough to suggest pancreatic cancer and by that time the cancer has often spread into other parts of the body. Therefore, only 25% of the patients survive the first year and one of the first targets for metastases is the liver. However, the underlying biological process of the disease progression are yet poorly understood.

Together with the University Hospital Essen (AK Grüner), we continued with the characterization of the lipidome of isolated pancreatic cancer cells and matching cells of a liver metastasis. However, to get more insights into the lipid metabolism the cells were treated with different inhibitors to simulate a biological challenge similar to a therapeutic treatment in patients. Afterwards, lipids from the cells were extracted and analysed using the lipidomics workflow by means of LC-IM-qTOF-MS.

After feature analysis, data clean-up and identification 633 features out of a total of 1882 features have been found.



The figure on left side gives a brief overview of the different lipid classes as well as the diversity of lipids that have been identified in the samples. Currently, the data is analysed by bioinformatic and statistical means (e.g. pathway analysis) to identify changes in the lipid profile caused by the different inhibitors.

Figure: Overview about the different lipid classes analyzed by LC-IM-qTOF-MS

Collaborative Project – Project Partner: Research group of B. Gruener from the University Clinic Essen (UKE) of the University of Duisburg-Essen

Characterization of the metabolome from *Pseudomonas aeruginosa* in biofilm as lung infection model

I. Ackermann, T. Köhler

Cystic fibrosis (CF) is an autosomal recessive inherited disease which leads to a production of thickened mucus in the airways. These conditions are conducive to poly-microbial infections, like chronic lung infection, in which *Pseudomonas aeruginosa* is the major pathogenic bacterium colonizing CF-lungs at the end of the lifetime of CF patients. This *in vitro* study uses a *P. aeruginosa* biofilm model under partly cystic fibrosis conditions, with a sampling of volatile extracellular metabolites. The gas sampling was done with thin film microextraction (TFME) and commercial polydimethylsiloxane (PDMS) films, whereas the analysis of loaded films was done by gas chromatography coupled to quadrupole mass spectrometry and thermodesorption (TD-GC-qMS). The TD-GC-qMS method was successfully used for standards of volatile metabolites which were known to be produced by *P. aeruginosa*. Limits of detection and quantification for middle and less polar compounds in low nanomolar range (0.5 nM and 1.5 nM) were achieved. The determined LODs and LOQs are within the clinically relevant concentration range for sputum samples. The developed method was finally applied to investigate the extracellular volatile metabolites produced by biofilms of the strain *P. aeruginosa* DSM 50071 under aerobic and anaerobic conditions. It was shown in this study, that different oxygen conditions (aerobic and anaerobic) resulted in emitting different extracellular volatile metabolites. Specific metabolites, like 1-undecene (aerobic) and 2-undecanone (anaerobic), could be identified. The results are promising, that the biofilm model may be applicable for the identification of P. aeruginosa under clinical conditions. Furthermore, the model could be the basis for studying extracellular volatile metabolites from different mono or co-cultures of various bacteria, as well as the implementation of pulmonary conditions, like these in CF lungs. This possibility allows the development of a non-invasive "at-bedside" breath analysis method for CF patients in focus of various bacterial infections.

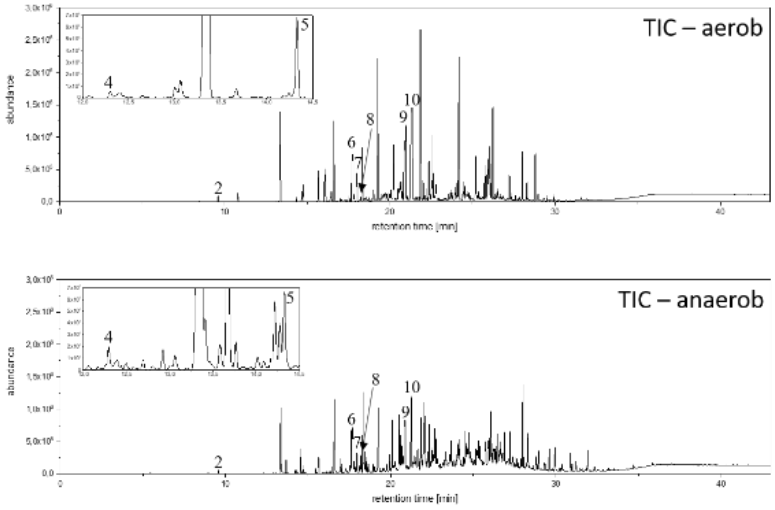


Figure: Comparison of aerob and anaerob cultivation

Collaborative Project – Project Partner: PD Dr. U. Telgheder, Dr. J. Wingender (University of Duisburg-Essen, Germany)

Funded by: DFG project number: 352241003

Development of a new GC-APCI ion source

Christian Lipok

Gas chromatography (GC) has been used since the early 1950 and is a well-known technique in analytical chemistry. The technology was usually coupled to thermal conductivity cells and flame ionization detectors (FID). Around 1960, the GC was frequently coupled to mass spectrometers (MS). Today, GC-MS is used in routine analysis using electron impact (EI) as ionization method. Normally, EI is used with 70 eV and the generated mass spectra show a high degree of fragmentation. Therefore, many substances show poor sensitivities and the determination of the molecular mass of the molecule can be challenging or even impossible. To improve the sensitivity and selectivity of GC-MS, the chemical ionization detector (CI) was developed. CI was firstly used at elevated pressures however it was desired to operate chemical ionization at higher pressures to increase ionization yield. In 1973, Hornig et al. introduced the chemical ionization at atmospheric pressure (APCI) technique. This soft ionization technique normally produces protonated molecular ions and hence, high sensitivity for a lot of substances is achieved. Due to missing databases and the lack of commercially available ion sources, APCI was not the first choice as ion source. This changed in 2006 when GC-APCI ion source was reported and coupled to a commercial liquid chromatography (LC) mass spectrometer. Nowadays, commercial APCI ion sources are available but some limitations must be adressed. The aforementioned ion sources show bad reproducibility with standard deviations (RSD) often higher than 20%. Therefore, this technology needs further improvement.

To improve the potential of the APCI ion source technology we selected a new design for the ion source chamber, which is based on a published GC-APPI design. The figure shows the schematic drawing of the new APCI ion source. The ion source was improved regarding sensitivity and repeatability. The column position, the make-up gas flow rate and its humidity was optimized. The make-up gas flow enhances the transportation of the analytes from the GC-transfer line exit port to the hexabore transfer capillary of the mass spectrometer. The new design improves the reproducibility of the APCI source and leads to relative standard deviation ≤ 2% for various chemical compound classes commonly analyzed by GC. Furthermore, the new APCI ion source was applied on real samples. Different pesticide classes in coffee beans were determined with RSD values between 2 and 7%.

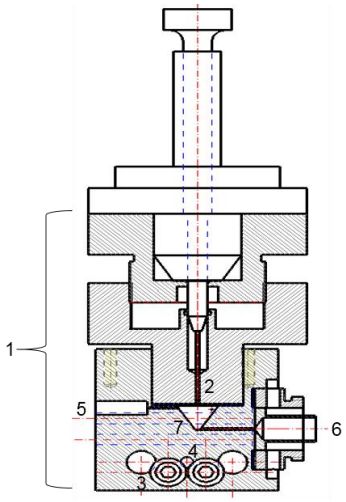


Figure: Ion source body (1); corona needle (2); heating cartridge (3); thermometer (4); GC column (5); transfer line (6); ionization volume (7)

Funded by: Agilent Technologies (Santa Clara, USA)

Thermogravimetry coupled to an atmospheric pressure photo ionization quadrupole mass spectrometry

Dominik Brecht, Florian Uteschil

The plasticizers of the ortho phthalic ester pose an emerging problem in the environment. The plasticizers are found in sediments and cosmetics due to the excessive use in the polymer industry. This became important in 2005 when the RoHS (Restriction of Hazardous Substances) regulation was established by the European Union. It regulates the use of plasticizers, flame retardants and heavy metals in plastics and electronic devices for the protection of the consumers. One candidate of the ortho phthalic esters is dibutyl phthalate which is restricted by the RoHS regulation. The development of a thermogravimetry coupled to an atmospheric pressure photoionization mass spectrometry (TG-APPI-MS) with a high temperature and flexible transfer line was realized. A method was developed to analyze plasticizers in solution which consist of a solvent evaporation step and subsequent evaporation of the analyte. These solutions of dibutyl phthalate (DBP) in hexane were used to investigate the repeatability (RSD: 3.6%) and linearity (R^2: 0.9995) of the new developed system. With the new device the detection of different phthalates in a standardized PVC (polyvinyl chloride) polymer was possible. On the example of Acetylsalicylic acid (ASA), the degradation of a pharmaceutical drug was investigated. The dimerization and the possible trimerization of ASA during the thermal degradation was shown. Ten tablets of different ASA manufacturers were analyzed with the new developed analysis platform. The active substance was found in every tablet. Differences in mass spectral data as well as the studying of the pack insert were used to assign the tablets to companies and their subsidiaries. A unique formulation of ASA was found to have a different mass pattern when analyzed with TG-APPI-qMS. The developed device is a promising tool for the product control and the identification of falsified drugs.

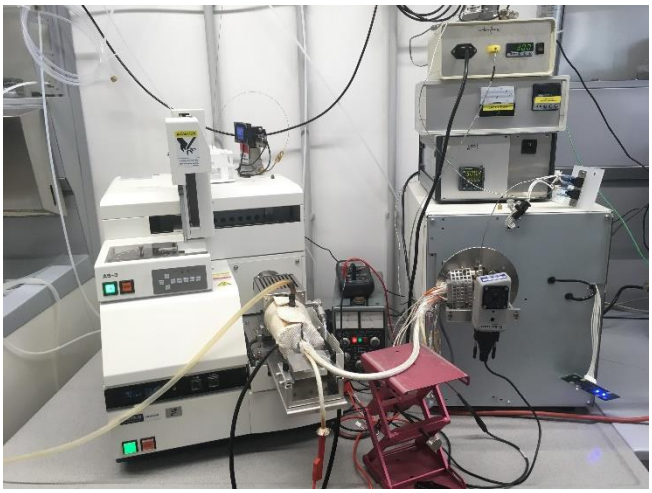


Figure: TG-APPI-qMS

Collaborative Project – Project Partner: Y. Terui and K. Maruoka (Hitachi High-Tech, Mito, Japan)

Funded by: Hitachi High-Tech (Tokyo, Japan)

Thermogravimetry coupled with mass spectrometry (TG-APPI-MS) for the analysis of honey

Maha Alhasbani, Florian Uteschil

The connection of thermogravimetry (TG) with mass spectrometry by using a transfer line and an APPI source opens a wide range of possible applications such as quality control of foods, drugs, polymers etc. In addition temperature-dependent reactions can be investigated.

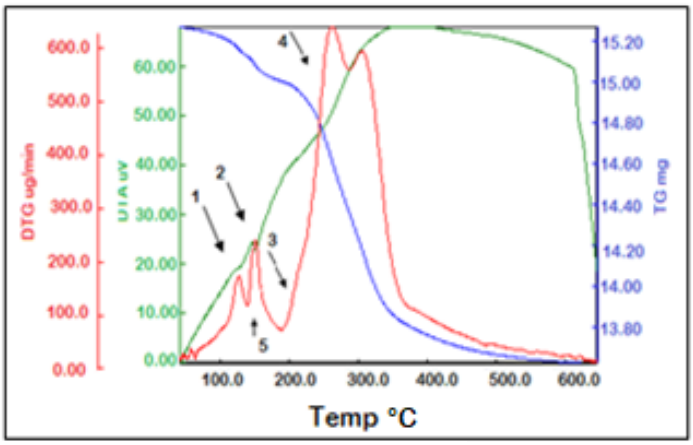


Figure 1: DTG curve of honey sample

In this work, several honey samples were analyzed for quality control. With thermogravimetry the moisture (1), degradations of component (2), carbonization (3) and oxidation of organic matter (4) of honey could be investigated. The melting point from the DTA curve was determined at 150.6°C (5).

The TG was coupled to an MS and the Figure 2 shows the results of one honey sample. In the near future, we will couple the TG to a high-resolution MS to increase the information content of the analysis.

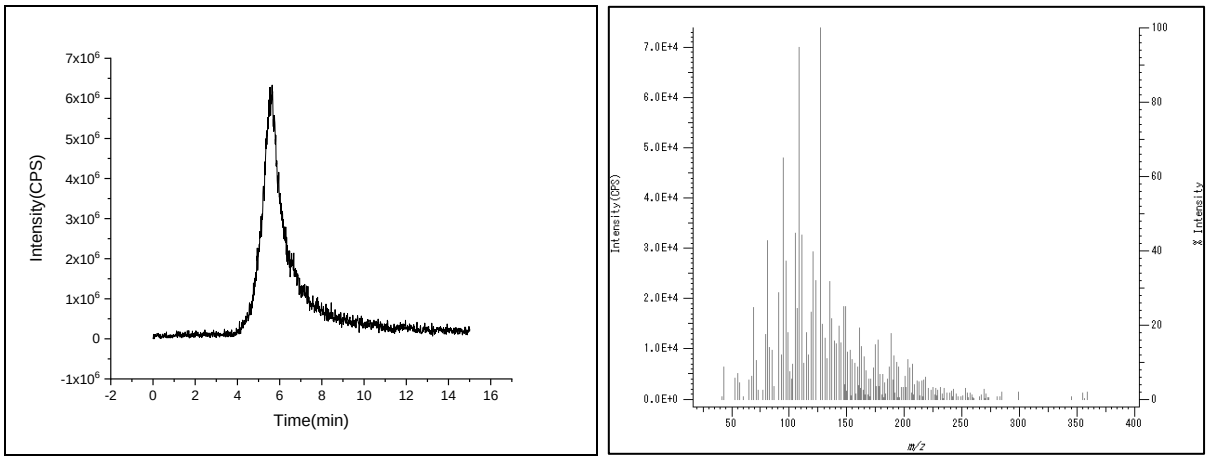


Figure 2: Chronogram recorded by MS and the whole time mass spectrum

Collaborative Project – Project Partner: Y. Terui and K. Maruoka (Hitachi High-Tech, Mito, Japan)
Funded by: Hitachi High-Tech (Tokyo, Japan)

Development of an ESI and APCI dual ionization source

Dominik Brecht, Florian Uteschil

The demand on high throughput methods for LC-MS is of growing interest in the field of analytical chemistry. Therefore, our group is working on solutions to increase the sample throughput in chromatographic analyses hyphenated mass spectrometry-based systems. A special attention lies on the development of ion sources for the mass spectrometry. In this work an ion source was developed which is capable to ionize the analyte molecules with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). This new ion source shall reduce the delay time of a mass spectrometer to increase the efficiency of mass spectrometric analyses. The challenge of this project is to construct an ion source which is comparable to single probe ion sources, but compromises must be made because ESI and APCI ionization are based on different ionization mechanism. ESI ionization takes place in the liquid phase and APCI in the gas phase, so there is a challenge to find the right temperature to use both probes as efficient as it is possible. Another challenge is the right choice of the gas flow rates which influence the ionization of the ESI and APCI drastically.

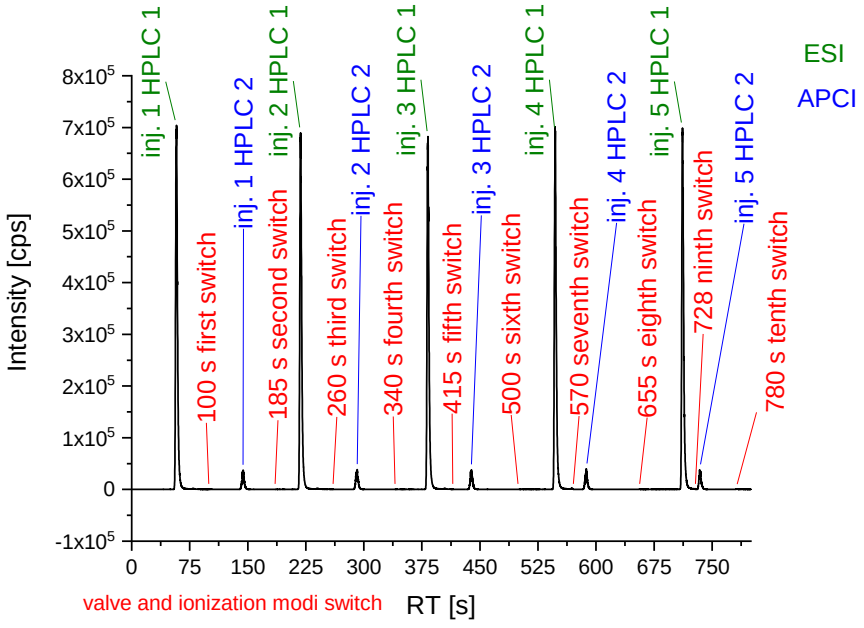


Figure: Mass spectra with ESI/APCI ionization mode change

The dual ion source was constructed by combining the ESI and the APCI probe in one ion source housing, resulting in two inlets for the eluent of the HPLC. The high sample throughput is realized by connecting two HPLCs with a six-port switching valve which switches between the two HPLCs and the two probes of the ion source. The figure shows the analyses of testosterone (HPLC1) and vitamin D_3 (HPLC2) with the new developed dual ion source. The HPLCs injected five times the analyte so that in total ten analysis in one run were performed. The Area of the ten peaks are reproduceable and it could be shown that the performance of a mass spectrometer can be increased by the dual ESI/APCI ion source.

Collaborative Project – Project Partner: Y. Terui and K. Maruoka (Hitachi High-Tech, Mito, Japan)
Funded by: Evonik AG and Hitachi High-Tech (Tokyo, Japan)

Development of a LTP ion source for LC-MS

Dominik Brecht, Florian Uteschil

The term LTP is the abbreviation for low temperature plasma which can be explained to its ambient temperature compared to the inductive coupled plasma like it is used in the ICP. The LTP is based on a discharge in the presence of noble gases (e.g. Helium, Argon) or in environmental gases like nitrogen, oxygen or air. This LTP is a non-equilibrium plasma which is usually ignited in a dielectric barrier discharge (DBD) by a sine wave voltage function or by high voltage pulses at defined pulse widths and frequencies. In a DBD at least one electrode is covered by a dielectricum (glass, quartz) to prevent the discharge from arcing. There are many applications in the industry for the LTP – for example surface modifications of polymers or medical applications like sterilisation of bacterial grow media or wounds. Therefore it is obvious that the LTP is generating a diverse ion chemistry which can also be used as ion source for mass spectrometry.

Until now there are only a few publications coupling the LTP with liquid chromatography to a mass spectrometer. Here we demonstrate the coupling of an LC-LTP-MS/MS on the example of caffeine (see figure). The two photographs present the operation of the LTP ionization probe during the analysis. The plasma is recognizable at light and even better in the dark. The plasma is ignited at the inner elctrode and is evolving from the ignition point towards the ambient atmosphere. The plasma at the outside of the dielectricum is called after glow and is here especially pronounced. An outstanding perfomance of the LTP is shown by the dilution series of caffeine with an instrumental limit of detection of 10 ng/L (ppt).

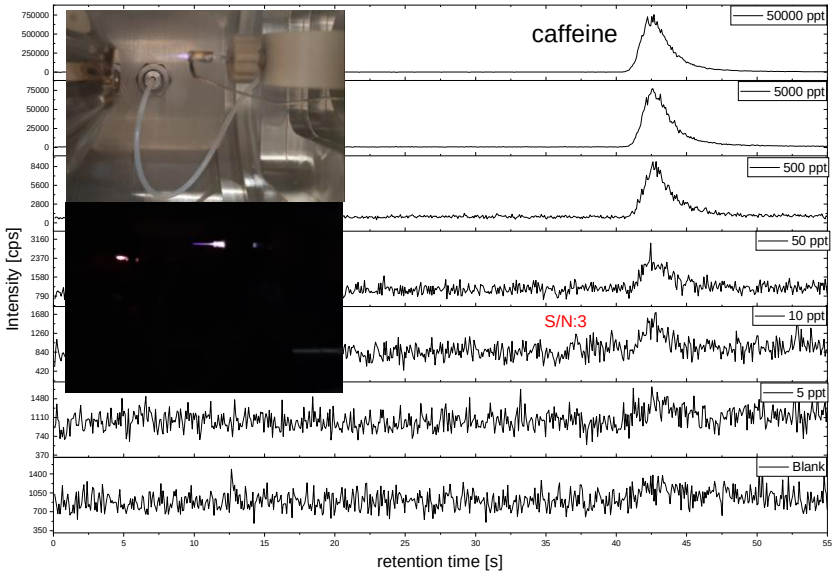


Figure: Sensitivity of LTP ionization for caffeine

Collaborative Project – Project Partner: Y. Terui and K. Maruoka (Hitachi High-Tech, Mito, Japan)

Funded by: Evonik AG and Hitachi High-Tech (Tokyo, Japan)

Origin of Life – Origin of Life in Deep-Reaching Tectonic Faults

Oliver J. Schmitz

The origin of life is still an unsolved mystery in science. Hypothetically, prebiotic chemistry and the formation of protocells may have evolved in the hydrothermal environment of tectonic fault zones in the upper continental crust, an environment where sensitive molecules are well protected against degradation induced e.g. by UV radiation. The composition of fluid inclusions in minerals such as quartz which have grown in this environment during the Archean period might provide important information about the first organic molecules formed by hydrothermal synthesis.

In fluid inclusions of Archean quartz minerals from Western Australia we found a variety of organic compounds such as alkanes, halocarbons, alcohols and aldehydes which unambiguously show that simple and even more complex prebiotic organic molecules have been formed by hydrothermal processes. Stable-isotope analysis confirms that the methane found in the inclusions has most likely been formed from abiotic sources by hydrothermal chemistry. Obviously, the liquid phase in the continental Archean crust provided an interesting choice of functional organic molecules. We conclude that these organic substances could have made an important contribution to prebiotic chemistry which might eventually have led to the formation of the first living cell.

Figure: Drill core from Wehrer Kessel

To support this thesis, we have now taken part in a drilling in the Wehrer Kessel in the Vulkaneifel, Germany. This is a region in Germany, that is defined to a large extent by its volcanic geological history. Characteristic of this volcanic field are e.g. volcanic tuffs, lava streams and volcanic craters like the Laacher See. The Volcanic Eifel is still volcanically active today. We had taken a 18 m long drill core (between 950 and 968 m depth). In this region, the transition from CO_2 supercritical to CO_2 subcritical should have taken place. According to our theory, in this transition substances that have formed at depth must have precipitated due to the loss of solubility at the transition to CO_2 in the subcritical temperature range. We now hope to be able to detect substances similar to those found in the quartz samples from Western Australia.

Figure: Calcite layer in the core

Collaborative Project – Project Partner: Prof. U. Schreiber and Prof. C. Mayer (University of Duisburg-Essen)

Origin of Life – Molecular Evolution in a Peptide-Vesicle System

Yildiz Danisan, Martin Sulkowski

Based on a new model of a possible origin of life, we propose an efficient and stable system undergoing structural reproduction, self-optimization, and molecular evolution. This system is formed under "realistic" conditions by the interaction of two "cyclic" processes. These two interrelated, different processes (vesicles as the structural environment and supplying peptides from a variety of amino acids as versatile building blocks) strengthen each other synergistically. We demonstrate that structures growing in a combination of both cycles have the potential to support their own existence, to undergo chemical and structural evolution, and to develop unpredicted functional properties. The key mechanism is the supposable mutual stabilization of the peptides by the vesicles and of the vesicles by the peptides together with a constant production and selection of both. The development of the proposed system over time would not only represent one of the principles of life, but could also be a model for the formation of self-evolving structures ultimately leading to the first living cell. The experiment yields clear evidence for a vesicle-induced accumulation of membrane-interacting peptide that could be identified by liquid chromatography combined with high-resolution mass spectroscopy. We found that the selected peptide has an immediate effect on the vesicles, leading to reduced vesicle size, increased vesicle membrane permeability, and improved thermal vesicle stability.

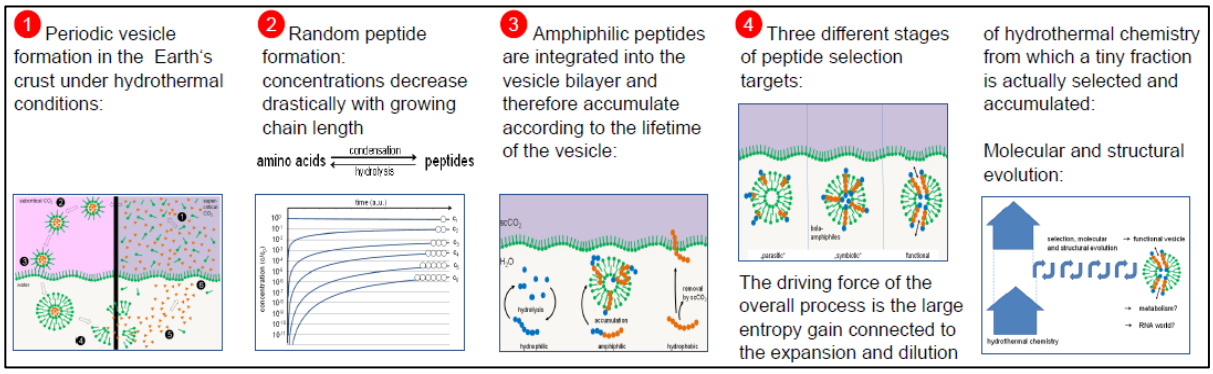


Figure 1: Idea and simulation

The experiment confirmed the underlying theory for the formation of precursors of life. It is an evolutionary process in which peptides are formed from amino acids. This process works synergistically with the stabilization of vesicles, which in turn stabilize longer-chain peptides. At the same time, the life time of the peptides increases due to the strapping. Although the great variety of possibilities of the underlying amino acids to form peptides can lead to different peptides over time. A set of preferred peptides should be formed over a long period of time (far beyond the capabilities of the experiment), which can serve as starting material for emerging life.In this context, the vesicles are of particular importance, as they stabilize the system and thus support the formation of a platform that can ultimately lead to a RNA world.

Collaborative Project – Project Partner: Prof. U. Schreiber and Prof. C. Mayer (University of Duisburg-Essen)

Ozone Stress Effect on the Intracellular Metabolites from *Cobetia marina* by Two-dimensional Gas Chromatography with Mass Spectrometer

Junjie Li

In general, microorganisms could optimally survive and reproduce due to the adaption to the normal environments. However, the balance in such optimum condition could be broken by any extreme change, which was considered as a kind of stress and might lead to lag time increasing, growth rate reducing, even cell death. Those stresses might include cold or heat shock, hyperosmotic pressure, acid or organic solvent stress, and oxidative stress. Compared to others, oxidative stress works not physically but leads to oxidative damage via the accumulation of reactive oxygen species (ROS), which influences on the lipids, nucleic acids and proteins then causes cell toxicity. In this work a GCxGC-MS system was employed with the column set (non-polar x mid-polar) for the metabolic non-target analysis of *Corbetia marina*, the model bacteria for biofouling. *C. marina* was treated with ozone to investigate the intracellular metabolic state change under oxidative stress. A minimal inhibitory concentration test was involved to guarantee that the applied ozone dosages were not lethal for the cells. In this study, non-target analyses were performed to identify the metabolites according to the NIST database. As a result, over 60 metabolites were detected under normal living condition. By comparison of ozone treated and non-treated samples, eight compounds were selected, to describe obvious trends between the contour plots. The oleic acid exhibited a slight growth by increasing ozone dosage. In contrast, other metabolites such as amino acids L-proline and L-Isoleucine showed less abundance after ozone treatment, which was more evident once ozone dosage raised. Thus, this work could provide a hint for searching up/down regulating factors in such environmental stress condition for *C. marina*.

Figure: Scheme of the ozonization

Collaborative Project – Project Partner: Prof. W. Schuhmann, Prof. A. Rosenhahn (Ruhr University Bochum, Germany) and Prof. T. Schmidt (University of Duisburg-Essen, Germany)
Funded by: Mercator Research Center Ruhr

LCxLC and LC+LC coupled to mass spectrometry for the analysis of chemical constituents in *Buddleja davidii* root

Jiang Luo, Enise Bekar, Lidia Montero, Yingzhuang Chen

The focus of this study was the analysis of the complex chemical composition from different parts of *Buddleja davidii*, whose species are commonly known as ornamental plants and herbal medicines in many countries. As herbal medicine, it has been utilized for stroke treatments, headache, wound healing, neurological disorder, etc. However, the understanding of its chemical matrices is still insufficient. Therefore, an online two-dimensional reversed phase liquid chromatography x hydrophilic interaction liquid chromatography (RPLCxHILIC) system coupled with mass spectrometry was applied for further detailed investigation of the chemical constituents in *Buddleja dividii*. In this 2D-LC method, a new at-column dilution (ACD) modulator was introduced in the 2D-LC system to solve the incompatibility problem of the mobile phase between two dimensions (RPLC/HILIC), which resulted in a 2D-LC analysis with high orthogonality. For the root extract, as one of the analyzed samples, the optimization of the 1st D and 2nd D gradients was carried out carefully. Whit this new modulator a much better separation with more peaks and better peak shapes was achieved compared to two-dimensional liquid chromatography system using traditional standard (TS) modulator. With the similar approach, the other four parts of *Buddleja davidii* were well separated. Comparing the different analysed parts, flowers and leaves showed the most complex profiles. MS and MS/MS data were obtained successfully, which demonstrated the potential of the proposed RPLCxHILIC-MS system in the constituents' analysis of herb medicine.

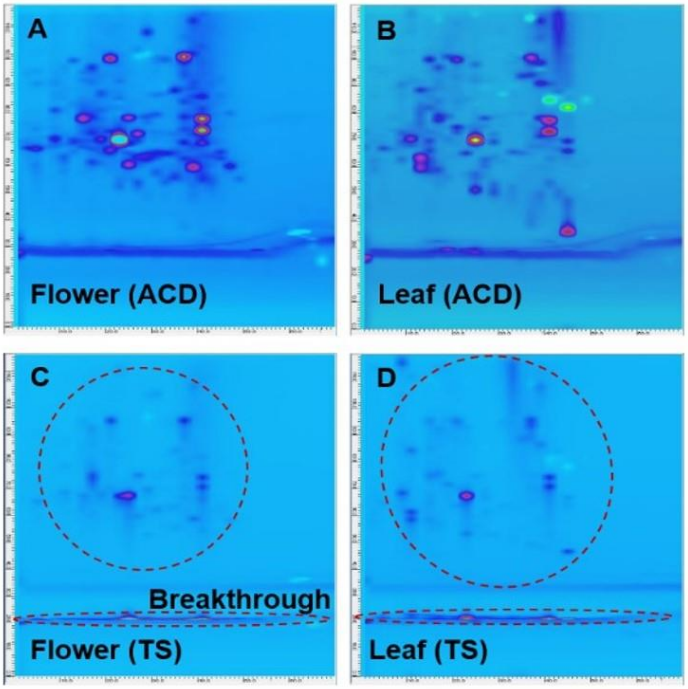


Figure: Contour plots of RPLCxHILIC with ACD modulation or with TS modulation for different parts of *Buddleja davidii.* Flower with ACD modulation (A), leaf with ACD modulation (B), leaf with TS modulation (C), flower with TS modulation (D), stem with ACD modulation (E), fruit

Funded by: Agilent Technologies (Waldbronn, Germany) and China Scholarship council (CSC)

A novel five-dimensional µLC+LC-IM-qTOF-MS/MS separation method for the analysis of complex proteomic samples

Annika Funck, Lidia Montero

A five-dimensional analytical method was applied by coupling a novel continuously multi heart-cutting two-dimensional liquid chromatography (LC + LC) method to an ion mobility quadrupole time-of-flight tandem mass spectrometer (IM-qTOF-MS/MS) for a non-target analysis of complex proteomic samples. A combination of two reversed phase (RP) columns was used for the miniaturised µLC + LC method. The RPLC + RPLC separation was coupled to an IM-qTOF-MS. An additional dimension was obtained by applying tandem MS (MS/MS) for the identification of the peptides. Tryptically digested peptides of both bovine serum albumin (BSA) and Escherichia coli (E. coli) were analysed with the system in order to demonstrate the features of this multidimensional method. For the four-dimensional µLC + LC-IM-qTOF-MS analysis a peak capacity of about 1300 was obtained. The fragmentation data obtained from the µLC + LC-IM-qTOF-MS/MS analysis was sufficient to demonstrate the identification of the amino acid sequence of a peptide. Moreover, the separation of isobaric peptides, which differ in their constitution or configuration, was proven. A total peak capacity of almost 4000 was achieved for the separation of E. coli digest.

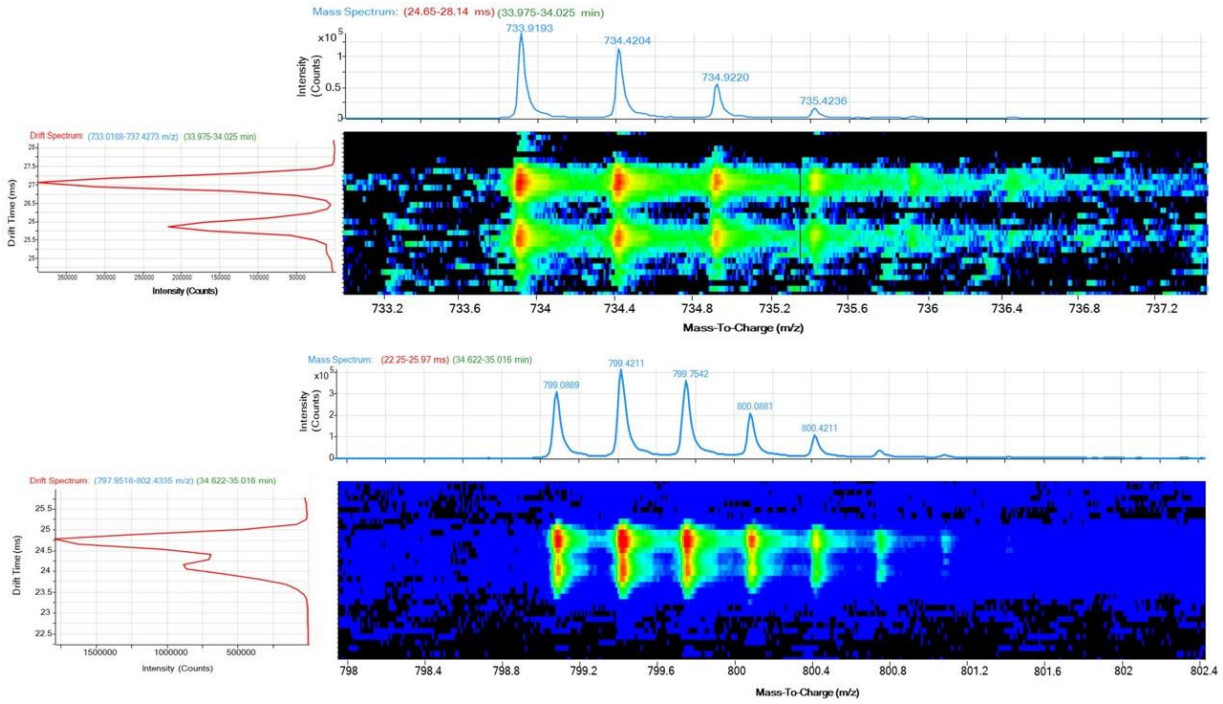


Figure: µLC+LC-ESI-IM-qTOF-MS/MS analysis of protein digest

Funded by: Agilent Technologies (Waldbronn, Germany)

Application of Comprehensive 2D-LC Coupled to MS using Mixed-Mode in the First Dimension and Reversed Mode in the Second Dimension for the Separation of Polar Compounds in Real Food Samples

Katharina Hellmann, Lidia Montero

Currants are appreciated berries for their attractive colors and for their acid-sweet flavor balance. They can be consumed fresh, but they are commonly used in the industry for juices or jams production as well. Interestingly, the organoleptic properties are related to the composition of this berries. The typical color of the red and black currants is associated to anthocyanidins while the content on organic acids like ascorbic, malic, citric, tartaric acids as well as phenolic compounds is the responsible for the acid-astringent flavor.

Studying the whole phenolic compound profile of currants is challenging due to the complex composition of the samples. Phenolic acids, flavonols, anthocyanidins and even polymeric procyanidins are the main compounds that make up the phenolic profile of black and red currants. For this reason, the use of multidimensional liquid chromatography is essential to resolve the complexity of these samples.

In this work, the comparison of the phenolic profile of commercial and natural black and red currants is carried out using a LCxLC-DAD-qTOF method. To resolve very polar acids besides different other phenolic compounds we used a mixed mode separation in the first dimensions. This consisted of an ion exchange and a reversed phased mechanism by coupling in series an anion exchange column with a penta-fluoro-phenyl column for increased pi-pi interactions. In the second dimension, a reversed phase C18 column was used. The method provided a very high orthogonality, besides, a very high peak capacity of 1824 was achieved allowing a complete evaluation of the black and red currant samples. Results reveled a complex phenolic profile of every sample. Interestingly, commercial and natural juices showed similar composition however, the natural juices presented higher intensities of the phenolic composition.

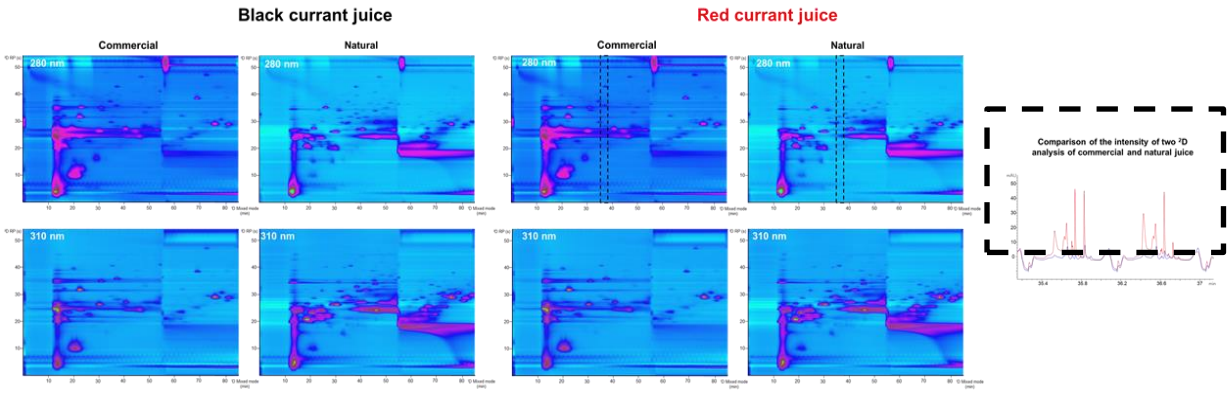


Figure: Comparison of the intensity of two 2D analysis of commercial (blue) and natural (red) juices.

Funded by: Agilent Technologies (Waldbronn, Germany)

Mass spec based immuno-capture assay enables full characterization of therapeutic antibodies after injection *in-vivo*

Annika Doell

An understanding of what happens to therapeutic antibodies *in-vivo* after subcutaneous injection is of high interest. Therefore, we applied the open flow microperfusion technique to extract interstitial fluid from the subcutaneous tissue. In order to analyze those biological samples a specific and sensitive workflow was required. In this study, a complete workflow that enables full characterization of therapeutic antibodies after subcutaneous injection was developed . Compared to classical pharmacokinetic approaches where only a limited number of peptides are detected, our workflow provides full sequence coverages and even enables the identification of potential quality attributes. The efficiency to purify therapeutic antibodies from biological matrices of two different antibody capture molecules and two types of magnetic beads was compared. Furthermore, several desalting protocols were tested in the development of a miniaturized peptide map procedure. Best results were achieved using a commercial anti-human capture mAb fragment in combination with streptavidin coated magnetic beads providing capture efficiencies of 90-100%. The optimized peptide map protocol that requires <1µg mAb includes two desalting steps and showed sequence coverages of 95-100%. The final method was successfully used for analysis of interstitial fluid and serum samples after a subcutaneous injection of a therapeutic antibody into a domestic pig.

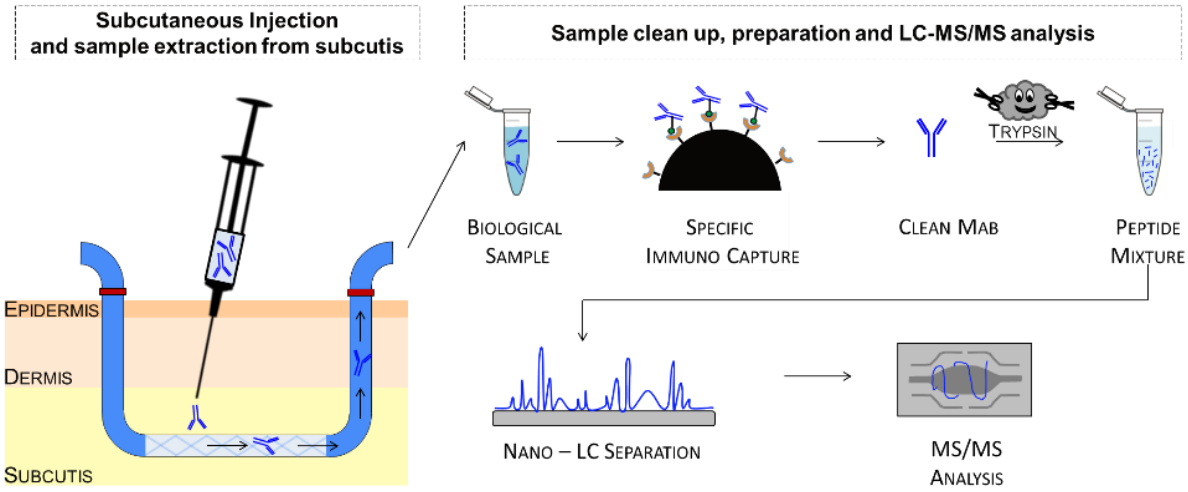


Figure: Scheme of the developed analytical method

Collaborative Project – Project Partner: Dr. Markus Hollmann, Abbvie GmbH & Co.KG

Doctoral Theses accomplished 2019

Dr. Annika Doell (external PhD student)

In-vivo characterization of therapeutic antibodies after subcutaneous injection using a LC-MS based immuno-capture assay

Therapeutic antibodies have become increasingly important in the treatment of autoimmune diseases and different types of cancer. During production and storage the protein molecules can undergo modification due to different degradation reactions, which may decrease biological activity and/or bioavailability of the antibody. Therefore, the analysis of protein modifications on the peptide level using liquid chromatography coupled to mass spectrometry (LC-MS) is a common approach to characterize protein therapeutics. While analysis of *in-vitro* samples is performed routinely, the full characterization of protein therapeutics after *in-vivo* administration is highly challenging due to the low protein amount that is available for analysis. The work described in this thesis focuses on the development of a complete workflow that enables full characterization of therapeutic antibodies after subcutaneous injection. At first, a suitable capture protocol to purify the therapeutic antibody from complex biological matrices was developed in order to remove matrix proteins that may interfere with the mass spectrometric analysis. Best results were achieved using a commercial anti-human capture mAb fragment in combination with streptavidin coated magnetic beads providing capture efficiencies of 90-100%. The obtained results further demonstrated that the protocol works in an unbiased way and allows capturing both, stressed and unstressed material. To localize then the sites of particular modifications, it is a common approach to apply enzymatic digestion followed by LC-MS analysis (peptide map). This peptide map procedure is routinely performed using 250 µg antibody for digestion. However, for *in-vivo* samples only a few hundred nanogram (up to 1 µg) are available for analysis. Therefore, a part of this thesis is also focused on the development of a special digestion protocol suitable for low sample amounts. The final workflow requires less than 1 µg antibody, includes two desalting steps and shows sequence coverages of 95-100%. Thus, compared to standard *in-vitro* approaches a 250-fold reduction of the required amount of antibody was achieved. With the implementation of a nano-LC-MS platform the required sensitivity for the analysis of *in-vivo* samples was realized. Thus, compared to a standard flow LC using 2.1 mm columns, the developed nano-LC platform offers a 300-fold sensitivity increase. Finally, the workflow was successfully applied for the characterization of a therapeutic antibody after subcutaneous injection in both, serum and interstitial fluid. This is the first time that a therapeutic mAb has been extracted from the subcutis of a pig after subcutaneous injection and got fully characterized. Overall, the results revealed the same modifications for both sample types and therefore it can be concluded that the antibody is not modified in the subcutaneous tissue after injection within the first ten hours. The ability to specifically capture and fully characterize therapeutic antibodies from biologic matrices is a major improvement and of high importance for the development of biotherapeutics. This workflow described here lays the foundation for future experiments to answer the question of what happens to antibodies after injection *in-vivo*.

Dr. Bing Peng (external PhD student)

Novel strategies for targeted lipidomics in complex biological system

Lipids are fundamental biomolecules deeply involved in numerous biological processes. To elucidate the roles of lipids in biological systems, the study of lipidomics emerged with support from state-of-the-art analytical techniques. However, the high complexity of lipids resulted in many analytical challenges to obtain analyses of whole lipidomes. Therefore, a strategy called targeted lipidomics focuses on scanning and quantifying of selected groups of lipids only. However, one of the main challenges of targeted lipidomics is the lack of suitable bioinformatics tools and workflows to set up a targeted assay for the huge variety of lipid classes. Thus, the aim of this thesis is to improve the targeted lipidomics workflows, including the creation of targeted assays and the parameters for separation, and apply and validate developed workflows to different model systems such as the RAW 264.7 cell line (a community model) or platelets as an *ex-vivo* model.

Firstly, an interim strategy was developed to adapt Skyline (a targeted proteomics software package) for targeted lipidomics analyses. Secondly, LipidCreator was developed, the first open source software fully supporting the targeted lipidomics assay development. LipidCreator not only delivers the computation of fragment masses of over 60 lipid classes, it also provides the functionality to define fragments, introduces stable isotope labeling of lipids for targeted assays, provides an optimization module for collision energy and generates *in-silico* spectral libraries. This software can either be used as a standalone tool or with Skyline. On the basis of LipidCreator, the high performance chromatography/mass spectrometry-based targeted lipidomics workflows including extraction optimization, lipid category tailored gradients and fragmentation rules in mass spectrometry were established, leading to significantly increased accuracy for the analysis of targeted lipids.

Finally, the established targeted approaches were utilized in conjunction with shotgun lipidomics, enabling us to create a first quantitative draft of the platelet lipidome covering almost 400 lipids species derived from 28 lipid classes and a dynamic abundance range of seven orders of magnitude. The result of the resting platelet lipidome indicated that only 15 lipids comprise 70% of the whole lipid content of platelets. In addition to this, a systematic assessment of the lipidomics network revealed that 80% of the platelet lipidome is unaffected by the change from resting to the activated state, indicating the feasibility of quantitative and differential comprehensive lipidome analyses. The strategies were further applied to a rare disease model (Niemann-Pick A/B) which displays clotting and bleeding issues. For this disease model, it was investigated whether lipids are responsible for this phenotype. The results showed a strong upregulation of sphingosylphosphorylcholine (SPC) and a direct pathophysiological effect of SPC on the platelet activation and thrombus formation.

Master Theses accomplished 2019

Alam Ashraful
Analysis of pesticides by GC-QqQ-MS

Karim Chowdhory
Use of thermogravimetry coupled to a mass spectrometer

Martin Dißner
Database development for GC-MS of derivatized metabolome standards

Bachelor Theses accomplished 2019

Imke Ackermann
Metabolome analysis of *P. aeruginosa*

Enise Bekar
Analysis of Buddleja davidii with LC+LC and ACD modulation

Annika Funck
A novel five-dimensional µLC+LC-IM-qTOF-MS/MS separation method for the analysis of complex proteomic samples

Jasmin Heine
Analysis of fatty acids in biological samples with GC-QqQ-MS

Katharina Hellmann
Application of Comprehensive 2D LC Coupled to MS using Mixed-Mode in the First Dimension and Reversed Mode in the Second Dimension for the Separation of Polar Compounds in Real Food Samples

Laurin Domenic Grabler
Liquid extraction of TFME films and their use for the metabolome analysis of *Pseudomonas aeruginosa* from the lung infection model using GCxGC-qMS

Jiang Luo
RPLCxHILIC coupled to mass spectrometry for the analysis of chemical constituents in *Buddleja davidii* root

Simon Jan Schastok
Development of a method for quantification of pesticides in green tea using online SPE-HPLC-QqQ-MS

Kevin Schulz
Determination of CCS values of lipids from human plasma using LC-IM-qTOF-MS/MS

Philipp Swiderski
Method development for metabolome analysis with gas chromatography and high-resolution time-of-flight mass spectrometry

Kübra Temel
Analysis of the metabolome of *Pseudonomas aeruginosa* in a biofilm model with different cultivation parameters using thermodesorption GC-MS

Jasmin Maria Turkowski
Analysis of fatty acids from biological samples using GC-QqQ-MS

Pia Wittenhofer
Characterization of the lipid profile of biological samples by SFC

Scientific Publications 2019

Original Paper / Peer-reviewed

B. Peng, D. Kopczynski, B. S. Pratt, C. S. Ejsing, M. Hermansson, D. Schwudke, S. W. Meckelmann, O. J. Schmitz, B. MacLean, O. Borst, N. Hoffmann, R. Ahrends **LipidCreator: A workbench to probe the lipidomic landscape,** submitted to Nature Methods

T. Koehler, I. Ackermann, D. Brecht, F. Uteschil, J. Wingender, U. Telgheder, O. J. Schmitz **Analysis of volatile metabolites from in vitro biofilms of Pseudomonas aeruginosa by TD-GC-qMS**, submitted to Analytical and Bioanalytical Chemistry

Q. A. Ngo, D. T. Hoang, T. Duc, H. A. Duong, O. J. Schmitz, H. V. Pham **Characterization of volatile components from Ethyl acetate extract of Stixis suaveolens (Roxb.) by comprehensive two-dimensional gas chromatography hyphenated with a time-of-flight mass spectrometer**, accepted in Vietnam Journal of Chemistry

Y. Chen, L. Montero, J. Luo, J. Li, O. J. Schmitz **Application of the new at-column dilution (ACD) modulator for the two-dimensional RP×HILIC analysis of Buddleja davidii**, accepted in Analytical and Bioanalytical Chemistry

Y. Chen, L. Montero, O. J. Schmitz **Advance in on-line two-dimensional liquid chromatography modulation technology**, Trends in Analytical Chemistry (2019) 120: 115647-115655

Y. Chen, J. Li, O. J. Schmitz **Development of a at-column dilution modulator for flexible and precise control of dilution factors to overcome mobile phase incompatibility in comprehensive two-dimensional liquid chromatography,** Analytical Chemistry (2019) 91:10251-10257

A. Doell, O. J. Schmitz, M. Hollmann **Shedding light into the subcutis – A mass spec based immuno-capture assay enables full characterization of therapeutic antibodies after injection *in-vivo*,** Analytical Chemistry (2019) 91: 9490-9499

D. Brecht, F. Uteschil, O. J. Schmitz **Thermogravimetry coupled to an atmospheric pressure photo ionization quadrupole mass spectrometry for the product control of pharmaceutical formulations and the analysis of plasticizers in polymers**, Talanta (2019) 198:440-446

C. Koch, M. Nachev, J. Klein, D. Koester, O. J. Schmitz, T. Schmidt, B. Sures **Degradation of the polymeric brominated flame retardant "Polymeric FR" by heat and UV**, Environmental Science & Technology (2019) 53:1453-1462

C. Hellmann, O. J. Schmitz **How to deal with mercury in sediments ? A critical review about used methods for the speciation of mercury in sediments**, Chromatographia (2019) 82: 125-141

K. Jooß, S. W. Meckelmann, J. Klein, O. J. Schmitz, C. Neusüß **Capillary zone electrophoresis coupled to drift tube ion mobility-mass spectrometry for the analysis of native and APTS-labeled *N*-glycans**, Analytical and bioanalytical chemistry (2019) 411:6255-6264

Books

A.-F. von Trotha and O. J. Schmitz, **Qualitätskontrolle in der TCM** (2019) Springer-Verlag, Berlin, ISBN-13: 978-3662592557

Poster Presentations

D. Brecht, F. Uteschil, O. J. Schmitz, **Development of an ESI-APCI dual ionization source for LC-MS analyses**, anakon, Muenster, Germany, March 2019

D. Brecht, F. Uteschil, O. J. Schmitz, **Investigation of drugs using a novel thermogravimetry atmospheric pressure photo ionization mass spectrometry coupling (TG-APPI-qMS)**, anakon, Muenster, Germany, March 2019

C. Lipok, O. J. Schmitz, **A new design for atmospheric pressure chemical ionization ion sources**, anakon, Muenster, Germany, March 2019

K. Rentmeister, L. Montero, A. Funck, S. W. Meckelmann, S. Buckenmaier, O. J. Schmitz, **A Novel 4D-Analytical Platform for Omics Sciences**, anakon, Muenster, Germany, March 2019

M. Meyer, S. W. Meckelmann, O. J. Schmitz, **Optimization and development of a SPE protocol for the enrichment of plant phenolic compounds by Design of Experiment**, anakon, Muenster, Germany, March 2019

K. Rentmeister, L. Montero, A. Funck, S. W. Meckelmann, S. Buckenmaier, O. J. Schmitz, **A Novel 4D-Analytical Platform for Omics Sciences**, 5th International Ion Mobility Seminar, Bordeaux, France, March 2019

K. Rentmeister, T. Koehler, A. Schubert, K. Schulz, O. J. Schmitz, S. W. Meckelmann, **Characterization of the human plasma lipidome using LC-IM-qTOF-MS**, 5th International Ion Mobility Seminar, Bordeaux, France, March 2019

Y. Chen, J. Li, O. J. Schmitz, **Development of an At-Column Dilution Modulator for Flexible and Precise Control of Dilution factors to Overcome Mobile Phase Incompatibility in Comprehensive Two-Dimensional Liquid Chromatography,** analytica Vietnam, Ho Chi Minh City, March 2019

J. Klein, S. W. Meckelmann, O. J. Schmitz, **Analysis of complex samples using multidimensional separation and detection techniques**, analytica Vietnam, Ho Chi Minh City, March 2019 3nd International Poster Prize

L. Kuschmierz, M. Meyer, B. Meyer, S.-V. Albers, C. Bräsen, J. Wingender, O. J. Schmitz, B. Siebers, **Archaeal biofilms: Composition of extracellular polymeric substances, exopolysaccharide synthesis and transport in *Sulfolobus acidocaldarius*,** Gordon Research Conference Archaea, Les Diablerets, Switzerland, July 2019

M. Meyer, L. Kuschmierz, B. Siebers, J. Wingender, O. J. Schmitz, **Analysis of exopolysaccharides from archaeal biofilms by supercritical fluid chromatography coupled to mass spectrometry**, 48th HPLC, Milano, Italy, June 2019

K. Rentmeister, P. Wittenhofer, O. J. Schmitz, S. W. Meckelmann, **Analysis of the HepG2 lipidome by means of supercritical fluid chromatography coupled with IM-qTOF-MS(/MS)**, 48th HPLC, Milano, Italy, June 2019

Y. Danisan, M. Sulkowski, U. Schreiber, C. Mayer, O. J. Schmitz, **Origin of Life - Experimental evolution of functional vesicles in hydrothermal environments**, 48th HPLC, Milano, Italy, June 2019

J. F. Ayala-Cabrera, C. Lipok, E. Moyano, O. J. Schmitz, F. J. Santos, **Feasability of atmospheric pressure ionization sources for the analysis of polychlorinated naphthalenes by gas chromatography-high resolution mass spectrometry**, The 39th International Symposium on halogenated persistent organic pollutants (Dioxin 2019), Kyoto, Japan, August 2019

Invited Lectures / Oral Presentations

Prof. Oliver J. Schmitz

Ion mobility mass spectrometry as a powerful tool for target, suspected target and non-target environmental analysis
36th Forum Analytik 2019, Vienna, Austria, February 2019

From 1D-LC with 1000-mm columns to LC+LC-IM-qTOF-MS
Agilent´s Innovative Separation Strategies for Enhanced MS Analysis European tour, Duesseldorf, Germany, February 2019

2D-LC-IM-qTOF-MS as a generic analytical method for the analysis of complex samples
5th International Ion Mobility Spectrometry (IMS) Meeting, Bordeaux, France, March 2019

How using an at-column dilution (ACD) Mmodulator can improve 2D-LC Analysis
Anakon 2019, Münster, Germany, March 2019

The power of chromatography in combination with ion mobility-mass spectrometry
6th analytica Vietnam conference, Ho Chi Minh City, Vietnam, April 2019

The power of chromatography in combination with ion mobility-mass spectrometry
25th International Symposium on Separation Science, Lódz, Poland, September 2019

Use of SFC-IM-qTOF-MS for the analysis of sugars and lipids
Agilent LC/MS Seminar, Düsseldorf, Germany, October 2019

Challenges in single-cell analysis with mass spectrometry
18th International Beijing Conference and Exhibition on Instrumental Analysis (BCEIA), Beijing, China, October 2019

Mass spectrometry: What needs to be improved?
Tsinghua University, Peking, China, October 2019

Chinese Herbs Analysis: The Power of Chromatography in Combination with Ion Mobility-Mass Spectrometry
3rd International Symposium on Advances in Pharmaceutical Analysis (APA), Xi´an, China, October 2019

Dr. Lidia Montero

2D-LC-IM-qTOF-MS as a generic analytical method for the analysis of complex samples
49th HPLC 2019, Milano, Italy, September 2019

2D-LC analysis as a powerful tool for Food authenticity, bioactivity and proteomics
Agilent Analytik-Forum 2019, Hannover, Germany, June 2019

Julia Klein

Analysis of complex samples using multidimensional separation and detection techniques
29th PhD seminar of the Working Group Separation Science of the Section for Analytical Chemistry of the GDCh, Hohenroda, Germany, January 2019

Kristina Rentmeister

Characterization of human plasma lipidome using LC-IM-qTOF-MS
29th PhD seminar of the Working Group Separation Science of the Section for Analytical Chemistry of the GDCh, Hohenroda, Germany, January 2019

Timo Köhler

Bacterial lung infections: How thermodesorption GC-MS can help develop early detection
Gerstel User Seminar, Mülheim an der Ruhr, Germany, May 2019

Miscellaneous

Conference Organization

Prof. Oliver J. Schmitz, Chairman of the 6th analytica Vietnam conference in Ho Chi Minh City (Vietnam, April 2019), together with Prof. Viet Pham.

Prof. Oliver J. Schmitz (together with Kristina Rentmeister and Timo Köhler), Organization of the 29th PhD seminar of the Working Group "Separation Science" of the Section for Analytical Chemistry of the GDCh in Hohenroda (left photo shows the participants (> 150) and right the speakers of the PhD seminar).

Dr. Lidia Montero was a Lecturer about Chestnut European Market in the seminar "Chestnut, more than a fruit: a food that increases the traditions, the entrepreneurship and the innovation", organized by the University of Concepción (Campus Chillán) in Chile, November 2019. In addition, Dr. Montero gave two workshops about "Being in a safe place - Guidance to maintain the security in the lab" and "Practice-orientated seminar for laboratory users - Introduction to the laboratory" at the 6th International Trade Fair for Laboratory Technology, Analysis, Biotechnology and Diagnosis (Analytica Vietnam, April 2019) and at International Trade Fair for Laboratory Technology, Analysis, Biotechnology and Diagnosis (Analytica Lab Africa, Johannesburg, South Africa, July 2019), respectively.

Editorial Tasks by Prof. Oliver J. Schmitz

- 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
- Deputy Chairman of the Working Group Separation Science of the Section for Analytical Chemistry of the GDCh

Awards

Professor Oliver J. Schmitz was awarded the 2019 Professor Andrzej Waksmundzki medal by the Committee on Analytical Chemistry of the Polish Academy of Sciences during the 25th International Symposium on Separation Sciences in Lódz (Poland, 15-18th September 2019) in recognition for his important achievements in the field of separation, and especially, chromatographic techniques as well as for their implementation.

In April 2019, during the 6th analytica conference in Ho Chi Minh City, Vietnam, Julia Klein was awarded with the 3rd International Poster Prize of the symposium.

Institute Colloquium

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

Prof. Dr. Uwe Karst from the University of Muenster visited the Applied Analytical Chemistry (AAC) at University of Duisburg-Essen. He was one of the speakers at the Analytical Chemistry-Colloquium held in cooperation with the research group of Prof. Torsten Schmidt (IAC).

We would also like to thank all our other guests who participated in our colloquium:

Dr. Andrey Shevchenko, MPI of Molecular Cell Biology and Genetics, Germany, Shotgun lipidomics: analytical principles and biomedical applications, 21.01.2019

Prof. Dr. Kevin Pagel, FU Berlin, Germany, Sugars in the Gas Phase – Novel Techniques to Unravel the Glycocode, 22.05.2019

Dr. Peter Boeker, University of Bonn, Germany, Hochdurchsatz-Gaschromatographie mit negativem Temperaturgradienten: Messungen, Simulationen und Mehrdimensionalität, 03.06.2019

Prof. Dr. Philipp Weller**, HS Mannheim, Germany,** Authentizitätsanalyse mittels chemometrischer Verfahren, 01.07.2019

Dr. Björn Meermann, BAM Berlin, Germany, 04.11.2019

Prof. Dr. Uwe Karst, University of Muenster, Germany, Hyphenated techniques to solve (bio)medical questions, 18.11.2019

Prof. David Sedlak, UC Berkeley, 04.12.2019

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

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

Lecture Anaytical 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 Environmental Chemistry: Pollutants (in English, Prof. Dr. O. J. Schmitz)

Tutorial Environmental Chemistry: Pollutants (in English, 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)

Environmental Toxicology (M.Sc.)

Lecture Applied Analytical Chemistry (in English, Prof. Dr. O. J. Schmitz)

Tutorial Applied Analytical Chemistry (in English, Prof. Dr. O. J. Schmitz)

Lecture Environmental Chemistry: Pollutants (in English, Prof. Dr. O. J. Schmitz)

Tutorial Environmental Chemistry: Pollutants (in English, Prof. Dr. O. J. Schmitz)

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)

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

Course 2: Advanced Course Liquid Chromatography (2D-LC, LCxLC, SFC)

Course 6: Spectroscopy and Capillary Electrophoresist (in German, Prof. Dr. O. J. Schmitz)

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2019