# 9th Swiss Pharma Science Day 2016

## CONFERENCE REPORT and ABSTRACTS

**Prof. Dr. Rudolf Brenneisen, Secretary General SAPhS**
**Andreas Schittny, PhD student, University of Basel[1]**

At its inception nine years ago, the Swiss Pharma Science Day (SPhSD) was intended as a gathering of Swiss pharmaceutical scientists working in academia and industry, with a special focus on the support of young scientists. This idea has been met with enthusiasm by pharmaceutical scientists and the support by the pharmaceutical industry and regulatory authorities. The organizers were thus highly motivated to make this year's event again a success for the participants, and pharmaceutical sciences in Switzerland as such.
Here is our report on the 9th SWISS PHARMA SCIENCE DAY of August 31, 2016 held again at the Pathology Langhans Auditorium of the University of Bern and the House of University of Bern. The SPhSD organizers Prof. Dr. Gerrit Borchard, president of the Swiss Academy of Pharmaceutical Sciences (SAPhS) opened the conference attendend by about 120 colleagues from the University of Basel (including FHNW), University of Geneva-EPGL, ETH Zurich (including University of Zurich and ZHAW), industry, and other countries, respectively.

Attendees checking the SPhSD program

Dr. Christine Moll, vice-president, and Prof. Gerrit Borchard, president of SAPhS

[1] See also: A. Schittny, Aktuelle Forschung in den Pharmazeutischen Wissenschaften, pharmaJournal 22 | 11.2016

Prof. Ursula von Mandach, Univ. Hospital Zurich, and Prof. Stefan Mühlebach, Vifor Pharma

Prof. Bruno Gander, ETH Zurich; Dr. Heinz Schmitter, pharmacist in Zurich; Prof. Jürg Gertsch, Univ. of Bern

No legend necessary

Peter Tiefenböck, ETH Zurich

Starting the SPhSD with coffee and croissant


Prof. Gerrit Borchard, President SAPhS

Prof. Christian Leumann, new rector of the University of Bern and former director of pharmaceutical studies at the University of Bern, emphasized in his welcome address the important role of pharmaceutical sciences as interface between natural sciences and medicine in the translational research. He identified translational medicine as promising field of today's science, especially at the University of Bern. He also recognized the responsibility of pharmacists in the society and therewith justified the decision to re-establish in near future the full pharmacy curriculum at the University of Bern.

Prof. Christian Leumann, Rector University of Bern

## Morning Session, Lectures 1-3

The morning session was chaired by Prof. Ursula von Mandach, SAPhS senate's board member, introducing the keynote speaker ***Prof. Dr. Johannes Khinast*****,** Graz University of Technology, Austria.


Prof. Ursula von Mandach, SAPhS senate's board member, University Hospital Zurich, introducing Prof. Johannes Khinast

Prof. Khinast is currently Head of the Graz University of Technology, Prof. of Pharmaceutical and Process Engineering, Head of the Institute for Process Engineering, Scientific Director of the Research Center for Pharmaceutical Engineering, and Director of the University Life-Long-Learning Curriculum Clean Room Technology. 1995, he got his PhD in Chemical Engineering at the Graz University of Technology, followed 1996-98 by a Post Doc stay at the University of Houston TX. His research interests are pharmaceutical engineering and process development, formulation of nano-structured drugs, process analytic technology, heterogeneous catalysis and crystallization, modeling and simulation of complex processes and systems. He was honored with several awards, such as Bristol-Myers Squibb Young Faculty Development Award, DuPont Young Professor Award, and the Styrian Innovation Award.

His lecture was entitled ***«Rational Approaches to the Development of Advanced Pharmaceutical Manufacturing Systems»*****.** New trends in pharmaceutical research and new requirements for drugs demand new production systems, too. Whereas pharmaceutical research develops more and more complex galenic forms and release systems, the pharma industry is faced with the call for higher flexibility in production capacity as well as personalized medicine. However, new formulations always result in many unknowns of the production process and therefore represent a risk, which is usually not taken by pharma companies at the end of drug development. So, nowadays innovative formulation systems only seldomly appear in the market, although innovative ideas are existing. This problem is approached by the Research Center for Pharmaceutical Engineering of the Graz University of Technology and his scientific director Prof. Khinast. His institute is focussing on three core areas: modelling and prediction, advanced formulations and release, as well as process and production sciences. Numerous close cooperations with partners in industry and university contribute to the knowhow of the research center. Thereby, the institute is relying on the concept of "Advances Pharmaceutical Manufacturing (APM)". Thus, every manufacturing process must be controllable, mechanistically understandable, robust, scalable, fundable, and increasable. To implement these goals the so called continuous production plays more and more an important role. Thereby, pharmaceutical products are manufactured in a single production line from the raw material to the final product, without interruption and within few hours. This saves time and increases flexibility and production capacity. Essential is the continuous

process monitoing. For this the research center developed suited methods, usually imaging techniques. For example they could manage to in-process control the coating thickness of tablets or the agent concentration of every produced tablet. These developments go hand in hand with more and more improved modeling methods. For instance, mixing processes, weat powder flow or filling of capsules can be simulated by calculating the properties of thousands of particles. This contributes to the understanding of processes as well as their optimization before actually beginning with manufacturing. Besides technical projects the institute of Prof. Khinast is also involved in organisational requests concerning formulation projects of companies and in regulatory matters. Therefore, it is the vision of Prof. Khinast that the institute is also a “center of centers” for pharmaceutical engineering.

*Speaker’s Abstract:*

In the last years, significant changes have occurred in the way we make pharmaceuticals. One major trend is a shift towards continuous manufacturing as a means to increase quality of products and to have more flexibility in terms of manufacturing. Another trend is the increasing need to make personalized or individualized drugs, possibly “on demand”. Finally, highly complex drug products that have been developed by pharmaceutical scientists have to be manufactured as well, with the same high quality as “simple” products. These complex medicines include nano-based systems, biopharmaceuticals or non-biological complex drugs. Here, entirely new processes are essential that need to be tested and validated. In the presentation I will highlight rational approaches to the development of a new manufacturing paradigm, called “advanced pharmaceutical manufacturing”. Better understanding of material properties and the interaction of materials, on-line real-time analyzers for the state of the process and advanced design-, optimization-, and control-methods are critical elements in the advanced manufacturing context. Finally, our work in the field of Discrete Element Modeling (DEM), Finite Element Modeling (FEM) and the coupling of DEM with Computational Fluid Dynamics (CFD) will be highlighted as tool to rationally design processes and to derive control strategies.

The second speaker was ***Prof. Dr. Jennifer Keiser***, Swiss Tropical and Public Health Institute (TPH) Basel. Jennifer Keiser is an Assistant Professor at the Department of Medical Parasitology and Infection Biology at the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland. She studied pharmacy at the University of Basel (1990-1995). Her PhD at Swiss TPH (1997-1999) was followed by postdoctoral work at Princeton University (2000-2003). She is heading the Helminth Drug Development Unit since 2006. Jennifer Keiser has published more than 230 scientific publications and is an associate editor of 4 journals. Her research has been large supported by funding schemes of the Swiss National Science Foundation (Marie Heim-Vögtlin program (2004-2006), Swiss National Science Professorship (2006-2012)), and the European Research Council (ERC Consolidator Grant 2014-2019). Research interests of her team include in vitro and in vivo evaluation of anthelminthic activities of compounds (for which an unique set of helminth rodent models is maintained), pharmacokinetic studies, and clinical trials in endemic countries.

Prof. Keiser was presenting the topic ***«Towards Better Treatment Options for Neglected Helminth Infections: From Drug Discovery to Development»***

Infections with the helminths *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm are widespread in humid tropical areas and often occur at the same time. However, nowadays no therapy effective against all three worms species is existing. A meta-analysis showed that the standard medication albendazole and mebendazole are efficacious against infections with *A. lumbricoides* while against hookworm only albendazole reveals high efficacy. The research group of Prof. Keiser works at the TPH on the development of an efficient treatment for all three worm infections. It is her goal to control worm infections worlwide and eliminate locally. She is developing an *in vitro* method to test on *T. muris* larvae of mice the efficacy of anthelmintic drugs, including compounds originally developed for the veterinary medicine. Oxantel showed *in vitro* as well in the mouse model very good trichuricidal efficacy. To test oxantel in a clinical study on children first a suitable formulation was required, as this drug was no longer formulated. Consequently, an adequate preparation was developed by the research group of Pharmaceutical Technology at the University of Basel. In a first clinical efficacy and safety study performed in Tansania the effect of oxantel on *Trichuris* could be confirmed with average side effects. To closer examine the option of a co-medication oxantel plus albendazole the research

group performed interaction assays *in vitro* and in rats. No relevant interactions were observed. In a further clinical study oxantel plus albendazole were significantly more efficacious than the standard treatment currently also recommended by WHO. Finally, a dose- and side effect-finding study showed that children of age 7 to 14 should get a fixed and not body weight-based dose. Therefore, it was possible to develop an efficacious, safe, and comprehensive treatment for worm infections in children. This could have a direct impact on the health in tropical regions.

*Speaker's Abstract:*

In large parts of the humid tropics, the three soil-transmitted helminths (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) are widespread and often occur concurrently. The standard treatments albendazole and mebendazole are efficacious against infections with *A. lumbricoides* while against hookworm only albendazole reveals high efficacy. Both treatments show low efficacy against *T. trichiura* infection in regimens used in large-scale control programs. In more detail, a recent network meta-analysis determined low cure rates of 32.4% (95% CI; 22.9-43.7) and 47.0% (95% CI; 35.0-59.2) for albendazole and mebendazole against *T. trichiura*, respectively. New broad spectrum therapeutic products should therefore be developed to improve control and potentially achieve local elimination of soil-transmitted helminth infections. We have developed a simple and cost-effective drug sensitivity assay using *T. muris* first-stage larvae ($L_1$). This assay is routinely used in our laboratory and provides accurate and reproducible drug effect data *in vitro*. Following a "low hanging fruit" approach we also tested several veterinary anthelminthics. Oxantel pamoate revealed an excellent trichuricidal activity *in vitro* and in mice and performed superior than the standard drugs albendazole or mebendazole, while lacking activity against hookworm. Oxantel pamoate might therefore be an interesting partner drug in combination chemotherapy to cover all major soil-transmitted helminth infections. However, oxantel pamoate is not produced for human use any longer. In collaboration with the Department of Pharmaceutical Technology at the University of Basel a child-friendly tablet formulation of oxantel pamoate was therefore developed, which laid the cornerstone for three randomized controlled trials on Pemba Island, Tanzania. We evaluated the efficacy and tolerability of oxantel (20 mg/kg) and an oxantel pamoate (20 mg/kg)-albendazole (400 mg) combination in school-aged children infected with *T. trichiura* and concomitant soil-transmitted helminth infections. Oxantel pamoate-albendazole showed a higher cure rate and egg reduction rate against *T. trichiura* compared to the standard.

The moderator Prof. Ursula von Mandach acknow-ledging with Swiss chocolate the lecture of Prof. Keiser

The third lecture on a topic in molecular biology was given by ***Prof. Dr. Michael Hall*** of the University of Basel.

Michael N. Hall was born (1953) in Puerto Rico and grew up in South America (Venezuela and Peru). He received his Ph.D. from Harvard University and was a postdoctoral fellow at the Pasteur Institute (Paris, France) and the University of California, San Francisco. He joined the Biozentrum of the University of Basel (Switzerland) in 1987 where he is currently Professor and former Chair of Biochemistry. Hall is a pioneer in the fields of TOR signaling and cell growth control. In 1991, Hall and colleagues discovered TOR (Target of Rapamycin) and subsequently elucidated its role as a central controller of cell growth and metabolism. TOR is a highly conserved, nutrient- and insulin-activated protein kinase. The discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. As a central controller of cell growth and metabolism, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, diabetes, and obesity. Hall is a member of the US National Academy of Sciences, has received numerous awards, including the Louis-Jeantet Prize for Medicine (2009), the Marcel Benoist Prize for Sciences or Humanities (2012), the Breakthrough Prize in Life Sciences (2014), and the Canada Gairdner International Award (2015), and has served on several editorial and scientific advisory boards. He and his wife Sabine (née Carrère) live in Basel with their daughters Zoé and Léa.

The title of Prof. Hall's lecture was ***«mTOR Signaling in Growth and Metabolism»*.** When he first described in 1991 the mTOR-protein (mTOR: mechanistic target of rapamycin) he did not assume that this would open a research field with nowadays 2'500 publications per year. At the beginning it was found that sorolimus, also called rapamycin, indirectly binds to mTOR. Further research showed that mTOR plays a central role in cell growth and metabolism and is influenced by nutrients, growth factors and stress. Thus, mTOR is also involved in cancer, obesity, diabetes, and cardiovascular diseases and plays a role in aging processes. Meanwhile, the function of mTOR in the regulation of cell growth is well understood. However, it is not clear how in details the protein is influencing deseases or the full body size. It is already known that mTOR has a signal effect beyond its own cell and even own tissue. More research is necessary to fully elucidate these highly complex mechanisms. Still after more than 15 years of research Prof. Hall and his team is engaged in this topic with blood, sweat and tears.

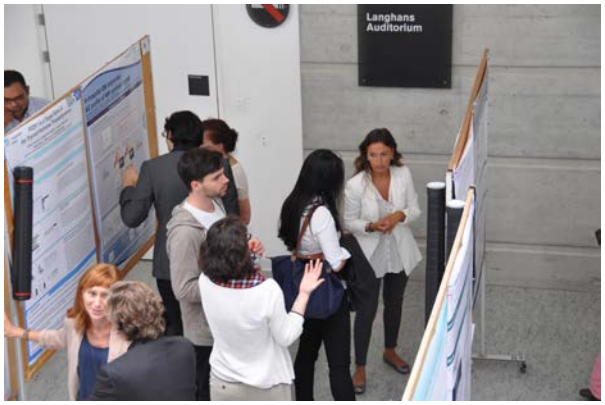
*Speaker's Abstract:*

TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, cellular energy, and stress. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. TOR is found in two structurally and functionally distinct multiprotein complexes termed TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth.

As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.
While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Data on the role of mammalian TORC1 (mTORC1) and mTORC2 in controlling cellular processes and in specific tissues will be presented.

## Lunch Break and Poster Session

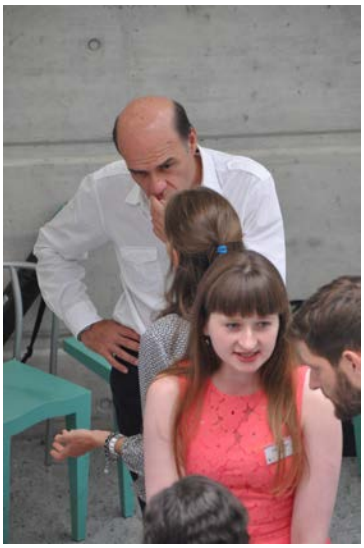
After the lunch break with an excellent Chinese buffet, served by Gastronomy Inselspital, the participants were moving to the poster session exchanging scientific knowledge and experience with young academics. The break was also ideal for socializing and professionally networking. The abstracts of 59 posters can be found at the end of this report. As in previous years, 6 authors were evaluated by the reviewing board to receive awards for outstanding poster presentations. These awards were sponsored by Mundipharma Medical Company, the AKB Foundation, GSIA Foundation, Pharmaceutical Society of Zurich, Glatt Group, Zeller Söhne AG, and Vifor Pharma.




Poster session beginning

Prof. Bruno Gander (ETHZ, SAPhS senate's board member), poster reviewer

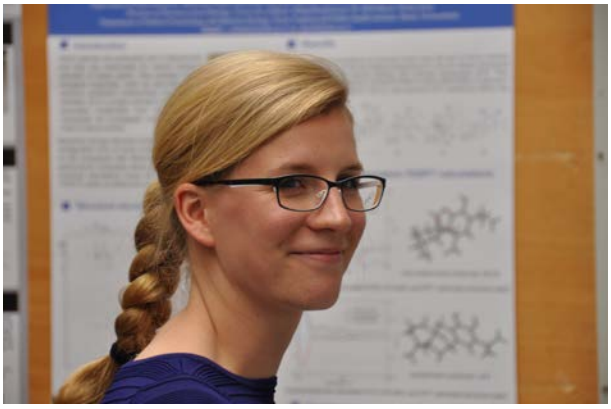
Prof. Stefan Mühlebach (Vifor Pharma, vice-president SAPhS), poster reviewer, talking to Dr. Fabienne Böni, University of Basel



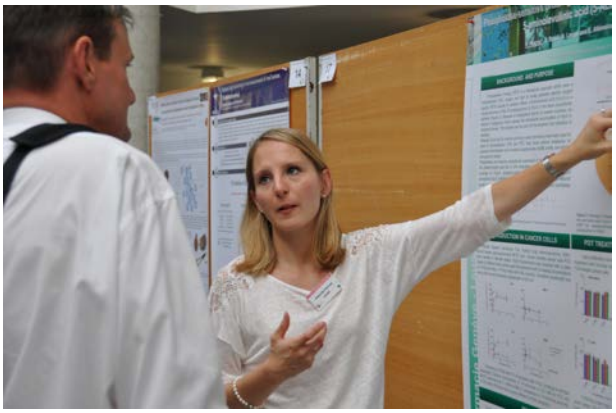
Prof. Georg Imanidis, FHNW,
SAPhS senate's board member

Dr. Ursula Hirter-Trüb, Museum of Pharm. History Basel


Dr. Isabelle Arnet, University of Basel



Poster presenter (anonymous)



Viktorija Herceg, University of Geneva, explaining Prof. Jörg Huwyler (University of Basel), poster reviewer, the goals of her project

Prof. Stefan Mühlebach, reviewing the poster of Albulena Lutfija, University of Geneva

Prof. Georg Imanidis, FHNW, talking to colleagues

Prof. Oliver Germershaus, FHNW, and the poster presenter Simon Bachler, ETHZ

Philippe Tschopp, SAPhS senate's board member, photographer of the SPhSD (left) chatting with two colleagues

Dr. Andreas Stöckli, Mundipharma Medical Comp., waiting for the afternoon session

## Afternoon Session, Lectures 4-5

Prof. Jörg Huwyler, University of Basel, moderating the afternoon session and introducing …

***Dr. med. Liat Fishman, Patient Safety Switzerland.*** Liat Fishman completed her studies of medicine at the University of Heidelberg and Charité - Universitätsmedizin Berlin. She subsequently worked for seven years at the Agency for Quality in Medicine in Berlin as a scientific collaborator and project manager in the areas of evidence-based medicine, clinical guidelines and patient safety programmes. In January 2014, she joined the Patient Safety Foundation Switzerland at its offices in Zurich to coordinate the pilot programme progress! Medication Reconciliation, which aims to improve medication safety at interfaces of care. Her lecture on a Public Health topic was entitled

***«progress! Medication Reconciliation: A National Pilot Program to Improve Medication Safety at Transitions in Care»***

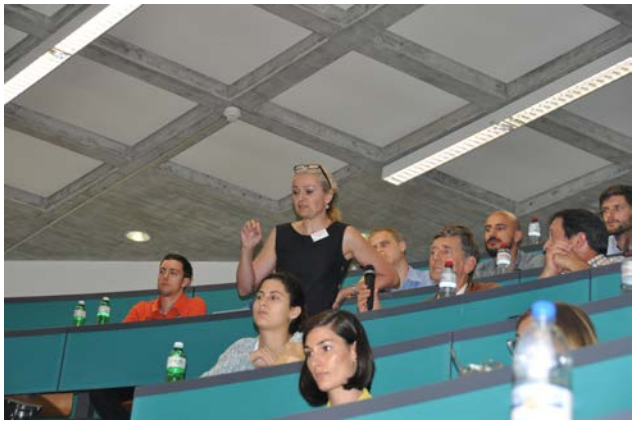
*Speaker's Abstract:*

Transitions in care such as admission to and discharge from the hospital put patients at risk for errors due to poor communication and inadvertent information loss. Studies have shown that in up to half of patients the medication history at admission contains errors. This may lead to unintended omissions or duplications of medications in hospital, or to incorrect dosages. Discrepancies remain common at discharge, further posing a threat to patients. By implementing Medication Reconciliation, health care organisations can prevent such medication errors. Medication Reconciliation is the process of systematically creating a complete list of all medications a patient is currently taking, and consistently comparing this list against admission, transfer and/or discharge medication orders. The basis for the medication list should be a structured patient/carer interview, if possible. The goal of Medication Reconciliation is to ensure that accurate and reliable information about medications is communicated across all transitions of care. Medication Reconciliation as a patient safety measure has become a widespread practice in many countries, but is still largely uncharted territory in Switzerland. With the national pilot program *progress! Medication Reconciliation* the Patient Safety Foundation Switzerland aims to promote the implementation of this practice in acute care hospitals in Switzerland. The program was launched in March 2015 and is mainly funded by the Federal Office of Public Health. Its two axes are an awareness campaign and a pilot project with eight hospitals from all over Switzerland. Within the awareness campaign recommendations for implementing Medication Reconciliation at admission, hospital transfers and discharge were published. The pilot project focuses on the implementation of a best possible medication history (BPMH) at admission, which is the basis of a safe prescription process in the hospital and of effective medication reconciliation at the subsequent transitions of care. Each hospital in the pilot project is testing a process for a BPMH within an internal medicine ward, with the aim of integrating the new process into routine care once the pilot is concluded at the end of 2016. For the duration of the project four workshops are

held where the implementation experiences are discussed between the interprofessional hospital project groups. In addition, process as well as qualitative data is being collected to better understand the feasibility and barriers of implementation. Important conditions of successful implementation seem to be obtaining additional resources in order to guarantee rigorous implementation, the adaptation of workflow processes and of supporting electronic tools, the continuous sensibilisation and supervision of frontline staff and the clear definition of roles and responsibilities while promoting a culture of interprofessional collaboration. Medication Reconciliation is regarded as an important patient safety measure but is a challenge to implement sustainably. With *progress! Medication Reconciliation* the Patient Safety Foundation Switzerland hopes to contribute to "MedRec" becoming an indispensable part of routine care of patients in Switzerland.

Questions from the audience

Dr. Liat Fishman



***Prof. Dr. Renato Zenobi, ETH Zurich***, was finishing the SPhSD lecture program with a topic in the field of Analytical Chemistry. Renato Zenobi is Professor of Analytical Chemistry at the Organic Chemistry Laboratory of the Swiss Federal Institute of Technology (ETH) Zurich. Born in Zurich in 1961, he received a M.S. degree from the ETH Zurich in 1986, and a Ph.D. at Stanford University in the USA in 1990. This was followed by two postdoctoral appointments at the University of Pittsburgh (1990 - 1991) and at the University of Michigan (1991). Renato Zenobi returned to Switzerland in 1992 as a Werner Fellow at the EPFL. He joined ETH Zurich in 1995, where he has been since. In 2010 he was appointed Associate Editor of *Analytical Chemistry* (American Chemical Society). He has chaired the 2014 International Mass Spectrometry Conference in Geneva, Switzerland. Renato Zenobi has received many awards for his scientific work, most recently the Thomson Medal (International Mass Spectrometry Foundation, 2014), the 2014 RUSNANO prize, and the 2015 Fresenius Award (German Chemical Society/GDCh). Zenobi's research areas include laser-based

analytical chemistry, electrospray and laser-assisted mass spectrometry, ambient mass spectrometry, and near-field optical microscopy and spectroscopy. He has made important contributions to the understanding of the ion formation mechanism in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, and to ambient ionization methods. He is well known for the development of analytical tools for the nanoscale, in particular TERS (tip-enhanced Raman spectroscopy), a spectroscopic methodology with ≈ 10 nm spatial resolution.

The title of Prof. Zenobi's presentation was ***«Exhaled Breath as a Window to Body Metabolism: From Medical Diagnosis To Pharmacokinetics».*** The research group of Prof. Zenobi at the ETH Zurich has developed a method allowing to detect organic substances in the breath. Breath is directly transferred to the analytical instrument where the molecules are ionized with secondary electrospray and then the molecular weight determined by high resolution mass spectrometry. Molecules can be detected up to 1000 Da with a sensitivity in the range of ppt (1 part/billion). This non-invasive and very fast analytical method allows an unexpected number of applications. Provided the right molecules are analyzed in the spectrum, it is possible to acquire human finger or breath prints. These are individual and partly depending on daytime. In addition, they can potentially be used as diagnostic tool. In a patient study suffering from chronic sleep apnoea syndrome (OSAS) a statistically significant changement in the profile of particular substances could be measured, in case the patients were no longer treated with compressed air during the night. In another study significant differences in the breath prints of patients with light COPD, heavy COPD, healthy smokers and healthy non-smokers could be shown. It can be assumed that this method is also applicable to the diagnosis of non-lung diseases. Such studies are ongoing. However, the challenge is to find among the exhaled molecules the right markers for particular diseases allowing to get characteristic breath prints. The required statistics and structure elucidation by mass spectrometry is complex and complicated. Therefore, additional research is needed to improve the methodology. The method could even be used for drug development in animals. For example it was possible to determine the pharmacokinetics of ketamine and its metabolites in the breath of mice. This would allow to considerably reduce the number of animal experiments. In humans it could be possible to use the method for therapeutic drug monitoring or compliance control. Last but not least, because of the trend to always smaller and cheaper mass spectrometers Prof. Zenobi is confident that his method can in future find its way into hospitals, doctor's practice and pharmacies.

*Speaker's Abstract:*

Exhaled breath contains relevant information on a person's health status. Our vision is to use real-time and completely non-invasive chemical analysis of exhaled breath for applications such as medical diagnosis, monitoring progress and treatment of diseases, drug compliance, pharmacokinetics, and others. The methodology we use to analyze breath in real time is based on secondary electrospray ionization coupled to high-resolution mass spectrometry (SESI-HRMS). It affords ppb … ppt limits of detection, and analysis of compounds with molecular weights up to 1000 Da. Extensive statistical and chemometric evaluation of the large data sets is another important element for the success of this approach. A number of interesting

questions can now be addressed via on-line mass spectrometric analysis of exhaled breath: is there a core pattern for individual phenotypes visible in mass spectrometric "breathprints"? Can diurnal changes be monitored via exhaled breath? Can diseases be diagnosed via exhaled breath, and if yes, which ones? Can proper drug use (or drug abuse) be detected via analysis of the chemical composition of exhaled breath? The presentation will focus on several examples in medical diagnosis, including the detection of novel biomarkers for diseases such as obstructive sleep apnea (OSA) [1] and chronic obstructive pulmonary disease (COPD) [2]. Monitoring of drug compliance and pharmacokinetics via real-time SESI-MS will also be shown [3,4].

References:


[1] E.I. Schwarz, P. Martinez-Lozano Sinues, L. Bregy, T. Gaisl, D. García-Gómez, M.T. Gaugg, Y. Suter, N. Stebler, Y. Nussbaumer-Ochsner, K.E. Bloch, J.R. Stradling, R. Zenobi, and M. Kohler, *Effects of CPAP Therapy Withdrawal on Exhaled Breath Pattern in Obstructive Sleep Apnoea*, Thorax 71, 110-117 (2016).

[2] P. Martinez-Lozano Sinues, L. Meier, C. Berchtold, N. Sievi, G. Camen, M. Kohler, and R. Zenobi, *Breath Analysis in Real Time by Mass Spectrometry in Chronic Obstructive Pulmonary Disease*, Respiration 87, 301-310 (2014).

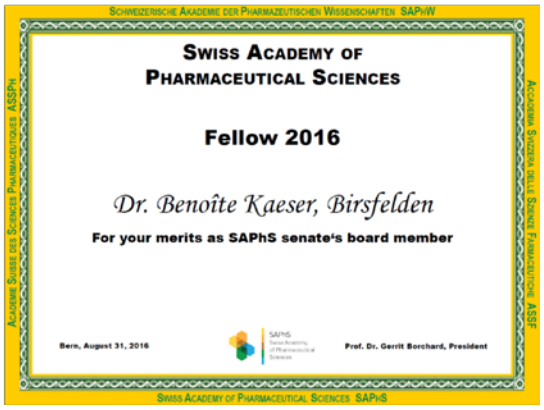
[3] X. Li, P. Martinez-Lozano Sinues, R. Dallmann, L. Bregy, M. Hollmén, S. Proulx, S.A. Brown, M. Detmar, M. Kohler, and R. Zenobi, *Drug pharmacokinetics determined by real-time analysis of mouse breath*, Angew. Chem. Int. Ed. 54, 7815-7818 (2015); *Pharmakokinetik von Medikamenten durch Echtzeit-Analyse der Atemluft von Mäusen*, Angew. Chem. 127, 7926-7929 (2015).

[4] G. Gamez, L. Zhu, A. Disko, H. Chen, V. Azov, K. Chingin, G. Krämer, and R. Zenobi, *Real-time, In-vivo Monitoring and Pharmacokinetics of Valproic Acid via Analysis of Exhaled Breath*, Chem. Comm. 47, 4884-4886 (2011).


## Recognitions and Awards – Fellows of SAPhS and Poster Prizes

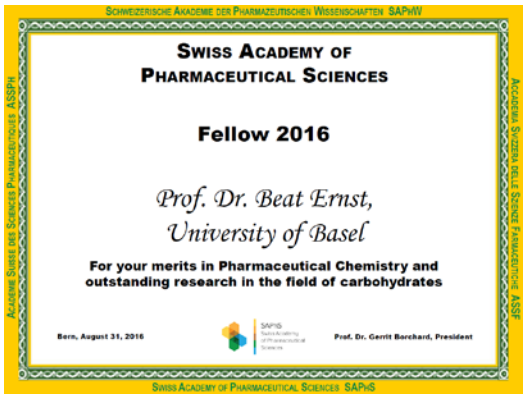
The SAPhS nominates every year scientists who are distinguished by their outstanding research and professional contributions in the field of Pharmaceutical Sciences in Switzerland. The following four distinguished colleagues were awarded **"Fellows of the SAPhS 2016"**:

***Dr. Benoîte Kaeser*****,** Birsfelden, SAPhS senate's board member**:**
***„For your merits as a SAPhS senate's board member".***

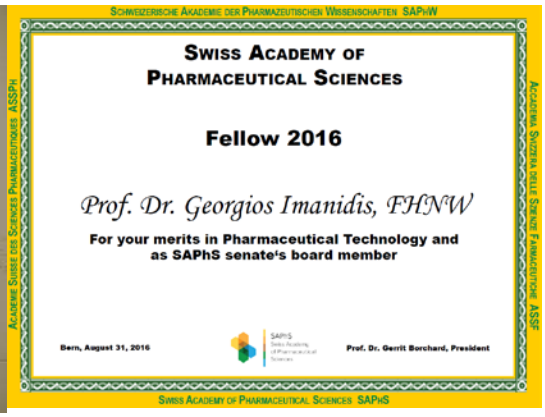


***Prof. Dr. Beat Ernst***, Dept. of Pharmaceutical Sciences, University of Basel:
*„For your merits in Pharmaceutical Chemistry and outstanding research in the field of carbohydrates".*

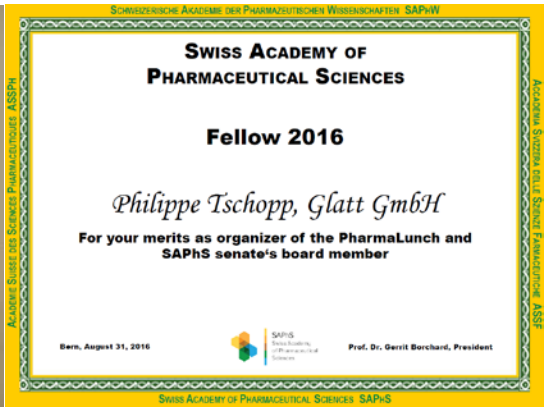
(Could not be present)




***Prof. Dr. Georgios Imanidis***, University of Applied Sciences and Arts Northwestern Switzerland FHNW, SAPhS senate's board member:
*„For your merits in Pharmaceutical Technology and as a SAPhS senate's board member".*




***Philippe Tschopp***, Glatt Group, SAPhS senate's board member:
*„For your merits as organizer of the PharmaLunch and SAPhS senate's board member".*



The following six **Poster Prizes** were awarded:

**1st prize** (CHF 1‘500), sponsored by Mundipharma Medical Company Basel, presented by Dr. Andreas Stöckli to

***Elena Moroz***, ETH Zurich (group Prof. Leroux), poster P-23:
*“Carrier-Free Gene Silencing by Amphiphilic Nucleic Acid Conjugates in Differentiated Intestinal Cells”*

**2nd prize** (1’000), sponsored by the Foundation of the Association of Bernese Pharmacists (AKB), presented by the AKB past president Michele Bordoni to

***Janine Hussner***, University of Basel (group Prof. Meyer zu Schwabedissen), poster P-46:
*„Species Specific Differences in Structure and Function of the Organic Anion Transporting Polypeptide 2B1”*

The awardee already left the SPhSD before the ceremony

**3rd prize** (500), sponsored by the Pharmaceutical Society of Zurich (PharmGZ), presented by Prof.Gander to

***Katrin Fuchs***, University of Geneva (group Prof. Jordan), poster P-15:
*„Mapping of Antiangiogenic Drug Distribution in a Rabbit Model of Liver Cancer“*

**Prize for the best poster in Pharmaceutical Technology** (1’000), sponsored by Glatt Group, presented by Philippe Tschopp to

***Philip Grossen***, University of Basel (group Prof. Huwyler), poster P-38:
*„Functionalized PEG-b-PCL Nanoparticles to Target Human Brain Capillary Endothelial Cells in vitro“*

**Prize for the best poster in Pharmaceutical Biology** (1’000), sponsored by Zeller Söhne AG, to

***Joëlle Houriet***, University of Geneva (group Prof. Wolfender), poster P-35:
*„Investigation of the Anti-Obesity Effect of Pueraria montana var. lobata”*

**Special Prize** (500), sponsored by Vifor Pharma, presented by Prof. Stefan Mühlebach to

***ElHadji Assane Diop***, University of Geneva (group Prof. Rudaz), poster P-48:
*"Low-Cost Analytical Device for Detecting Counterfeits and Sub-Standards in Emerging Countries"*

Proud awardees, sponsors and organizers – all together

## Acknowledgements and invitation to the 10th Swiss Pharma Science Day 2017

The organizers would like to thank all speakers for their excellent presentations, the reviewer board (Proff. Cuendet, Gander, Germershaus, Huwyler and Mühlebach) for evaluating the poster prizes, the photographer Philippe Tschopp, and all colleagues of the SAPhS senate's board who helped to realize the SPhSD 2016.

Everybody is welcome to participate in the **10th Anniversary SPhSD**, which will take place on **Tuesday August 22, 2017**, at the Ettore Rossi Auditorium, Childrens Hospital, Inselspital-University Hospital Bern.

## Sponsors of SPhSD 2016

- AKB-Stiftung zur Förderung des Pharmazeutischen Nachwuchses
  Platin Sponsor, sponsoring Second Poster Prize, keynote lecture of Prof. Khinast and lecture of Dr. Fishman

- Stiftung der Gesellschaft Schweizer Industrieapotheker (GSIA)
  Gold Sponsor, sponsoring lecture of Prof. Keiser

- Mundipharma Medical Comp. Basel
  Gold Sponsor, sponsoring First Poster Prize and lecture of Prof. Hall

- Actelion Pharma
  Gold sponsor, sponsoring lecture Prof. Zenobi

- CSL Behring Bern

- Max Zeller Söhne AG
- Sponsoring prize for best poster in Pharmaceutical Biology

- Glatt Group
  Sponsoring prize for best poster in Pharmaceutical Technology

- Pharmazeutische Gesellschaft Zürich (PharmGZ)
  Sponsoring Third Poster Prize

- Vifor Pharma AG
  Sponsoring Special Poster Prize

- Galexis AG

- Medworker

## Closing the SPhSD 2016 at the House of the University of Bern

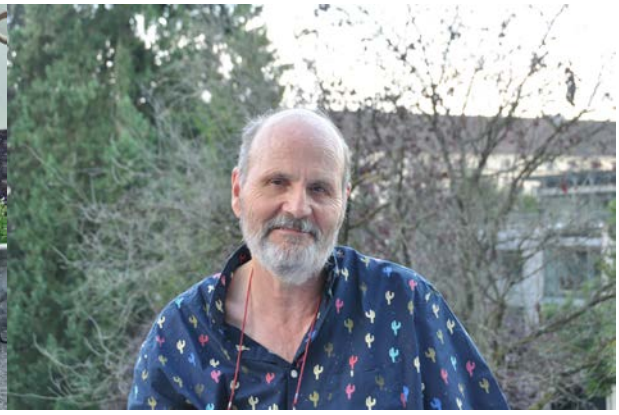
It is a tradition to finish the Swiss Pharma Science Day with an apéro at the historic House of the University of Bern, as usual on a beautiful and warm summer evening. Participants enjoyed tasteful Swiss wines and snacks facilitating socializing and networking.











See you all again at the 10. SPhSD 2017!

## P-1

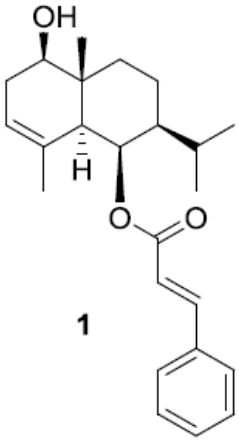
## Eudesmane Sesquiterpenes from *Verbesina lanata* with Inhibitory Activity Against Major Agricultural Pathogens

**J. Ramseyer[1], B. Thuerig[2], M. De Mieri[1], H.-J. Schärer[2], T. Oberhänsli[2], M. P. Gupta[3], L. Tamm[2], O. Potterat[1], M. Hamburger[1]**

[1] *Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
[2] *Research Institute of Organic Agriculture FiBL, 5070 Frick, Switzerland*
[3] *Centro de Investigaciones Farmacognósticas de la Flora Panameña, Facultad de Farmacia, Universidad de Panamá, Panamá, República de Panamá*

**Introduction:** Copper is still permitted in organic farming to control plant fungal diseases. However, due to its unfavourable ecotoxicological profile, there is a growing demand for environmentally safer substitutes.
**Aims:** The aim of this study is to identify plant or fungal extracts with activity against major plant pathogens.
**Methods:** An in-house library of more than 3000 extracts from plant and fungal origin was screened *in vitro* against *Venturia inaequalis*, *Phytophthora infestans*, and *Plasmopara viticola*. The active constituents were tracked by a procedure referred to as HPLC-based activity profiling which combines biological activity data with chemoanalytical information. Active compounds were isolated by a combination of chromatographic techniques, including silicagel column chromato-graphy and preparative HPLC. Structure elucidation was performed by a combination of ESI-MS and NMR spectroscopy.
**Results:** As one of the hits, the ethyl acetate extract from the inflorescences of *Verbesina lanata* Rob. & Greenm. (Asteraceae) showed significant inhibitory activity *in vitro* against *V. inaequalis* and *P. viticola*, with $MIC_{100}$ values of 125 and 64 µg/mL, respectively. The activity could be correlated with a series of lipophilic compounds in the HPLC-UV chromatogram. Preparative isolation afforded several compounds which were identified as eudesmane sesquiterpenes. The major compound, 6β-cinnamoyloxy-1β-hydroxy-eudesm-3-ene (**1**), showed strong antifungal activity, with $MIC_{100}$ of 33 µg/mL against *V. inaequalis* and 4 µg/mL against *P. viticola*.
**Conclusions:** Our results demonstrate that plant-derived compounds could provide potential alternatives to copper in organic farming.

**Keywords:** Fungicides, organic farming, copper, *Verbesina lanata*, eudesmane sesquiterpenes.




**1**

**P-2**

## *In Vitro* Blood-Brain Barrier Permeability Predictions for $GABA_A$ Receptor Modulating Piperine Analogs

**M. Oufir[1], D. E. Eigenmann[1], C. Dürig[1], E. A. Jähne[1], M. Smieško[2], M. Culot[3], F. Gosselet[3], R. Cecchelli[3], H. C. Cederberg Helms[4], B. Brodin[4], L. Wimmer[5], M. D. Mihovilovic[5], M. Hamburger[1]**

[1] *Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
[2] *Molecular Modeling, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
[3] *Univ. Artois, EA 2465, Laboratoire de la Barrière Hémato-Encéphalique (LBHE), F-62300 Lens Cedex, France*
[4] *Drug Transporters in ADME, Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark*
[5] *Institute of Applied Synthetic Chemistry, TU Wien, A-1060 Vienna, Austria*

**Introduction:** The alkaloid piperine from black pepper was recently identified as a positive allosteric modulator of γ-aminobutyric acid type A ($GABA_A$) receptors interacting at a benzodiazepine-independent binding site [1, 2]. Subsequently, three generations of piperine analogs with improved pharmacological properties were synthesized. In order to reach the CNS, these compounds need to enter the brain by crossing the blood-brain barrier (BBB).
**Aims:** We here evaluated the BBB permeability of piperine and five selected analogs (SCT-66, SCT-64, SCT-29, LAU397, and LAU399) in three *in vitro* BBB models: a recently validated human model with immortalized hBMEC cells, a stem cell-derived human brain-like endothelial cells (BLEC) model, and a primary animal (bovine endothelial/rat astrocytes co-culture) model.
**Methods:** For each compound, reliable quantitative UHPLC-MS/MS methods in the range of 5–500 ng/mL in the corresponding matrix were developed, and permeability coefficients in the three BBB models were determined.
**Results:** *In vitro* predictions from the two human BBB models were in good agreement, while permeability data from the animal model differed to some extent, possibly due to protein binding of the screened compounds. In all three BBB models, piperine and SCT-64 displayed the highest BBB permeation potential. This was corroborated by data from *in silico* prediction. For the other piperine analogs (SCT-66, SCT-29, LAU397, and LAU399), BBB permeability was low to moderate in the two human BBB models, and moderate to high in the animal BBB model [3].
**Conclusions:** These results serve for selecting the most promising candidate molecule for the next cycle of medicinal chemistry optimization.

**Keywords:** $GABA_A$ receptor, human stem cells, immortalized cell line, *in vitro* blood–brain barrier (BBB) model, primary cells.

**References:**
[1] Zaugg J et al. J Nat Prod 2010; 73: 185-191.
[2] Khom S et al., Biochem Pharmacol 2013; 85: 1827-1836.
[3] Eigenmann DE et al. Eur J Pharm Biopharm 2016; 103: 118-126.

**P-3**

## The ME-NU Study - Does Multifaceted Nutritional Education Improve Malnutrition Management in Hospitals? Project with Emphasis on Educational Interventions and the Ethics Committee Submission

**P. Cadisch[1], C. Aeberhard[2], Z. Stanga[2], S. Mühlebach[1]**

[1] *Department of Clinical Pharmacy and Epidemiology, University of Basel, 4031 Basel, Switzerland*
[2] *Department of Diabetes, Endocrinology, Nutritional Medicine and Metabolism, Bern University Hospital-Inselspital, and University of Bern, 3010 Bern, Switzerland*

**Introduction:** Disease-related malnutrition (DRM) is a condition that results from a lack of uptake or intake of energy and nutrients. It leads to an altered body composition with diminished function and a negative clinical outcome. DRM is a worldwide problem that is highly prevalent (20-50% of the admitted patients in hospitals). It has negative effects on health and is often associated with an increased risk of complications, including increased morbidity and mortality rates with concomitant economical impact. Nutritional education of physicians is vital in treating and preventing DRM and must be improved as doctors tend to lack the awareness and knowledge necessary to adequately manage patients' malnutrition.
**Aims:** The aim of the master thesis was to design and plan a study that examines the effect of multifaceted nutritional education for resident and senior physicians on their knowledge and its translation to clinical practice. A specific aim was to submit the study project to the Ethics Committee for approval. Additionally, the objective was to generate and conduct pilot trials with medical students using different educational strategies and ultimately evaluate their performance.
**Methods:** After an intensive literature research, the Medical Education-Nutrition (ME-NU) study was designed and planned. The required documents for the Ethics Committee were prepared, reviewed in various expert rounds and submitted. Two educational tools (interactive case presentation and online case vignettes) were developed and tested with medical students.
**Results:** The documents submitted were analyzed by the Ethics Committee and evaluated as not underlying their competence, therefore the ME-NU study is ready to be conducted. The interactive case presentation and online case vignettes improved the students' knowledge by 10.7% (7 students, p-value = 0.061) and 1.2% (27 students, p-value = 0.691), respectively. Both educational tools increased the awareness of clinical nutrition to the students and were rated as instructive. Students are interested in clinical nutrition, but lack of knowledge, time pressure and unclear responsibility negatively impact the issue.
**Conclusions:** The students' initial knowledge about the management of malnutrition is poor, due to little emphasis laid on clinical nutrition in medical education and training. The interactive case presentation represents an appropriate method to educate students. The contribution of the online case vignettes can be further improved. Knowledge is an eminent factor to ameliorate malnutrition management in hospitals. Furthermore, it is important to clearly define the responsibilities and to develop optimized processes in order to overcome lack of time to manage malnutrition in hospitals.

**Keywords:** Disease-related malnutrition, malnutrition management, nutritional knowledge, medical education.

**P-4**

## Nanocrystals of Kartogenin Loaded Particles for Cartilage Regeneration

**P. Maudens, L. Neff, C. Thauvin, O. Jordan, E. Allémann**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction:** 15 % of the world's population suffers from osteoarthritis (OA), the most common type of joint disease that mostly affects cartilage. Development of new drug delivery systems for intra-articular (IA) administration is a must to treat OA.
**Aims:** The aim of this study was to develop Nanocrystals-Polymer Particles (NPPs) as highly drug-loaded particles with extended release properties for a local and potent osteoarthritis treatment and to test this concept *in vivo*.
**Methods:** Kartogenin, a CBFβ-RUNX1 pathway activator, has been selected as a very potent cartilage promoting active pharmaceutical ingredient [1]. The different steps of the study were, (i) to formulate nanocrystals of Kartogenin by wet-milling and to protect them against a crystalline regrowth (assessed by SEM, X-ray diffraction, DSC and DLS characterization), (ii) to synthesize a fluorescent polymer allowing intravital tracking of particles, (iii) to encapsulate a high payload of nanocrystals in polymeric microparticles (obtained by spray drying and characterized by laser diffraction, SEM, UHPLC), (iv) to assess *in vitro* drug release, (v) to evaluate cytotoxicity of particles on human synoviocytes (MTT test) and (vi) to investigate *in vivo* the activity of the particles on an OA mouse model induced by a destabilization of the medial meniscus (DMM) (evaluated by X-ray microtomography, Multiplex ELISA and histology) [2].
**Results:** Kartogenin nanocrystals were produced with a monomodal size distribution by a wet-milling process and optimally stabilized at least over 4 weeks by Vitamin E TPGS. NPPs obtained by spray-drying had a mean size of 13.6 µm with a high drug loading of 31.5% w/w and extended drug release of 62% over 3 months. NPPs were non-toxic to human synoviocytes at 100×$EC_{50}$. *In vivo* experiments on DMM murine model showed a good retention of NPPs in the joint over a 2-months period, revealed an effect on bone density and cartilage repair (Fig. 1). Serum levels of OA biomarkers (e.g. IL-1β and VEGF) were also investigated.

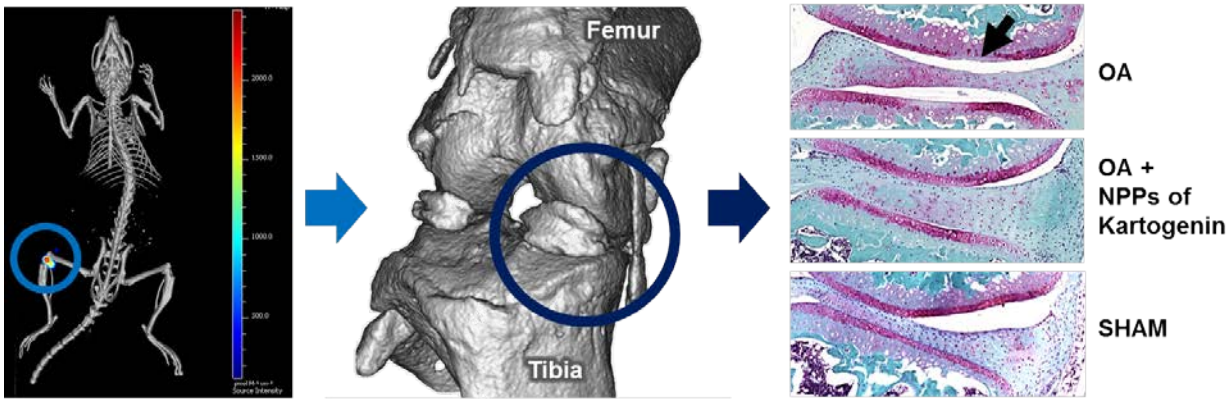



**Fig. 1.** Micro-CT and intravital imaging of mouse (left), Micro-CT of knee (center) and histological evaluation of cartilage thickness with Safranin O / Fast Green stain (right).
**Conclusions:** A new pharmaceutical particle technology has been successfully developed to offer a controlled and extended release of Kartogenin from biocompatible polymeric particles for cartilage repair. Its bioactivity in an osteoarthritis mouse model was shown. This innovative drug delivery system provides locally a high Kartogenin dose over a long period of time. It shows promise as an efficient treatment of OA.
**Keywords:** Kartogenin, nanocrystals, microparticles, cartilage, osteoarthritis.
**References:**
[1] Johnson K et al. Science 2012; 336: 717-21.
[2] Glasson SS, Blanchet TJ, Morris EA. Osteoarthritis & Cartilage 2007; 15: 1061-69.

**P-5**

## Home Parenteral Nutrition (SwissHPN-II Study): The Design of a Follow-On Investigation with Emphasis on the Evaluation of Catheter-Related Complications, on Patients' and Physicians' Questionnaires and on the Ethics Committee Submission

**C. Jeiziner[1], C. Aeberhard[2], Z. Stanga[2], S. Mühlebach[1]**

[1] *Department of Clinical Pharmacy and Epidemiology, University of Basel, 4031 Basel, Switzerland*
[2] *Department of Diabetes, Endocrinology, Nutritional Medicine and Metabolism, Bern University Hospital-Inselspital, and University of Bern, 3010 Bern, Switzerland*

**Introduction**: Home parenteral nutrition (HPN) is a long-term intravenous administration of nutrients and water to patients not able to meet their nutritional requirements by the oral and/or enteral route in a home setting [1]. It concerns only a few patients in Switzerland (4 in 1 million habitants [2]). A central venous access is necessary for HPN. Catheter related complications (CRC) are relevant and therefore measures to prevent are important in this population. A most common problem is the catheter-related infection (CRI). A first nationwide prospective study over a 3-months (SwissHPN [2]) took place in 2013/2014. A follow-on study over a longer period of time (SwissHPN-II) is intended and the documentation for the Ethics Committee submission is therefore needed.
**Aims**: The approval of the follow-on study SwissHPN-II with the submission of the necessary documents to the Ethics Committee of the University Hospital of Bern is aimed. The evaluation of CRC will have a special focus. Additionally, a collaboration with the HAN&CIF special interest group of the European Society for Clinical Nutrition and Metabolism (ESPEN) is intended. The learnings for a master thesis student will be evaluated.
**Methods**: An extensive literature search on CRC was undertaken. The questionnaires and the study protocol were processed according to the literature search and by using internal expert evaluation rounds. A proof of concept in pilot interview study using the established questionnaires was conducted. On March 1st 2016 – CIF Action Day - the actual HPN patients of the University Hospital of Bern were assessed according to a given structured database.
**Results**: The final questionnaires as a result of the multi-professional evaluation was defined and the study protocol with all the corresponding documents was submitted on 27th April 2016. A total of 5 patients and 5 physicians were included in the pilot study. Infections were the most frequent CRC.
**Conclusions**: This master thesis allowed a most important learning experience on HPN and a first approach to see the different HPN patient characteristics, the underlying diseases and therapy indications and their living situation. With the help of a multi-professional team useful question-naires for maximum structured information to characterize and evaluate the Swiss HPN patients in the SwissHPN-II study were created. From the pilot study resulted that a more mentally than physically good quality of life in HPN patients is possible. CRI are frequent. Successful prevention and treatment of CRC can be achieved by improving knowledge in care and handling of the catheters.

**Keywords**: Home parenteral nutrition, quality of life, catheter-related complications.

**References :**

[1] Staun M et al. Clin Nutr 2009; 28: 467-479.
[2] Aeberhard C et al. Ann Nutr Metab 2015; 67: 210-217.

**P-6**

## Statin Use and Risk of Gallstone Disease in Switzerland: A Case-Control Study Based on Swiss Claims Data

**F. A. Biétry[1,2], O. Reich[3], M. Schwenkglenks[4], C. R. Meier[1,2]**

*[1] Basel Pharmacoepidemiology Unit, Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, 4031 Basel, Switzerland*
*[2] Hospital Pharmacy, University Hospital Basel, 4031 Basel, Switzerland*
*[3] Department of Health Sciences, Helsana Insurance Group, 8081 Zürich, Switzerland*
*[4] Institute of Pharmaceutical Medicine (ECPM), University of Basel, 4056 Basel, Switzerland*

**Introduction:** Gallstone disease is a heavy economic burden in the Western world. Use of statins has previously been associated with a lower risk of developing cholesterol gallstones. So far, no studies on this association have been conducted using data from the Swiss ambulatory setting.
**Aims:** To examine the association between use of statins and the risk of cholecystectomy.
**Methods:** We conducted a matched case-control study using claims data from the Helsana Group, a large Swiss health insurance provider, to identify cases with cholecystectomy (as proxy for gallstone disease) between 2013 and 2014. We identified four random controls for each case, matched on age, sex, index date, and canton. Every patient was required to be enrolled with the Helsana Group constantly from 2008 on. We categorized patients into current (last prescription recorded within 180 days prior to the index date) or past statin use (last prescription recorded more than 180 days prior to the index date). Additionally, we categorized medication use by duration of use prior to the index date (short-term, 1-4; medium-term, 5-19; long-term, ≥ 20 prescriptions). We applied conditional logistic regression analysis to calculate relative risk estimates as odds ratios (ORs) with 95% confidence intervals (CIs). We adjusted the ORs and CIs for history of diabetes, ischemic heart disease, stroke and transient ischemic attack, use of opposed or unopposed oestrogens, use of fibrates, and other lipid-lowering agents.
**Results:** We identified a total of 2,220 cholecystectomy cases and 8,880 controls. Compared with non-use of statins, the adjusted OR (aOR) of undergoing a cholecystectomy was 0.85 (95% CI 0.74-0.99) for current statin users. Short-term current statin use was not associated with a statistically significantly altered risk estimate for cholecystectomy (aOR 1.34, 95% CI 0.99-1.83), while long-term use of statins was associated with reduced ORs (5-19 current statin prescriptions; aOR 0.77, 95% CI 0.65-0.92). The cholecystectomy risk was not affected either by short-term current statin use or by past statin use, irrespective of the duration of exposure.
**Conclusions:** Long-term use of statins was associated with a reduced risk of cholecystectomy.

**Keywords:** Cholecystectomy, claims data, gallstone disease, risk, statins.

**P-7**

## Benzodiazepine Use and Risk of Developing Alzheimer's Disease: A Case-Control Study Based on Swiss Claims Data

**F. A. Biétry[1,2], A. Pfeil[3], O. Reich[4], M. Schwenkglenks[3], C. R. Meier[1,2]**

*[1] Basel Pharmacoepidemiology Unit, Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, 4031 Basel, Switzerland*
*[2] Hospital Pharmacy, University Hospital Basel, 4031 Basel, Switzerland*
*[3] Institute of Pharmaceutical Medicine (ECPM), University of Basel, 4056 Basel, Switzerland*
*[4] Department of Health Sciences, Helsana Insurance Group, 8081 Zürich, Switzerland*

**Introduction:** A possible association between benzodiazepine use and Alzheimer's disease (AD) has been hypothesized in previous studies. We explored the relation between benzodiazepine exposure and the risk of developing AD in the Swiss ambulatory setting.
**Aims:** To estimate the relative risk of developing AD in relation to previous benzodiazepine use.
**Methods:** We conducted a matched case-control study using claims data from the Helsana group, a large Swiss health insurance provider, to explore benzodiazepine use in cases with AD in 2013 or 2014 and in controls. We identified cases via recorded incident use of acetylcholinesterase inhibitors or N-methyl-D-aspartate receptor antagonist memantine, based on recorded anatomic therapeutic chemical classification codes for these drugs. For each case, we identified at random one control patient with no prescriptions for one of the above mentioned AD-specific drugs but matched on age, sex, index date and canton. Every patient was required to be constantly insured in the Helsana Group from 2008 on. The date of the first prescription for an AD-specific drug was referred to as the "diagnosis date". Because of the assumption that first-time prescription of a benzodiazepine close to the diagnosis date may be due to symptomatic treatment of prodromal symptoms of early dementia, we shifted the index date to 2 years before the diagnosis date. We conducted conditional logistic regression analyses to calculate relative risk estimates as odds ratios (ORs) of developing AD with 95% confidence intervals (CIs), adjusted for use of antidepressants.
**Results:** The adjusted OR (aOR) (95% CI) of developing AD for those who started benzodiazepines in the year before diagnosis was 1.36 (0.94-1.96). After accounting for benzodiazepine use initiated during the prodromal phase by shifting the index date, use of benzodiazepines was not associated with an increased risk of developing AD 0.84 (0.72-0.97). Long-term benzodiazepine use yielded an aOR of 0.82 (0.58-1.17).
**Conclusions:** Benzodiazepine use was not associated with an altered risk of AD after taking into consideration a prodromal phase of 2 years.

**Keywords:** Alzheimer's disease, benzodiazepines, case-control study, claims data, risk.

**P-8**

## Wound Healing Through Nano-Complexes

**V. Patrulea[1], L. A. Applegate[2], V. Ostafe[3], O. Jordan[1], G. Borchard[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] University Hospital of Lausanne (CHUV-UNIL), Department of Musculoskeletal Medicine, 1066 Epalinges, Switzerland*
*[3] West University of Timisoara, Department of Chemistry, Timisoara, 300115, Romania*

**Introduction**: Dermal wound healing is a complex process, which includes four overlapping stages: inflammation, migration, proliferation and maturation [1]. Tissue repair refers to healing in which new growth restores damaged tissue to the normal state. The ability of skin to repair itself after a minor injury is remarkable, but this is not the case for difficult-to-healing, chronic and ulcer wounds. Therefore, there is a growing need for developing biomaterials promoting wound healing to improve tissue regeneration. Wound regeneration needs to be guided by biological cues, such as Arg-Gly-Asp (RGD), a peptide known to induce cell adhesion and migration [2]. Nanocomplexes based on polyelectrolyte self-assembly are suitable carriers for these cues in order to accelerate the healing and improve the completeness of the final repair.
**Aims**: Our focus is to develop different formulations of polyelectrolyte nanocomplexes for topical wound application: a sprayable suspension of nanocomplexes, nano-structured hydrogels and freeze-dried foams, which would hydrate upon exudate absorption. Formulations are based on the chitosan derivative O-carboxymethyl-N,N,N-trimethyl-chitosan (CMTMC) grafted with RGDC peptide.
**Methods**: CMTMC was functionalized with RGDC through a 6-carbon spacer (1,6-diaminohexane, DAH), leading to RGDC-DAH-CMTC. Nano-sized polyelectrolyte particles were prepared by complexation of the cationic chitosan derivative with anionic chondroitin sulfate. Hydrogels were obtained by mixing RGDC-functionalized chitosan with hyaluronic acid (HA) at a 1:1 (v/v). Foams were produced by lyophilization of the previously prepared hydrogels. Both nanocomplex suspensions and hydrogels were formulated and tested for their potential to induce human dermal fibroblast (HDF) adhesion, migration and subsequent wound healing.
**Results**: The synthesis process allowed for controlled covalent binding of RGDC at a high peptide substitution degree (15.3 µg of peptide per mg of chitosan). Nano-sized polyelectrolyte particles were obtained with a size of about 200 nm as confirmed by differential light scattering (DLS) and scanning electron microscopy (SEM). Hyaluronic acid gels embedding RGDC-DAH-CMTMC nano-gels were prepared, with viscosities adapted for topical patient application. Upon lyophilization, freeze-dried foam bandages were also obtained.
The new extra-cellular matrix based on chitosan derivatives was shown to be useful both as a carrier of RGDC peptide and for complete closure of wounds after 24 h. *In vitro* bio-adhesion assay showed that HDF treated with formulations based on RGDC-derivatized chitosan showed a spread phenotype (instead of round cells) and increased motility compared to CMTMC treated control cells. These results were attributed to the presence of the adhesion peptide.
**Conclusions**: Overall, adhesion peptide-bearing nano-formulations promoted HDF survival, motility and migration. They have the potential to accelerate cell migration *in vivo* and promote healing of chronic wounds.

**Keywords**: Wound healing, tissue repair, chitosan, bioadhesion peptide.

**References:**
[1] Patrulea V et al. Eur J Pharm Biopharm 2015; 97: 417-26.
[2] Patrulea V et al. Carbohydr Polym 2016; 142:114-23.

**P-9**

## The *In Vitro* Cytotoxic Effect of Withanolides in Multiple Myeloma Cancer Stem Cells and Tumoral Cells

**M. E. Issa[1], S. Barta[1], A. Monteillier[1], E. M. K. Wijeratne[2], A. A. L. Gunatilaka[2], M. Cuendet[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] Natural Products Center, The University of Arizona, 250 E. Valencia Rd, Tucson, AZ 85706-6800, USA*

**Introduction:** Despite recent therapeutic advances, multiple myeloma (MM) remains an incurable malignancy. This is partly attributed to cancer stem cells (CSCs), a rare cancer subpopulation believed to cause the fatal relapses that affect the vast majority of MM patients. CSCs are equipped with metabolic tools that render them highly resistant to conventional therapies. Thus, compounds capable of eradicating MM-CSCs may significantly improve the prognosis of MM patients.
**Aims:** The aim of this project was to investigate the cytotoxic effect of a series of withanolides in MM-CSCs and tumoral cells.
**Methods:** Highly tumorigenic MM-CSCs ($CD44^+$, $CD166^+$ and $CD138^-$), RPMI-8226, dexamethasone-sensitive (MM1-S) and dexamethasone-resistant (MM1-R) myeloma tumoral cells were used to study the growth inhibitory effects of withanolides using MTT/XTT assays. In MM-CSCs and RPMI 8226 cells, the effects of the most potent withanolide (G5) on cell cycle distribution, viability/mortality and apoptosis were assessed using DNA-specific staining, calcein/ethidium homodimer staining, and annexin V/propidium iodide staining, respectively. In order to identify a possible mechanism of action that underlies the growth inhibition of withanolides, a cell-based NF-κB inhibition assay was employed.
**Results:** Withanolides inhibited the growth of sensitive and resistant MM cells. Withanolide G5 resulted in an accumulation in $G_2$ phase, induced cell death and apoptosis in MM-CSCs and RPMI 8226 cells. Preliminary results indicated that the tested withanolides inhibited NF-κB.
**Conclusions:** Withanolides exhibited anticancer effects in MM cells, likely through NF-κB inhibition. These results warrant further investigation of G5 and other withanolides in additional MM-CSCs models and potentially in *in vivo* models.

**Keywords:** Multiple myeloma, withanolides, cancer stem cells.

**P-10**

## Compatibility and Stability of Sandimmun® in Commercial All-in-One Parenteral Nutrition Admixtures

**N. Bilang[1], C. Aeberhard[2], Z. Stanga[2], S. Mühlebach[1]**

*[1] Department of Clinical Pharmacy and Epidemiology, University of Basel, 4031 Basel, Switzerland*
*[2] Department of Diabetes, Endocrinology, Nutritional Medicine and Metabolism Bern University Hospital-Inselspital and University of Bern, 3010 Bern, Switzerland*

**Introduction:** To treat or prevent malnutrition parenteral nutrition (PN) is indicated in patients where the gastrointestinal tract does not work sufficiently anymore. These patients often suffer from severe underlying diseases which have to be treated by i.v. medications. Co-administration of a drug and PN or even admixing the drug inside the PN is generally not recommended. Due to the complex formulation of the normally used AiO PN admixture the addition of drugs could lead to interactions between the components which might result in reduced efficacy or even adverse effects in the patient. A medication used on a long-term period and potentially indicated in such PN patients is the critical dose drug cyclosporin A (Sandimmun®), an immunosuppressant. The i.v. formulation of cyclosporin A (CsA) is a micellar solution with potential interactions with the PN. Physicochemical stability tests are required to document compatibility and stability of Sandimmun® but also of the PN admixture over the time of administration.
**Aims:** The aim of this study was to test the physicochemical stability and compatibility of CsA in two often used AiO PN O/W-emulsion admixtures (NuTRIflex® Omega Special and NuTRIflex® Lipid Special). A specific focus was given to assess whether the emulsifier of Sandimmun® has an influence on the lipid emulsion stability of PN admixtures.
**Methods:** Physicochemical stability of CsA within the PN was tested with a quantitative LC-MS/MS method used for therapeutic drug monitoring (TDM) and adapted to this specific analysis in a PN admixture matrix. The PN emulsion stability was comparatively assessed by lipid droplet measuring in a critical size ≥ 1 μm by a validated oil immersion light microscopic method in a defined sampling and measuring procedure, and by visual inspection. The check was completed by pH measurements. The microscopic method was also used to determine the effect of the drug solubilizer on the lipid droplet characteristics. All analytical tests were performed for different CsA concentrations (~1.4 mg/kg, ~5 mg/kg, ~10 mg/kg), over a period of one week and under different storage conditions (4°C, ± 23°C, 37°C).
**Results:** Both lipid emulsions were stable with and without Sandimmun® over seven days according to the specifications since the mean of the largest lipid droplets was always below 4.5 µm and the standard deviation for the largest lipid droplets never exceeded ± 2.0 µm. The LC-MS/MS results for the CsA concentrations showed no consistency. The deviation from the target values were between 23% and 44% for all samples tested. No temperature-dependent concentration changes were detected. Adding the solubilizer Kolliphor EL® (included in a medium SAN concentration of ~5 mg/kg CsA) alone increased the PN lipid stability significantly which could have negative impact on the lipid clearance as previously shown [1].
**Conclusions:** Further improvement for repeatability of the drug assessment is necessary to get reliable data on Sandimmun®-PN stability. To correlate with therapeutic effectiveness of such an admixed drug, further investigations should also consider pharmacokinetic aspects of CsA in patients (TDM).

**Keywords:** Physicochemical stability, co mpatibility, all-in-one PN, cyclosporin A, lipid emulsion characterization.

**Reference:**

[1] Schmid U, Parenterale AiO-Nährmischungen als Träger für Medikamente? Ciclosporin A (Sandimmun® i.v.) als lipophile Modellsubstanz, 2002, Diss. ETH Nr.14635.

**P-11**

## Search for Effective Antiepileptic Natural Products by *In Vivo*-Bioguided Isolation

**T. Brillatz[1], E. F. Queiroz[1], L. Marcourt[1], M. Jacmin[2], A. D. Crawford[2], J. L. Wolfender[1]**

[1] *Phytochimie et Produits Naturels Bioactifs, Ecole de Pharmacie Genève-Lausanne Section des Sciences Pharmaceutiques, University of Geneva, 1211 Geneva, Switzerland*
[2] *Luxembourg Center for Systems Biomedicine, University of Luxembourg, L-4367 Belvaux, Luxembourg*

**Introduction:** Epilepsy is a chronic disorder characterised by recurrent seizures. It is the most widespread neurological disease in the world and approximately 50 million people worldwide are affected. Patients who get pharmaceutical treatments are often subject to side effects and one third develop therapy resistance. Thus, there is a clear need for new antiepileptic drugs (AEDs). Compounds from natural origin can be sources for new AEDs but they are present complex biological matrices and require adapted strategies for the isolation [1].
**Aims:** Consequently, we present a simple and efficient way to isolate active compounds in mg-scale by innovative analytical techniques coupled to a miniaturised *in vivo assay* [2]. For this we investigate medicinal plants for their anticonvulsant activity thanks to the *Danio rerio* (zebrafish) larvae model with seizures induced by the $GABA_A$ antagonist pentylenetetrazol (PTZ).
**Methods:** Bioactive crude natural extracts are submitted to a metabolite profiling and dereplication for a first estimation of their chemical composition by UHPLC-TOF-MS/UV-ELSD. After microfractionation of the extract at the µg-scale, microfractions are screened for their anticonvulsant activity. Then, an upscale fractionation is performed by MPLC-UV-ELSD in order to isolate the peaks identified for their bioactivity. Therefore, a gradient transfer is used to upscale the analytical conditions to preparative conditions to maintain the same chromatographic selectivity and target the bioactive peaks only. In addition, semi-preparative HPLC is used to purify fractions which are still unpure. Finally, pure compounds are obtained and screened for their anticonvulsant activity and identified by HRMS and NMR.
**Results:** Fish movements from the zebrafish are screened and give data converted in bar plots. Thanks to control treatments, these results can be interpreted as anti or proconvulsants.
**Conclusions:** These strategies represent a substantial acceleration for the discovery of new AEDs of natural origin thanks to the zebrafish anticonvulsant PTZ model.

**Keywords:** Medicinal plants, epilepsy, anticonvulsant, zebrafish.

**References:**
[1] Challal S et al. Planta Med 2015; 81: 1636-1643.
[2] Challal S et al. ACS Chem Neurosci 2015; 15: 993-1004.

**P-12**

## Biological Activities of Plants from Niger Used in Traditional Medicine

**N. Saraux, P. Christen, M. Cuendet**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction**: In Niger, traditional medicine plays an important role in the health care system. Populations frequently use plants as a first-line medication against different illnesses such as cancer or parasitic diseases, and delay the treatment in health centers [1]. In this type of sicknesses, current drugs suffer from numerous side effects. Therefore, it is important to find new and effective treatments. In this context, plants, which are known to be rich sources of active molecules [2, 3], should be investigated. In traditional medicine of Western Africa, several species of the genus *Ficus* are extensively used against various health problems such as fever and parasitic diseases [1].
**Aims**: Raw extracts of plants collected in Niger were screened on several targets to identify active compounds in two different fields: cancer prevention and parasitic diseases.
**Methods**: To isolate and identify active metabolites, 38 plants were extracted in solvents of increasing polarity (dichloromethane, methanol and water). Then, extracts were tested on different cancer chemopreventive targets such as NF-κB, quinone reductase (QR) and multiple myeloma cancer stem cells [3]. Moreover, in collaboration with the Swiss Tropical and Public Health Institute in Basel, extracts were tested on*Trypanosoma brucei brucei* STIB 900, *Leishmania donovani* MHOM-ET-67/L82, *Trypanosoma cruzi* Tulahuen C4 and *Plasmodium falciparum* NF54. The most active extract was selected for further analysis.
**Results**: The dichloromethane extract of the stem bark of *Ficus polita* Vahl showed activities on *L. donovani*, *P. falciparum* and QR induction. Hence a dereplication by means of UHPLC-HRMS was performed on this extract. A bioassay-guided fractionation is in progress to identify the compounds responsible for those activities.
**Conclusions**: This screening points out the *in vitro* activity of some traditional medicinal plants on cancer chemoprevention and parasitic diseases. *Ficus polita* showed promising activities on QR induction, leishmaniosis and malaria. To our knowledge, the antimalarial and antileishmanial activities of this plant have not been reported yet. The process to identify the metabolites responsible for those activities is undergoing.

**Keywords**: Ethnopharmacology, screening, *Ficus polita*, parasitic diseases, quinone reductase induction.

**References**:
[1] Eklu-Natey RD, Balet A. Pharmacopée africaine, Dictionnaire et monographie multilingues du potentiel médical des plantes africaines. Edtions d'en bas, ed.: Genève, 2012; Vol. 2.
[2] Ndjonka D et al. Int J Mol Sci 2013; 14: 3395-439.
[3] Kinghorn AD et al. Planta Med 2004; 70 (8): 691-705.

**P-13**

## Modern Tools to Analyse Museum Samples of Curare and Psychoactive Preparations Used by Amazonian Tribes

**S. Cretton[1], P.-M. Allard[1], I. Garcia-Gomez[2], K. Anheuser[2], C. Durand[2], B. Wastiau[2], J.-L. Wolfender[1], M. Cuendet[1], P. Christen[1]**

[1] *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
[2] *Museum of Ethnography, 1205 Geneva, Switzerland*

**Introduction:** Indigenous tribes of the Amazonian rainforest have used a vast array of poisons and psychoactive drugs from plant origin for centuries. There is a great variety of species used and each group has their own recipes to prepare plant mixtures [1]. Arrow poisons are made of alkaloids extracted mainly from the Menispermaceae (*Chondrodendron* spp., *Abuta* spp., *Curarea* spp.), as well as the Loganiaceae (*Strychnos* spp.). The resin obtained from these plants is named curare. For shamanic ceremonies, *Virola theiodora* (Spruce ex Benth.) Warb., *Banisteriopsis caapi* (Spruce ex Griseb.) Morton, *Psychotria viridis* Ruiz & Pav. and *Anadenanthera peregrina* (L.) Speg. are plants commonly used and known to produce psychoactive indole alkaloids.
**Aims:** Objects containing hunting poisons such as curare pots, blow gun darts, arrows, quivers, and ceremonial vessels holding powders with hallucinogenic constituents have been collected by museums such as the Museum of Ethnography in Geneva (MEG) for decades. To assess the chemical content of these nearly one hundred year old samples, analyses by ultra-high pressure chromatography - high-resolution tandem mass spectrometry (UHPLC-HRMS/MS) were performed.
**Methods:** The samples belonging to the MEG collection were extracted by methanol, centrifuged, and then the supernatant was injected into a hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive Plus, ThermoFischer Scientific). The dereplication strategy was based on the creation of a molecular network [2], which groups molecules according to their structural similarities deduced from their MS/MS fragmentation pattern. A subsequent comparison of the experimental fragmentation spectra with an extensive *in silico* MS/MS database of natural products was performed [3]. The putative identification of constituents was then confirmed by injection of standards when available.
**Results:** Bioactive compounds were detected in a majority of the 16 samples. D-tubo-curarine and its derivatives chondrocurine and cycleanine, and alkaloids encountered in the genus *Strychnos* were detected in curare preparations. *N*,*N*-Dimethyltryptamine (DMT) and derivatives, such as bufotenine (5-HO-DMT) and 5-MeO-DMT, were present in snuffs for shamanic ceremonies but also unexpectedly in arrow poisons.
**Conclusions:** UHPLC-HRMS/MS analysis led to the detection and identification of alkaloids present in museum samples without damaging them due to the high sensitivity of the method. In addition, these results demonstrated the stability of these compounds and are valuable for curators who handle ancient collector's items which may still remain hazardous.

**Keywords:** Curare, hunting poison, natural product dereplication, molecular networking.

**References:**
[1] Prance G. J R Coll Physicians Lond 1999; 33: 368-376.
[2] Yang JY et al. J Nat Prod 2013; 76: 1686-1699.
[3] Allard P-M et al. Anal Chem 2016; 88: 3317-3323.

**P-14**

## Differential Light Scattering (DLS) for Size Measurement of Iron Sucrose: A Validated Method

**T. Di Francesco, G. Borchard**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction:** Iron sucrose (IS) is a colloidal drug used to increase iron blood levels in iron deficiency anemia. Over the last decade, several intended copies of IS were approved following the generic paradigm approach. However, clinical data provided evidence that patients receiving either IS or one of its copies were showing different clinical outcomes [1]. In order to assess the pharmaceutical quality of both IS and its copies, the European Directorate for the Quality of Medicines & HealthCare (EDQM) installed the non-biological complexes (NBC) working party. One of the main tasks of the working party is to draft a monograph to prove unequivocally the quality of IS.
**Aim:** We suggest an ICH validated analytical procedure based on the use of dynamic light scattering (DLS) to determine the hydrodynamic diameter of these suspensions.
**Methods:** IS underwent a 50-fold dilution in Milli Q water starting from the concentrated solution. Hydrodynamic diameter of IS was successively determined as size distribution in Number using a Zetasizer Nano S (Malvern, UK). The scattering angle was set at 173° and the He-Ne laser beam was used at $\lambda = 633$ nm. The refractive index was set at 1.3341 [2]. Each sample was introduced into a disposable polystyrene cell and the test was carried out after an equilibration time of 60 sec.
**Results:** The hydrodynamic diameter of IS was identified as equal to $7.0 \pm 0.1$ nm ($n = 3$). In order to prove the reliability of the assay, a validation of the analytical procedure was performed. Following ICH guideline Q2 (R1) [3], the specificity of the procedure was assessed using negative controls, positive controls and different stress tests. All the assays carried out showed a relative standard deviation lower than 15%.
**Conclusions:** We successfully established an analytical procedure to determine the hydrodynamic diameter of IS suspensions. ICH validation revealed the robustness and reliability of our DLS protocol. Authorities might further use this protocol to characterize IS and evaluate the quality of its intended copies.

**Keywords:** Iron sucrose (IS), dynamic light scattering (DLS), ICH validation.

**References:**
[1] Agüera ML et al. PLoS One 2015; 10: e0135967.
[2] Koralewski M et al. J Nanopart Res 2013; 15: 1-20.
[3] ICH 2005; www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf.

**P-15**

## Mapping of Antiangiogenic Drug Distribution in a Rabbit Model of Liver Cancer

**K. Fuchs[1], A. Kiss[2], P. E. Bize[3], R. Duran[3], A. Denys[3], G. Hopfgartner[2], G. Borchard[1], O. Jordan[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] Life Sciences Mass Spectrometry, University of Geneva, 1211 Geneva, Switzerland*
*[3] Centre hospitalier universitaire vaudois (CHUV), 1011 Lausanne, Switzerland*

**Introduction:** One treatment option of hypervascular solid tumors is the occlusion (or embolization) of the tumor by hydrogel beads which are injected into the vascular bed of the tumor. These beads are additionally evaluated for the local delivery of chemotherapeutic agents. Furthermore, the delivery of antiangiogenic drugs to the tumor tissue would be advantageous as it would prevent the tumor from growing new blood vessels, thus improving the efficacy of the treatment.
**Aims:** We studied the local delivery of the anti-angiogenic sunitinib via beads, and spatial distribution of the drug and its metabolites into the tumor and liver tissue over time.
**Methods:** Left liver lobes implanted with VX2 tumor and contralateral lobes without tumor of New Zealand white rabbits were imaged between 1 day and 14 days after chemoembolization with sunitinib-eluting beads [1]. Tumor and contralateral liver sections were subjected either to fluorescence micros-copy or to mass spectrometry imaging.
**Results:** Due to the fluorescence of sunitinib, fluorescence microscopy of the tumor and liver sections showed drug distribution resulting from release and diffusion. Sunitinib was found localized around beads 1-7 days after treatment. At 12-14 days, the drug was still retained by the necrotic tumor tissue, showing high homogeneous tumor drug concentration, but almost completely eliminated from the contralateral liver tissue. The quantitative mass spectrometry imaging data of the tumor tissue confirmed the distribution of sunitinib.
Mass spectrometry imaging discriminated between parent drug and its metabolites. Several of the drug's metabolites were detected in the tumor tissue of adjacent sections over 14 days, among which was the pharmacologically active desethyl metabolite.
**Conclusions:** Sunitinib was selectively delivered to the tumor by drug-eluting beads at high, therapeutic levels. The drug was distributed from the beads, possibly reaching most of the VX2 tumor, during at least 14 days. This matches the time span during which the antiangiogenic effect is required [2].

**Keywords**: Drug-eluting beads, sunitinib, transarterial chemoembolization, hepatocellular carcinoma, drug biodistribution.

**References:**
[1] Bize PE et al. Radiology 2016: 150361.
[2] Li X et al. World J Gastroenterol 2004; 10: 2878-82.

**P-16**

## Dynamic LC Retention Times Prediction for Marker Candidate's Identification: Steroidomics as a Case Study

**G.M. Randazzo[1], D. Tonoli[1,2,3], S. Hambye[1], D. Guillarme[1], F. Jeanneret[1,2,3], A. Nurisso[1], L. Goracci[4], J. Boccard[1], S. Rudaz[1,2]**

*[1] School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, 1211 Geneva, Switzerland*
*[2] Swiss Centre for Applied Human Toxicology (SCAHT), Universities of Basel and Geneva, 4055 Basel, Switzerland*
*[3] Human Protein Sciences Department, University of Geneva, 1211 Geneva, Switzerland*
*[4] Department of Chemistry, Biology and Biotechnology, University of Perugia, 06123 Perugia, Italy*

**Introduction:** Because several pathologies or environmental factors may potentially alter steroido-genesis (infertility, cancer, diabetes, obesity, toxic exposure, etc.), an extended monitoring of steroids based on UHPLC-HRMS untargeted detection (steroidomics) was developed. In order to improve steroid structure identification in biological matrices, a chemically driven feature selection based on database matching (HMDB, LipidMaps) was performed to extract steroid-related ion features from raw signals. Mathematical models based on the chromatographic linear solvent strength (LSS) theory were used to build a steroid database with the aim to predict retention times under any gradient conditions. A real case study involving the OECD reference cellular model for endocrine disruptors screening (H295R) was explored.
**Aims:** Predict the retention time to reduce the number of putative compounds and enable structure identification.
**Methods:** Steroid standards were obtained from different suppliers for database construction: Steraloids, Sigma Aldrich and Sterling. Analyses were carried out using a Bruker UHPLC-QTof Maxis 3G coupled to a Kinetex C18 TMS Endcap (2.1 x 50 mm x 1.7 um) column. Gradient elution was performed as follow: 95:5 water:acetonitrile (both with 0.1% of formic acid) with two different linear 5:95 gradients (15 and 60 min). The LSS $log\ k_W$ and $S$ model parameters were calculated with PyLSS.
**Results:** The data mining strategy was demonstrated as a relevant approach to extract and highlight biomarker candidates. However, as often in "omics" strategies, definitive identification remains the main bottleneck, even with the information of accurate mass. As example, an unknown steroid detected with an accurate mass of 305.2038 corresponds to the C19H28O3 molecular formula, and 19 possible isomers were found in databases. Due to the absence of characteristic diagnostic ions in MS/MS fragmentation spectra, the prediction of chromatographic retention times remained a decisive tool for identification. By taking analytical conditions into account, the prediction of retention times was successfully applied to help steroid identification by reducing the number of candidate compounds.
**Conclusions:** This work demonstrates that dynamic retention time prediction greatly facilitates the identification of isobars and isotopomers.

**Keywords:** Retention time prediction, QSAR, UHPLC-HRMS, steroidomics.

**P-17**

## An Innovative Screening Method to Identify Natural Inhibitors of *Chlamydiales* Growth

**C. Walter[1], M. de Barsy[3], M. Chambon[4], A. Frandi[2], P. Viollier[2], G. Greub[3], G. Turcatti[4], P. Christen[1], K. Perron[5], M. Cuendet[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] Institute of Genetics & Genomics in Geneva (iGE3), Department of Microbiology & Molecular Medicine, Faculty of Medicine / CMU, University of Geneva, 1211 Geneva, Switzerland*
*[3] Institute of Microbiology, University Hospital Center and University of Lausanne, 1011 Lausanne, Switzerland*
*[4] Biomolecular Screening Facility, Ecole Polytechnique Federale de Lausanne (EPFL), Station 15, 1015 Lausanne, Switzerland*
*[5] Microbiology Unit, Department of Botany and Plant Biology, University of Geneva, 1211 Geneva, Switzerland*

**Introduction:** *Chlamydiales* are obligate intracellular bacteria responsible for severe infections in humans, causing infertility and blindness. As the incidence of *Chlamydia* infections in the world's population is still increasing and the antibiotic resistance rises rapidly, it is essential to identify new drugs targeting the chlamydial developmental (intracellular replication) cycle. Therefore, new bacterial targets and active compounds need to be identified. Eight transcription factors (TF) are conserved in all members of the phylum *Chlamydiae* [1]. Out of them, the transcription factor EUO (Early Upstream Open reading frame) is predicted to be a major regulator of chlamydial growth. Due to its important role, a screen for EUO inhibitors was developed.
**Aims:** (i) To set-up a screening method allowing the measurement of the activity of the TF EUO in *Escherichia coli* (*E. coli*) and (ii) to identify natural products capable of impairing the EUO DNA binding activity.
**Methods:** Because *Chlamydiae* are strict intracellular bacteria, a heterologous system in *E. coli* was set up. For this, a promoter containing the EUO DNA binding domain (*prhs9*) was cloned upstream of a *lacZ* reporter gene. A second plasmid containing the EUO gene was used for the expression of EUO protein. Co-transformation of both plasmids in *E. coli* allows the expression of EUO protein and the binding to its cognate DNA consensus domain inhibiting *lacZ* expression. *LacZ* expression was measured by fluorescence using DDAOG, a novel fluorescent substrate for beta-galactosidase [2]. Compounds inhibiting the EUO DNA binding activity lead to the expression of the reporter gene and are considered active. The screening procedure was adapted to a 96 well plate format, which made it suitable for high-throughput screening (HTS). Then, 2640 natural products from the NCCR Chemical Biology collection provided by the BSF-ACCESS screening platform were tested for their effects on EUO activity.
**Results:** Out of all the compounds tested, 12 showed a EUO inhibitory activity above 10%. Importantly, one natural product with an inhibition of 8.5% has already been reported to have an anti-*Chlamydia* activity, thus validating the screening strategy [3].
**Conclusions:** Results showed that the method developed here is suitable for HTS. It can be used to screen natural products and has the advantage of using *E.coli* as heterologous screening system for intractable pathogens. The 12 compounds showing the most activity will be tested in dose-response studies. If the results are confirmed, the most active compound will be used for mechanistic studies such as EMSA or protein thermal shift assay.

**Keywords:** High-throughput screening, *Chlamydia*, EUO transcription factor, natural products.

**References:**
[1] Dommon D, Horn M. Mol Biol Evol 2015; 12: 3035-3046.
[2] Gong H et al. Anal Biochem 2009; 1: 59-64.
[3] Yamazaki T, Hara Y. Patent US 20020006447 A1 2002; US 09/910,499.

**P-18**

## Phosphatase-Sensitive Prodrugs for the Systemic Delivery of 5-Aminolevulinic Acid (5-ALA)

**V. Herceg, A. Babič, N. Lange, E. Allémann**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction:** Photodynamic therapy (PDT) is a therapeutic approach which uses a photosensitizer (PS), oxygen and light to locally generate reactive oxygen species (ROS) leading to cytotoxic effects. Upon the light activation of the PS, a part of the energy gets released in form of the fluorescence which can be used as a diagnostic tool for photodetection (PD). 5-ALA is a natural precursor of the PS protoporphyrin IX (PpIX) in the heme biosynthesis pathway. As a result of aberrant enzymatic activity in malignant cells and due to the bypass of the negative feedback control mechanism in which produced heme inhibits the synthesis of 5-ALA, an excess of exogenous 5-ALA causes the intracellular accumu-lation of PpIX. In the neutral pH found in the bloodstream, 5-ALA is in its charged, zwitterionic form which makes it unable to pass biological barriers. This, along with its short half-life, small volume of distribution and accumulation in the liver and kidneys makes 5-ALA unsuitable for systemic administration. Until today, among the attempts of successful chemical derivatization of 5-ALA, the most effective solution is the esterification of its carboxylic end. Nowadays, 5-ALA methyl ester (Metvix®) is used in the topical treatment of actinic keratosis and basal cell carcinoma, and the 5-ALA hexyl ester (5-ALA-Hex) (Hexvix®) has been approved for the PD of bladder cancer [1]. Although improved in the terms of lipophilicity, 5-ALA-Hex remains toxic with poor pharmacokinetic profile thus still not providing a solution for the systemic delivery of 5-ALA.
**Aims:** New phosphatase-sensitive 5-ALA prodrugs were synthesized in our group [2]. Here, the efficacy of these compounds was evaluated and compared to 5-ALA-Hex in 4 different cancer cell lines.
**Methods:** PpIX production was measured in cells over 24 h followed by the PDT. WST-1 assay was performed 24 h upon the PDT treatment in order to assess cell viability.
**Results:** PpIX production measurements and confocal microscopy results both show increased accumulation of PpIX in several cell types after the treatment with phosphatase-sensitive prodrugs of 5-ALA. Cytotoxic effects can be seen after the PDT treatment.
**Conclusions:** Among the tested compounds, we have selected one promising candidate for which the results show increased accumulation of PpIX in several cell types and potential cytotoxic effects after the PDT treatment.

**Keywords:** PDT, 5-ALA, PpIX.

**References:**
[1] Lange N et al. Br J Cancer 1999; 80:185-93.
[2] Babič A et al. J Control Release 2016; 235:155-64.

**P-19**

## Flash Chromatography MS-Targeted Isolation of Natural Products Under Normal Phase Conditions

**D. Righi, A. Azzollini, E. F. Queiroz, J.-L. Wolfender**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction:** Reversed phase liquid chromatography (RP-LC) is widely used for the metabolite profiling of complex natural extracts and is now more and more used for targeted MS isolation of biomarkers. Normal phase chromatography (NP-LC) is well suited for the purification of lipophilic secondary metabolites, also offering some advantages compared to RP, like low operating pressures and cheaper stationary phases.
**Aims:** The potential of NP-LC-APCI-MS for metabolite purification at the preparative scale using generic separation methods has been investigated on medium pressure preparative chromatography system (PuriFlash® - MS) in view of its application for targeted MS isolation of lipophilic secondary metabolite.
**Methods:** A mixture of three representative apolar natural products was used to optimize the experimentals conditions mimicking real isolation cases. All parameters were carefully optimized for both separation and detection (gradient system, split rate, flow rate, temperature, injection volume, column length, ionization source parameters). A special care was taken to find MS ionization and splitting conditions that provide good detection and preclude source contamination. Finally, a successful isolation of the apolar constituents of the dichloromethane roots extract of *Angelica archangelica* was performed.
**Results:** The HPLC analytical gradient was transferred to flash chromatography following a geometric gradient transfer method after calibration of the chromatographic systems. MS, in complement to UV detection, enabled the monitoring of NPs with weak and strong chromophores and the selectivity of MS was of great help for a precise collection of partially co-eluting compounds.
**Conclusions:** APCI-MS detection with optimized splitting and post-column elution of appropriate solvent was found robust and well-suited for purifications in normal phase mode. This strategy allows an efficient and rational targeted isolation of tens to hundreds mg of compounds for further structural identification or bioactivity characterization studies.

**Keywords**: Flash chromatography, APCI-MS, analytical method transfer, normal phase, reverse phase.

**P-20**

## Pollen Induced Asthma - Could Small Molecules in Pollen Exacerbate the Protein-Mediated Allergic Response?

**A. Bozicevic[1], M. De Mieri[1], C. Nassenstein[2], M. Hamburger[1]**

*[1] Division of Pharmaceutical Biology, University of Basel, 4056 Basel, Switzerland*
*[2] Institute of Anatomy and Cell Biology – Cardiopulmonary Neurobiology, Justus-Liebig-University, D-35385 Giessen, Germany*

**Introduction:** Plant pollen are known to be strong airborne elicitors of asthma in humans. *In vitro* data and clinical studies corroborate the involvement of small surface proteins present on the pollen grain. They modulate the immune system through IgE cross-linkage, thereby causing airway inflammation, and obstruction due to constriction of airways. At the physiological level, relaxation and constriction of airways is regulated by mechanisms involving proteins such as the lipid kinase PIP5Kγ [1] and the cation channel TRPA1 [2].
**Aims:** While the role of pollen surface proteins is well established, a possible contribution of small molecules present in pollen to the clinical outcome of asthma has not been explored up to now.
**Methods:** We analysed and compared the phytochemical profiles of pollen originating from 30 plant species causing varying degrees of pollen allergenicity. Profiling was performed by HPLC coupled with PDA, ESIMS, and ELSD detectors, off-line microprobe NMR spectroscopy, and spectrophotometric analysis. The presence of conjugated polyamines, such as $N^1$,$N^5$,$N^{10}$- tricoumaroylspermidine, $N^1$-caffeoyl-$N^5$,$N^{10}$-dicoumaroyl-spermidine and $N^1$,$N^5$,$N^{10}$,$N^{15}$-tetracoumaroylspermine was a characteristic feature of pollen from Asteraceae (*Ambrosia* and *Artemisia* ssp.). Compounds with Michael acceptor properties were also mainly present in Asteraceae pollen. Selected pollen extracts and pure compounds were tested for activation of TRPA1 in murine dorsal root ganglia and for the murine trachea constriction.
**Results:** Tetrahydrofuran extracts of selected pollen increased intracellular $Ca^{2+}$ at concentrations comparable to the prototypical TRPA1 activator cinnamaldehyde. Nevertheless, TRPA1 activators induced relaxation of pre-constricted murine tracheas and reduced the maximal MCh-induced tracheal constriction.
**Conclusions:** Pollen extracts and isolated electrophiles showed TRPA1 activation. Electrophilic sesquiterpene lactones parthenin and coronopilin induced tracheal dilatation, even though being TRPA1 activators. Other possible targets for electrophiles need to be identified and the tracheal stimulation with conjugated polyamines needs to be tested.

**Keywords:** Plant pollen, asthma, TRPA1 activation, murine trachea constriction.

**References:**
[1] Erle DJ and Sheppard D. J Cell Biol 2014; 205: 621-631.
[2] Avonto C et al. Angew Chem Int Ed 2011; 50: 467-471.

**P-21**

## Miniaturized X-Ray Powder Diffraction Calibration for Quantification of Hydrate Formation in Pharmaceutics

**W. Kirchmeyer[1,2], N. Wyttenbach[3], J. Alsenz[3], O. Grassmann[4], M. Kuentz[1]**

[1] *University of Applied Sciences and Arts Northwestern Switzerland, 4032 Muttenz, Switzerland*
[2] *University of Basel, 4056 Basel, Switzerland*
[3] *Roche Pharmaceutical Research & Early Development, Small Molecule Research, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, 4070 Basel, Switzerland*
[4] *Pharma Technical Development, F. Hoffmann-La Roche Ltd, 4070 Basel, Switzerland*

**Introduction:** The majority of pharmaceutical compounds show polymorphism. This can result in different bioavailability and behavior during processing [1]. It is therefore important to properly characterize the solid form of the drug. Sensitive analytical methods are required especially in case of polymorphic mixtures. A thorough understanding of potential solvent-mediated phase changes is needed for clinical candidates' selection as well as in-process controls [2] to avoid quality issues of the final drug product. An early solid-state screening is in these days typically conducted as part of pharmaceutical profiling since the suitable polymorph of a compound has to be selected early in development. Here, not only qualitative information is needed but also the quantification of different polymorphs that may occur is important.
**Aims:** To develop high throughput x-ray powder diffraction (XRPD)-calibrations in a miniaturized scale for obtaining quantitative information about solvent-mediated polymorphic transformations.
**Methods:** Three known hydrate-forming drugs were selected, i.e. caffeine, piroxicam and testosterone, and binary mixtures of hydrate and anhydrous material were prepared at 10% (w/w) intervals from 0 to 100% hydrate together with anhydrate (10 mg per sample) in a 96-well filter plate. The software STOE Win XPow Quant was used for quantitative analysis of x-ray diffractograms.
**Results:** XRPD patterns from binary mixtures were analyzed by considering the pure anhydrous, pure hydrated drug, and additionally the background as standard components. Calibration of the mixtures was measured in 5-35° 2-theta range and calculated compositions of the different binary mixtures were plotted against weight fraction. An adequate linearity was here obtained for all compounds and the obtained correlation coefficients differed slightly for the drugs studied.
**Conclusions:** This method is applicable for the determination of raw materials and is practical for industrial clinical candidates' selection. It is also useful to investigate the solvent-mediated phase transformations in different media to for example screen pharmaceutical additives. Other examples that could be studied with this approach in the future are amorphous compounds or metastable polymorphs.

**Keywords:** Pharmaceutics, polymorphism, X-ray powder diffraction (XRPD)-calibration.

**References:**
[1] Stahly G. Cryst Growth Des 2007; 7: 1007-1026.
[2] Gift AD et al. J Pharm Sci 2009; 98: 4670-4683.

**P-22**

## Development of an Injectable Formulation Forming an Implant in Situ for the Treatment of Prostate Cancer

**A. Lutfija, S. Ehrenberger, G. Borchard, O. Jordan**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction:** Prostate cancer is the most common cancer in men and the second cause of mortality related to cancer [1]. Existing treatments, including prostatectomy, radiotherapy and hormonotherapy or chemotherapy can show severe side effects. Alternative approaches to treat this kind of cancer are therefore required, such as thermotherapy, as established adjuvant in cancer treatment. Thermotherapy using superparamagnetic iron oxide nanoparticles (SPIONs) to apply local hyperthermia causing apoptosis of cancer cells takes advantage of SPIONs' capacity to dissipate heat under the effect of an external alternating magnetic field (AMF).
**Aims:** Our goal is to develop an injectable formulation containing SPIONs and polidocanol as an active agent, solidifying in contact with biological tissue as a minimally invasive treatment of prostate cancer. The entrapped SPIONs allow the application of magnetic local hyperthermia. Polidocanol causing fibrosis of the blood vessels at the injection site would block the blood supply to the tumor allowing to reach higher temperatures in the necrotic tumor and thus induce a more important level of apoptosis of cancer cells. Injectability, stability, drug release, and heating capacity are investigated in this project to ensure the feasibility of this formulation and its ability to reach the interval of 41-46°C for the use in moderate hyperthermia [2].
**Methods:** SPIONs coated with polymethyl methacrylate (PMMA) were suspended in a solution of mono/tri-iodo benzyl-ether polyvinylalcohol (MTIB-PVA) in DMSO. MTIB-PVA being a radiopaque, water-insoluble polymer, precipitates after injection in aqueous medium (or biological tissue). Different concentrations of PMMA-SPIONs and MTIB-PVA were evaluated in order to meet the criteria for rheology (in cone-plate configuration), syringeability (with a 21G syringe) and injectability (using texture analyzer and a prostatic model) of the suspension. Evaluation of the specific loss power (SLP) and the maximal temperature to reach of the solidified implant were studied by applying different magnetic fields and frequencies. Polidocanol was incorporated in the suspension at 32% w/w, corresponding to the clinically used dose. Drug release of the implant was investigated during 2 weeks following a dissolution test adapted from the European Pharmacopoeia. *In vitro* safety was evaluated by testing the viability of PC3 (human prostate cancer cells) when exposed to the implant in the absence of polidocanol and without applying AMFs with the cell proliferation reagent WST-1 in comparison to non-treated cells.
**Results:** Syringeability was appropriate for concentrations up to 25% w/w PMMA-SPIONs. Rheological studies showed pseudoplastic behavior. Injections performed in a prostatic model resulted in spherical and compact implants. The temperature measured after applying an alternate magnetic field was dependent on frequency, intensity of magnetic field, and concentration of PMMA-SPIONs. The final formulation, consisting of 25% w/w PMMA-SPIONs and 18% w/w MTIB-PVA, reached 43.5°C under clinically usable conditions (121 KHz and 6 mT). Drug release studies showed that 80% of polidocanol was released in only one day. No cytotoxicity was demonstrated when testing cell proliferation.
**Conclusions:** The formulation of 25% w/w PMMA-coated SPIONs in a solution of 18% w/w radiopaque polymer leads to an adequate injectability and forms a solid implant upon contact with a prostatic model. The heat dissipated by the implant reaches the threshold of 41°C for local moderate hyperthermia. No toxic effect of the implant (without polidocanol) was demonstrated *in vitro*. Drug release through the implant is appropriate to allow for the use of this formulation as a combination of hyperthermia and medical treatment.
**Keywords:** SPIONs, polidocanol, *in situ* forming implant, hyperthermia, PC3.

**References:**

[1] Ligue suisse contre le cancer www.liguecancer.ch
[2] Goldstein LS et al. Intern J Hyperthermia 2009;19:373.

**P-23**

## Carrier-Free Gene Silencing by Amphiphilic Nucleic Acid Conjugates in Differentiated Intestinal Cells

**E. Moroz[1], S. H. Lee[1], K. Yamada[2], F. Halloy[1], S. Martínez-Montero[2], H. Jahns[1], J. Hall[1], M. J. Damha[2], B. Castagner[1,3], J.-C. Leroux[1]**

[1] *Institute of Pharmaceutical Sciences, ETH Zurich, 8093 Zurich, Switzerland*
[2] *Department of Chemistry, McGill University, H3A 0B8 Montreal, Canada*
[3] *Department of Pharmacology & Therapeutics, McGill University, H3G 1Y6 Montreal, Canada*

**Introduction**: Nucleic acid therapy can be beneficial for the treatment of gastrointestinal (GI) diseases that currently lack appropriate treatments. The delivery of oligonucleotides (ONs) directly to the GI mucosa allows for high local concentrations with minimal systemic exposure and subsequent side-effects. Indeed, several ONs are currently progressing through clinical trials as potential treatments for inflammatory bowel diseases, although the uptake of free ONs by mucosal cells is relatively inefficient [1]. Therefore, strategies aimed at increasing the potency of orally administered ONs would be highly desirable. In the present work, we propose a novel nucleic acid delivery platform for intestinal cells based on highly resistant ONs derivatized with hydrophobic alkyl chain [2].
**Aims:** Synthesis of lipid-ON conjugates and evaluation of their *in vitro* silencing activity upon carrier-free transfection under various conditions mimicking the intestinal environment (inclusion of food-derived fats and digestive enzymes, transfection of differentiated intestinal epithelium).
**Methods:** Standard phosphoramidite solid-phase synthesis conditions were used for the synthesis of all ONs. Lipophilic moiety (docosanoic acid, $C_{22}$) was conjugated *via* an amino-hexanol-linker to the 5'-end of oligonucleotides. The lipid-ON conjugates were examined for their Bcl-2 mRNA silencing efficacy on 2 human colon cancer cell lines (proliferating and differentiated) in serum-free medium after overnight incubation. The gene expression levels of Bcl-2 were assessed by qRT-PCR and western blotting, as described previously [2, 3]. The silencing efficacy was also assessed in simulated intestinal conditions (after incubation with pancreatin or oil emulsion, inverted transfection setup).
**Results:** We screened a set of lipid-ON conjugates for the silencing of model Bcl-2 mRNA and selected 2′-deoxy-2′-fluoro-arabinonucleic acid modified ON bearing docosanoyl moiety (L-FANA) as the most potent candidate with lowest toxicity. The efficacy of L-FANA conjugate was preserved in simulated intestinal fluids and in the inverted transfection setup. Importantly, L-FANA conjugate was able to downregulate target gene expression at both mRNA and protein levels in a difficult-to-transfect polarized epithelial cell monolayer in the absence of delivery devices and membrane disturbing agents. This well-defined single-molecule-based approach proved to be superior to conventionally employed lipoplexes, which were ineffective for transfecting the epithelium.
**Conclusions:** We present an innovative and robust nucleic acid delivery platform for intestinal cells purely based on modified amphiphilic ON conjugates which efficiently knocks down a target gene at both mRNA and protein levels on difficult-to-transfect differentiated intestinal cells. This is the first system showing efficient target gene silencing in fully differentiated epithelial cells in the absence of complexation and membrane-disturbing agents, which could potentially improve the efficacy of ON enteral treatments currently under clinical investigation.

**Keywords:** Carrier-free transfection, 2′-deoxy-2′-fluoro-arabinonucleic acid (FANA), amphiphilic conjugate, differentiated monolayer, intestinal disease therapy.

**References:**
[1] Moroz E, Matoori S, Leroux J-C. Adv Drug Deliv Rev 2016; 101:108-121.
[2] Moroz E et al. Mol Ther Nucleic Acids 2016; accepted.
[3] Felber AE et al. Biomaterials 2012; 33: 5955-5965.

**P-24**

## Targeted Nanoparticles for Imaging and Treatment of Prostate Cancer Metastases in Lymph Nodes

**S. Ehrenberger, E. Sublet, G. Borchard, O. Jordan**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1205 Geneva, Switzerland*

**Introduction:** Detection of early metastases in lymph nodes is a key factor in cancer staging and treatment planning to decrease the risk of secondary tumors. Until now surgical removal of lymph nodes is often unavoidable for diagnosis, potentially leading to side effects like lymphedema. Being a prominent marker for lymph node and distant metastatic cells [1], the prostate-specific membrane antigen (PSMA) represents a potential option for active targeting of prostate cancer metastases.
**Aims:** The aim of this project is to develop PSMA-targeted superparamagnetic iron oxide nanoparticles (SPIONs) for detection of early prostate cancer metastases in lymph nodes. Besides the use of SPIONs as MRI contrast agent, the nanoparticles also dissipate heat when exposed to an alternating magnetic field. These two properties make them highly interesting as theranostic agent by combining MRI diagnostic and successive (co-)treatment by induced local hyperthermia.
**Methods:** A PSMA-targeting RNA-aptamer [2] was first tested for specific targeting by comparing its binding and internalization behavior in human, PSMA-positive prostate cancer cells (LNCaP) and human, PSMA-negative prostate cancer cells (PC3) by confocal laser scanning microscopy. The aptamer was then covalently attached to the SPIONs, previously coated with different biocompatible small molecules, by copper-free click chemistry under aqueous conditions. After purification, each reaction step was followed by changes in particle size and zeta potential. The functionalized nanoparticles were then tested *in vitro* for cytotoxicity using a modified WST-1 cell proliferation assay on PC3 cells.
**Results:** Binding to and internalization by PSMA-positive LNCaP cells were shown for the aptamer while PSMA-negative PC3 cells remained mainly unaffected. The aptamer was successfully coupled to SPIONs with different coatings, resulting in a zeta potential in the range of -32 to -36 mV and a hydrodynamic diameter of around 100 nm, forming a stable suspension. Based on the threshold of 80% cell viability in comparison to non-treated cells, no cytotoxicity of the functionalized SPIONs was shown *in vitro*.
**Conclusions:** Superparamagnetic iron oxide nanoparticles eligible for theranostic applications were successfully functionalized with a PSMA-targeting aptamer, presenting suitable properties for lymph node targeting. *In vivo* experiments are in preparation to evaluate the detection of prostate cancer lymph node metastases in rats using a clinical MRI scanner.

**Keywords:** SPIONs, aptamer, PSMA, theranostics.

**References:**
[1] Queisser Q et al. Modern Pathol 2015; 28: 138-145.
[2] Lupold SE et al. Cancer Res 2002; 62: 4029-33.

**P-25**

## Formation of a Self-Assembling Chitosan Hydrogel

**K. Kiene, F. Porta, J. Huwyler**

*University of Basel, Pharmaceutical Technology, 4056 Basel, Switzerland*

**Introduction:** We used the biopolymer chitosan to prepare a self-assembling hydrogel. By Michael addition between a sulfhydryl modified and a maleimide functionalized chitosan polymer we achieved a covalently crosslinked hydrogel. The microscopic aspect of the hydrogel matrix was investigated with scanning electron microscopy (SEM). A defined porous structure was observed with a pore diameter around 100 µm. Release kinetics were investigated using a model biomolecule, bovine serum albumin (BSA) with a molecular weight of 66 kDa. The gel showed sustained release properties. A post loading of BSA was also performed; however, in this case an evident burst release was observed. Lyophilization of the hydrogel after loading and self-assembly showed that the release properties of the hydrogel maintained unchanged.
**Aims:** The aim of this project was to develop a new method to form a self-assembling biocompatible hydrogel consisting of the biopolymer chitosan which can be used for drug delivery.
**Methods:** Scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), and UV-Vis spectrophotometry were used.
**Results:** The successful modification of chitosan with maleimide and sulfhydryl moieties, respectively, was proven by using FTIR. With SEM we observed a porous structure of the hydrogel with diameter of up to 100 µm. The self-assembling chitosan hydrogel showed sustained release profiles for the model protein BSA. In contrast, a post-loading of BSA led to burst release of protein. Lyophilization of the hydrogel after loading and self-assembly showed that the release properties of the hydrogel maintained unchanged which makes it very attractive in terms of stability.
**Conclusions:** Therefore, we can conclude that we synthesized a self-assembling biocompatible hydrogel with sustained release properties. Further studies must be performed to improve gelation time and release; however, we envision the application of this hydrogel for wound healing.

**Keywords:** Chitosan, hydrogel, drug delivery systems, Michael Addition.

**P-26**

## Modifying Cells Via Microinjection of Liposomes Carrying Biologically Active Cargo

**P. Tiefenböck, J.A. Kim, J.-C. Leroux**

*Institute of Pharmaceutical Sciences, ETH Zürich, 8093 Zürich, Switzerland*

**Introduction:** Microinjection (MI), well-established in cell biology, represents a powerful tool for the introduction of non-permeable macromolecules into cells [1]. By sparing their traffic through the endosomal pathway and thus preventing their degradation in the lysosomes, MI enables the delivery of sensitive molecules such as enzymes. Combining the MI technique with the versatile concept of cargo-encapsulating liposomes, we propose to engineer cells with new biological functions.
**Aims:** In this study, we established a MI-based platform to provide cells with exogenous organelle-like vesicles. Liposomes were microinjected in HeLa cells and the toxicity, stability, and dilution of the injected liposomes were characterized through different microscopy-based approaches. As a proof-of-concept, we used trypsin-bearing liposomes and showed their enzymatic activity inside the injected cells.
**Methods:** Four different liposomal formulations, differing in the main lipid and in the presence of poly(ethylene glycol) (PEG) chains on the surface, were tested for cytotoxicity with a propidium iodide exclusion assay. The stability of the liposomes was tested *via* a fluorescence dequenching microscopy-based assay, where the integrity of the injected liposomes was challenged at different time points using Triton X-100. The intracellular fate of the injected dye-labelled liposomes was analyzed microscopically, comparing their dilution upon cell division cycles to a dextran-based coinjection marker. To study the activity of trypsin-encapsulating liposomes injected into cells, the cleavage of a cell-permeable fluorogenic substrate was monitored. The successful enzymatic cleavage of the substrate by encapsulated trypsin was indicated by an increase in fluorescence.
**Results:** All tested liposomal formulations showed only a minor effect on cell viability, which was comparable to the coinjection marker alone. The liposomes were stable for up to 2 days inside the cells depending on the formulation injected. Interestingly, the dilution of the liposomes was non-linear during cell division as opposed to the coinjection marker, leading to some daughter cells with large amounts of liposomes and others with almost no liposomes at all. The activity of injected trypsin-encapsulating liposomes could be successfully shown by colocalization of liposomal marker with dequenched substrate.
**Conclusions:** MI of liposomes was shown to be well-tolerated by the cells, allowing to stably engineer them with cargo-containing vesicles for up to 2 days. The successful detection of enzymatic activity inside the liposomes in injected cells opens up exciting possibilities to generate semi-synthetic cells with interesting biological functions by employing other therapeutically relevant enzymes.

**Keywords:** Microinjection, liposome, enzymatic activity, semi-synthetic cell.

**Reference:**
[1] Zhang Y, Yu LC. BioEssays 2008; 30: 606-10.

**P-27**

## Therapeutic Protein Injection: Dendritic Cells Embedded in Hydrogels to Mimic Human Immune Subcutaneous Environment

**F. Groell, O. Jordan, G. Borchard**

*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*

**Introduction:** Injection site reactions (ISRs) caused by administration of therapeutic proteins into the subcutaneous tissue represent one of the main adverse events for these formulations. These ISRs can be due to: (i) an allergic reaction to the protein itself and/or excipient(s) of the formulation or (ii) the instability of the therapeutic protein leading to formation of aggregates that are recognized as foreign bodies by the immune system.
**Aims:** To better understand the formation of these protein aggregates in the subcutaneous space and their interaction with immune cells.
**Methods:** Stability studies were undertaken on a therapeutic protein, rhIFNα2b. The protein structure was characterized in its native state as well as after forced aggregation by circular dichroism (CD), fluorescence spectroscopy and dynamic light scattering (DLS). Aggregation was induced by stirring, metal-catalyzed oxidation, pH modification, thermal stress and freeze-thawing cycles [1]. We created a three-dimensional (3D) cell culture model of the subcutaneous space. For this, viscoelastic hydrogels forming under optimal cell culture conditions (37°C) and available as sterile and endotoxin-free products were selected. Elastic Young's modulus in compression was correlated between the hydrogels and *ex vivo* human subcutaneous tissue samples using a TA-XT*plus* Texture Analyzer (Stables Micro Systems Ltd). After a first cytocompatibility assessment with the human A-549 cell line, we reproduced the assay using a dendritic cell line. Dendritic cells were embedded in agarose, low gelling temperature, suitable for cell culture (BioReagent, Sigma-Aldrich®) at different concentrations (0.5, 0.35, 0.25%). Cells were maintained in culture for 3 days and their viability was monitored every day using cell proliferation reagent WST-1 (F. Hoffmann-La Roche Ltd).
**Results:** Stability studies of this therapeutic protein revealed different aggregation patterns depending on the stress applied. Orthogonal methods (CD, DLS, fluorescence) allowed us to obtain a good description of the nature of the aggregates. Agarose hydrogel at all the tested concentrations showed good cytocompatibility and enabled at least 1.5-fold proliferation increase after 3 days in 3D cell culture. Measured elastic Young's moduli on human subcutaneous fatty tissue were in accordance with literature [2] and our measurements on hydrogels (14 to 73kPa).
**Conclusions:** Hydrogels matching the elastic properties of human subcutaneous tissue were selected. Agarose was shown to be an adequate matrix for 3D dendritic cell culture. More hydrogels need to be tested in 3D cell culture along with protein aggregates to decipher their role in the initiation of an inflammatory reaction by dendritic cells.

**Keywords:** Therapeutic protein, aggregation, immunogenicity, 3D cell culture.

**References:**
[1] Joubert MK et al. J Biol Chem 2011; 286: 25118-33.
[2] Derler S, Gerhardt LC. Tribology Letters 2011; 45: 1-27.

**P-28**

## On Production of Fast-Dissolving Low-Density Powders for Deep Lung Deposition by Spray Drying of a Nanosuspension

**K. Simkova[1,2], B. Joost[2], G. Imanidis[1,2]**

*[1] Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
*[2] School of Life Sciences, University of Applied Sciences Northwestern Switzerland, 4032 Muttenz Switzerland*

**Introduction:** The main formulation requirement in pulmonary drug delivery for effective deep lung deposition is to have particles with aerodynamic particle size in the range of 1-3 µm. However, to evade phagocytic clearance by alveolar macrophages, the geometric particle size should exceed 3 µm. To fulfil these requirements particle density has to be smaller than 1 g/cm$^3$. Phagocytic evasion can further be ensured by fast drug dissolution, which, however, can be challenging especially for poorly water-soluble drugs given the small liquid amount in the lungs.
**Aims:** This work aimed at preparation of low-density particles with rapid dissolution by spray drying of an aqueous nanosuspension.
**Methods:** In this work nanomilling of budesonide, a drug used in pulmonary delivery, in a stirred media mill was employed to reduce particle size and improve dissolution rate. The resulting nanosuspension was spray-dried to engineer low-density particles using ammonium carbonate, albumin, or leucine as additives. The final powders were assessed in terms of their aerodynamic performance in a next generation impactor (NGI), geometric particle size, specific surface area, and flow behavior. Moreover, dissolution kinetics of aerodynamically classified powder fractions were measured.
**Results:** Wet nanomilling produced particles with median particle size of 320 nm. Depending on the additive used in spray drying, moderately to highly porous particles with specific surface area from 1.8 to 19.0 m$^2$/g were produced. While the median geometric particle size of the formulations varied only between 4.4 and 5.3 µm, the fine particle fraction ranged between 18.3 and 61.1%. One week after preparation, the products showed fast dissolution kinetics owing to the small particle size and probably also due to a partial amorphization of the drug.
**Conclusions:** The investigated approach, which combined nanomilling and spray drying of an aqueous formulation, provides the possibility to prepare low-density particles with fast dissolution, good aerodynamic performance, and geometric size favorable for phagocytic evasion.

**Keywords**: Dry powders for inhalation, spray drying, dissolution.

**P-29**

## Effect of Different $\alpha_2$-Receptor Agonists on the Ketamine Metabolism Assessed With Equine Liver Microsomes

**F. A. Sandbaumhüter[1], R. Theurillat[1], R. Bettschart-Wolfensberger[2], W. Thormann[1]**

*[1] Clinical Pharmacology Laboratory, Institute for Infectious Diseases, University of Bern, 3008 Bern, Switzerland*
*[2] Section of Anaesthesiology, Equine Department, Vetsuisse Faculty, University of Zürich, 8057 Zürich, Switzerland*

**Introduction:** The combination of ketamine and an $\alpha_2$-receptor agonist is often used in veterinary medicine. Four different $\alpha_2$-receptor agonists, medetomidine, detomidine, xylazine and romifidine, are available. They differ, based on their chemical structures, in selectivity for the $\alpha_2$-receptor and in their sedative and analgesic potency. These effects are used in the premedication of anaesthesia with the racemic NMDA-receptor antagonist ketamine. Previous *in vitro* studies with canine liver microsomes and *in vivo* studies with Beagle dogs showed that medetomidine decreases the formation of the active metabolite norketamine and has also an influence on the further metabolites 6-hydroxynorketamine (6HNK) and 5,6-dehydronorketamine (DHNK) [1,2]. In the daily veterinary practice the behavior of horses while recovering after anaesthesia with ketamine and an $\alpha_2$-receptor agonist is dependent on the selected $\alpha_2$-receptor agonist.
**Aims:** The goals of this work were to assess effects of the different $\alpha_2$-receptor agonists (i) on the N-demethylation of ketamine to *R*- and *S*-norketamine and (ii) on the formation of the further metabolites *RR*- and *SS*-6HNK and *R*- and *S*-DHNK in an *in vitro* system with equine liver microsomes.
**Methods:** Ketamine and equine liver microsomes were incubated with medetomidine, detomidine, xylazine or romifidine for 8 min at 37°C for determining the formation of norketamine and the inhibition parameter $K_i$ and $IC_{50}$. Metabolite formation was followed with incubations that lasted 5 h. All samples were extracted at alkaline pH and analyzed with enantioselective capillary electrophoresis with highly-sulfated γ-cyclodextrin as chiral selector using two different assays which are described in refs. [1] and [2]. The inhibition parameters were calculated with the four parameter logistic equation and the Cheng-Prusoff equation.
**Results:** Medetomidine was found to be the strongest inhibitor of the ketamine N-demethylation to norketamine followed by detomidine. Xylazine and romifidine showed almost no influence in the inhibition studies. With much longer incubation, however, also effects for xylazine and romifidine were detected. The formation of 6HNK and DHNK were influenced by all selected $\alpha_2$-agonists. Stereoselectivity was found for the inhibition parameters of xylazine, the elimination of ketamine and the formation of 6HNK in presence of all $\alpha_2$-receptor agonists.
**Conclusions:** All tested $\alpha_2$-receptor agonists, medetomidine, detomidine, xylazine and romifidine, have an effect on the ketamine metabolism. Medetomidine inhibits the formation of norketamine, 6HNK and DHNK. Further investigations have to show how these results can explain differences in the behavior of horses during recovery after an applied combination of ketamine and one of the four $\alpha_2$-receptor agonists.

**Keywords:** $\alpha_2$-receptor agonists, enantioselective capillary electrophoresis, equine liver microsomes, inhibition parameters, ketamine metabolism.

**References:**
[1] Sandbaumhüter FA, Theurillat R, Thormann W. Electrophoresis 2015; 36: 2703-2712.
[2] Theurillat R et al. Electrophoresis 2016; 37: 1129-1138.

## P-30

# Modernisierung der Monographien für Thymianfluidextrakt und Thymiansirup der Schweizer Pharmakopöe

**M. Steiner, S. Peter, E. Wolfram, B. Falch, B. Meier**

*Zürcher Hochschule für angewandte Wissenschaften, Fachgruppe Phytopharmazie und Naturstoffe, Institut für Chemie und Biotechnologie, 8820 Wädenswil, Schweiz*

**Einleitung**: Die Monographie für Thymiansirup in der Schweizer Pharmakopöe [1] ist nicht mehr zeitgemäss, da zur Standardisierung Thymol als Reinstoff zugesetzt wird. Als Wirkstoff enthält der Sirup einen Thymianfluidextrakt, dessen Herstellung sich ebenfalls als problematisch erwies, da mit der vorgeschriebenen Menge Lösungsmittel (3 Teile) keine Maische resultierte. Eine Revision der Monographie unter Einbezug der aktuellen Regularien zur Herstellung pflanzlicher Arzneimittel drängte sich deshalb auf.
**Ziel**: Es war abzuklären, wie ein Fluidextrakt oder eine Tinktur hergestellt werden muss, damit der in der Schweizer Pharmakopöe beschriebene Thymiansirup als Monographie nach zeitgemässen Kriterien weiter bestehen kann. Die Notwendigkeit des Einsatzes von Ammoniak und Glycerin als Bestandteil des Extraktionsmittels zur Optimierung der Thymolausbeute sollte überprüft werden. Angestrebt wurde eine möglichst geringe Ethanolkonzentration im Extraktionsmittel, damit der Sirup für Kinder applizierbar bleibt.
**Resultate**: Die Ausbeute an Thymol beim Mazerieren hängt ab vom Alkoholgehalt, wobei bei 40-60% Ethanol (V/V) das Maximum der Extraktionskurve erreicht wurde. Unterhalb von 30% ist die Thymolausbeute zu gering. Nach 30 min ist bereits eine maximale Ausbeute erreicht. Der Zusatz von Glycerin und Ammoniak hat keinen Einfluss auf die Ausbeute an Thymol. Um mit 30% Ethanol eine möglichst hohe Ausbeute zu erreichen wird eine Digestion bei 60°C empfohlen. Die Perkolationskurve mit 30% Ethanol verläuft sehr flach, sodass dieses Verfahren keine Alternative darstellt.

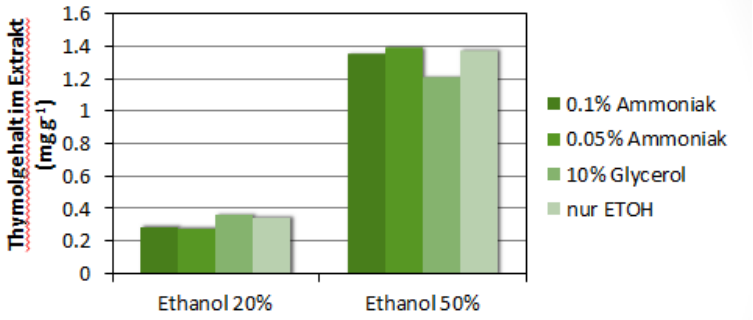



**Abb. 1**. Keinen Einfluss haben Glycerin und Ammoniak auf die Ausbeute an Thymol bei der Mazeration mit verschiedenen Ethanol-/Wassermischungen. Die Gehaltsbestimmung von Thymol erfolgt mit Gaschromatographie nach einem von der Monographie Thymi herba Ph. Eur. adaptierten Verfahren.

**Diskussion**: Es zeigte sich, dass ein Thymianfluidextrakt gemäss aktueller, allgemeiner Extrakt-Monographie der Ph.Eur. [2] nicht hergestellt werden kann. Es braucht mindestens fünf Teile Extraktionsmittel, um mit Thymi herba überhaupt einen Extrakt herstellen zu können, da die pulverisierte Droge sehr viel Extraktionsmittel aufsaugt. Ein Einengen auf einen Fluidextrakt (1 Teil Extrakt entspricht 1 Teil Droge) ist ohne aufwändige Rückgewinnungsverfahren infolge Thymolverlust nicht möglich. Der Fluidextrakt wurde durch eine 1:5-Tinktur ersetzt. Enthält die Tinktur 30% Ethanol kann ein kindergerechter Alkoholgehalt im Sirup (< 8%) bei adäquater Thymolkonzentration erreicht werden. Auf den Einsatz von Ammoniak und Glycerin bei der Extraktion konnte verzichtet werden, Glycerin wurde in die Sirup-Formulierung transferiert.

**Keywords:** Thymi herba, Extraktion, Thymiantinktur, Thymiansirup, Ph.Helv.

**Literatur**:

[1] Swissmedic (2012) Schweizer Pharmakopöe. Monographien CH272 Thymi extractum fluidum normatum und CH273 Thymi sirupus
[2] EDQM (2015) European Pharmacopeia. Monograph 07/2015:0765 Herbal drug extracts.

**P-31**

# Untersuchungen zum Extraktionsverhalten der Anthranoide in Sennae folium/ fructus unter Berücksichtigung der Aglyka

**K. Braun, S. Peter, E. Wolfram, B. Falch, B. Meier**

*Zürcher Hochschule für angewandte Wissenschaften, Fachgruppe Phytopharmazie und Naturstoffe, Institut für Chemie und Biotechnologei, 8820 Wädenswil, Schweiz*

**Einleitung**: Die Monographie Sennae folii extractum siccum normatum der Europäischen Pharmakopöe empfiehlt zur Herstellung Äthanol-/Wassermischungen von 50-80% V/V. Mit der Entwicklung von HPLC-Methoden [1,2] anstelle der aufwändigen photometrischen Methode nach einer adaptierten Bornträger-Reaktion ist es möglich geworden, das Extraktionsverhalten der Inhaltsstoffe selektiv zu untersuchen.
**Ziel**: Die Eignung der in der Monographie vorgeschriebenen Extraktionsmittel wurde anhand der Ausbeuten bei einer Mazeration untersucht. Zusätzlich wurde gemessen, ob bei der Extraktion aus den erwünschten Glykosiden, die als Prodrugs gelten, Aglyka entstehen.
**Methode**: Es wurden Extraktionsversuche mit Sennesfrüchten/-blättern im Labormassstab mit verschiedenen Äthanol-/Wassermischungen durchgeführt und die Ausbeute von Sennosid A und B sowie der Aglyka Rhein und Aloeemodin in Abhängigkeit von der Äthanol-Konzentration sowie mit 50% Äthanol in Abhängigkeit von der Zeit gemessen.
**Resultate**: Optimale Extraktionsergebnisse bezüglich Sennosid A und B wurden mit 40-70% Äthanol in Wasser erreicht (Abb.1). Die Extraktion der Sennoside erfolgte sehr rasch, schon nach 10 min erreichten die Ausbeuten den Bereich des Maximums von > 70%. Insbesondere bei der Extraktion der Blätter nimmt der Gehalt an Aglyka gegenüber der Droge nur in geringem Ausmass zu (maximal 1,8-fach). Bei den Früchten liegt dieser Koeffizient etwas höher (ca. 4-fach).

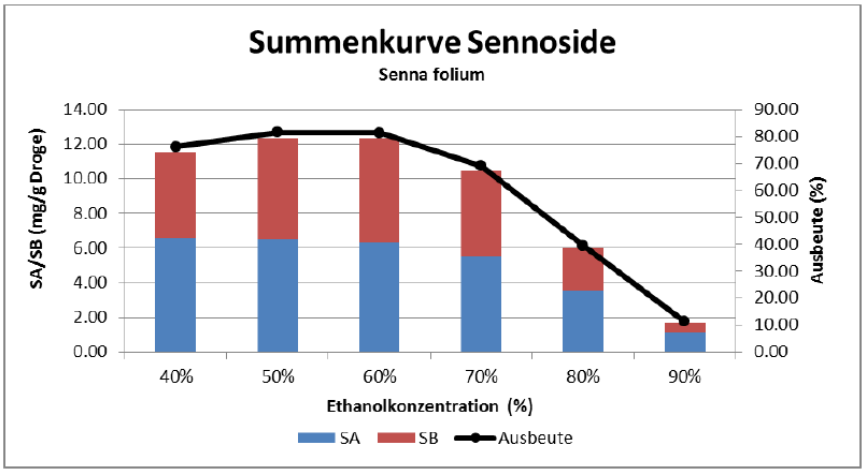



**Abb. 1**. Ausbeute Sennosid A und B bei der Extraktion mit wässrig/alkoholischen Extraktionsmitteln (Mazeration) bezogen auf die Droge in mg/g und in %. Maximum ist 100%.

**Diskussion**: Die Senna-Monographien der Ph. Eur. werden derzeit modernisiert. Für die Extraktmonographie würde es sich empfehlen, den erlaubten Lösungsmittelbereich auf bis zu 40% Äthanol zu erweitern und die obere Limite auf 70% zu senken. Für die Extraktionszeit braucht es keine Vorgaben. Im Vergleich zu anderen Anthranoiddrogen enthalten Sennesblätter und Sennesfrüchte wenig Aglyka, Aloeemodin ist gegenüber Rhein immer in geringeren Mengen (max. 25%) enthalten. Eine Limitierung der Aglyka drängt sich nicht auf, zumal keine massiven Umwandlungen der Gykoside in die Aglyka beobachtet wurden.

**Keywords:** Sennae folium/fructus, Extraktion, Anthranoidglykoside, Anthranoidaglyka.

**Literatur**:

[1] Rosenthal I, Wolfram E, Meier B. Pharmeuropa Bio&SN 2014; October, 92-102.
[2] EDQM (2015) Revision of the Monograph 0206 Sennae folium, Pharmeuropa 27/3 PA/PH/Exp. 13A/T (15) 19 ANP.

**P-32**

## Computergestützte Optimierung der UHPLC-Trennung von Anthranoiden in Sennae fructus und Sennae folium

**S. Peter, N. Meier, G. Josic, B. Meier, E. Wolfram**

*Zürcher Hochschule für angewandte Wissenschaften, Fachgruppe Phytopharmazie und Naturstoffe, Institut für Chemie und Biotechnologei, 8820 Wädenswil, Schweiz*

**Einleitung:** Die bisherigen unspezifischen spektrophotmetrischen Assays in Senna folium und fructus Monographien der Europäischen Pharmakopöe (Ph. Eur.) sollen durch moderne UHPLC-Methoden zur Quantifizierung der Anthranoide ersetzt werden.
**Ziel:** Entwicklung einer optimalen, robusten und validen UHPLC-Methode für Anthranoide in Sennae-Drogen.
**Methoden:** Senna-Schoten und -Blätter extrahiert mit ACN/0.1% v/v $NaHCO_3$ und aufgereinigt mit Solid Phase Extraction (SPE). Methoden [1] und [2] mit Nucleodur 100-3 $C_{18}$-Säule und $H_2O$/ACN/MeOH-Gradient mit Detektion bei 435 nm.
**Resultate:** Der Ph. Eur.-Methodenentwurf liefert 5-20% w/w höhere Gehalte an Anthranoiden berechnet als Sennoside B, vermutlich u.a. aufgrund von Koelution. Die Software Drylab® empfiehlt basierend auf 12 Experimenten innerhalb des Optimierungsraums (Temperatur, mobile Phase und Gradient) weitere Experimente. Die korrekte Peakzuordnung durch DAD und MS ist entscheidend. Abb. 1 (links) zeigt die optimale Methoden-Parameterregion. Durch die *in silico* Optimierung wurden folgende UHPLC-Parameter ermittelt: Phase A: $H_2O$, B: MeOH:ACN (34:66 v/v), beide mit 0.1% Ameisensäure; Stufengradient 0/20, 4/20, 8.63/30, 10/99 [min/%B], Säulentemperatur 43°C (Fig.1 rechts).

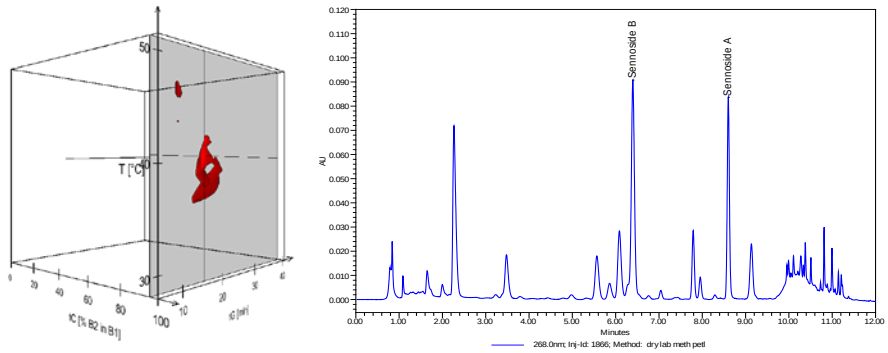



**Abb. 1**. Links: Der rot markiert Teil ist die Region der optimalen Chromatographie-Parameter für die Trennung der Anthranoide in Sennae. Rechts: Beispiel eines UHPLC-Profils von Sennae fructus angustifoliae mit dem optimierten Stufengradient.

**Schlussfolgerung:** Drylab® ist ein hilfreiches Instrument zur rationalen Methodenentwicklung, die Peakzuordnung bei Multikomponenten-Pflanzenextrakten ist eine Herausforderung.

**Keywords:** Cassia senna L., Ph. Eur., UHPLC, Anthranoide.

**Literatur:**
[1] Rosenthal I, Wolfram E, Meier B. Pharmeuropa Bio&SN 2014; October: 92-102.
[2] EDQM (2015). Senna draft monographs, Pharmeuropa 27.3. Strasbourg: EDQM.

**P-33**

## Hyperpolarized Xenon Biosensor as MRI Contrast Agent

**V. Vorobiev[1], A. Babič[1], J.-N. Hyacinthe[2], M. Pupier[3], E. Allémann[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] School of Health Sciences − Geneva, University of Applied Sciences and Arts Western Switzerland, 1206 Geneva, Switzerland*
*[3] Department of Organic Chemistry, University of Geneva, 1211 Geneva, Switzerland*

**Introduction:** A novel class of specific contrast agents (CA) for magnetic resonance imaging (MRI) based on hyperpolarized xenon (Xe) has been developed in recent years [1]. MRI principle is based on codifying the space of interest with magnetic field gradients and then transducing the nuclear magnetic resonance (NMR) signal of each voxel into a range of grey scale to create an image. Hyperpolarization enhances the NMR signal of Xe by several orders of magnitude, which permits its detection at a very low concentration. This gives an advantage over the actual marketed CA, which are detectable only at high concentrations. Moreover, commercialized CA have very low or no organ specificity and diffuse rapidly in tissues, so they have to be administrated intravenously at high doses just before imaging. Since Xe is a nontoxic inert gas with several interesting medical properties, such as activation of neuro and cardio protection, new CA using it would have promising perspectives first for precise diagnosis but potentially also for therapy.
**Aims:** To develop several new Xe-based CA for MRI with or without targeting ligands for different pathological conditions and testing them *in vitro* and *in vivo*.
**Methods:** Preliminary trials using non-hyperpolarized Xe NMR spectra where recorded on 300 MHz or 400 MHz NMR spectrometers and compared to the literature data [2]. We used the new broadband CHORUS excitation for easy and quantitative measurements [3]. We acquired spectra of pure Xe gas and Xe dissolved in several deuterated solvents: MeOD, $CDCl_3$, DMSO-$d_6$, $CD_2Cl_2$, $CD_3CN$, acetone-$d_6$, and $D_2O$. The following step of this preliminary study would require bubbling Xe into a solution of our new Xe host molecules and record NMR spectra of the caged and free gas. At this point hyperpolarized Xe will be used as the solubility of the host molecules is too low to trap sufficient amounts of Xe to detect without hyperpolarization. The best Xe host molecule will be attached to a targeting moiety with or without a linker. *In vitro* assays will be performed in the NMR-tube containing the cells and the signals of untrapped, trapped and Xe bound to cells will be recorded. Final stage would be *in vivo* trials with an image acquisition and reconstruction by small animal 3T MRI.
**Results:** First results by NMR spectroscopy are encouraging. We succeeded in detecting Xe without hyperpolarization. A signal was recorded in all tested solvents, even in water where Xe is very poorly soluble. Chemical shifts are comparable to literature [2] and control experiments confirmed the observation of Xe signals. It is interesting to note that the sensitivity of this method is high enough to detect non-hyperpolarized Xe rapidly after only 1 scan in 5 mm tube MeOD solution with liquid states NMR spectrometers.
**Conclusions:** Preliminary results show promise for the future steps of the project. Non-hyperpolarized Xe was detected by NMR in all tested solvents. The optimized pulse sequence will be easily used with the hyperpolarized gas in the next step of our experimentation. The synthesis of the new Xe host molecules has been achieved recently and they will be fully characterized by NMR spectroscopy. To confirm the proof of concept *in vitro* assays on cells will be conducted thereafter.

**Keywords:** Xenon, hyperpolarization, contrast agents, MRI.

**References:**
[1] Meersmann T, Brunner E. Cambridge UK: Royal Society of Chemistry; 2015.
[2] Cherubini A, Bifone A. Prog Nucl Mag Res Sp. 2003; 42:1-30.
[3] Power JE et al. Chem Commun 2016; 52: 2916-2919.

**P-34**

## Tailoring the Drug Release of Silk-Based Drug Delivery Systems Via Silk Modification

**K. Nultsch[1,2], O. Germershaus[1]**

*[1] Institute for Pharma Technology, School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, 4132 Muttenz, Switzerland*
*[2] Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*

**Introduction**: Natural silk comprises mainly two proteins, silk fibroin (SF) and silk sericin. While sericin is suspected of causing allergies in the presence of fibroin and therefore is removed by so-called degumming, the chemical modification of silk fibroin is a novel approach to control drug release. Tyrosine represents approximately 6% of the amino acids of SF and is evenly distributed within the protein [1]. These tyrosine residues can be modified by Copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC, click chemistry) with high reaction yield. By varying the degree of modification the release of high molecular weight model compounds can be controlled.
**Aims**: This work focuses on the controlled release of model compounds by altering the degree of modification.
**Methods:** SF films were prepared as previously described [2]. In brief, the silk cocoons were degummed for 1 h in 0.02 M sodium carbonate solution. After washing with ultrapure water the SF fibers were dried overnight. SF fibers were dissolved in Ajisawa's reagent (1:2:8 calcium chloride:ethanol:water) at 65°C. The SF solution was dialyzed against ultrapure water for 48 h and concentrated to approx. 6% SF solution (w/v). The SF solution was loaded with the model compounds by mixing it with 15 mg/mL model compound and poured in 6-well plates. After drying the films were treated with methanol to induce β-sheet formation. For the click chemistry the tyrosine residues on the surface of the films were coupled with an aniline derivative to form a diazonium salt. Then the aniline derivative was allowed to react with an alkyne-poly (ethylene glycol)-alkyne to crosslink the film surface. For drug release studies, phosphate buffered saline (PBS) was added and incubated at 37°C.
**Results**: The model compound release could be controlled by the alteration of the tyrosine residues via covalent binding of azide-alkine forming a cyclic cross linking. The degree of cross linking could be adjusted leading to a tunable sustained release system.
**Conclusions:** Silk fibroin is a promising material for drug delivery systems with. Via chemical modification the release can be controlled and additionally, the surface could be modified, e.g. a higher hydrophilicity.

**Keywords:** Silk fibroin, biopolymer, chemical modification, click chemistry.

**References:**
[1] Zhou CZ et al. Proteins: Struct., Funct., Bioinf. 2001; 44: 119-22..
[2] Hines DJ, Kaplan DL. Biomacromolecules 2011; 12: 804-12.

**P-35**

## Investigation of the Anti-Obesity Effect of *Pueraria montana* var. *lobata*

**J. Houriet[1], E. Buhlmann[2], C. Rudigier[3], E. Kiehlmann[3], J. Radtke[2], J. Heeren[4], T. Friedemann[2], S. Schröder[2], C. Wolfrum[3], J.-L. Wolfender[1]**

[1] *Phytochimie et Produits Naturels Bioactifs, Ecole de Pharmacie Genève-Lausanne Section des Sciences Pharmaceutiques, University of Geneva, 1211 Geneva, Switzerland*
[2] *HanseMerkur Center for Traditional Chinese Medicine, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg, Germany*
[3] *Institute of Food, Nutrition and Health, ETH Zurich, 8603 Schwerzenbach, Switzerland*
[4] *Institute for Biochemistry and Molecular Cell Biology, Center for Experimental Medicine, University Medical Center Hamburg-Eppendorf, D-20246 Hamburg, Germany*

**Introduction:** According to the World Health Organisation (WHO), the increase in the prevalence of overweight/obese people worldwide has reached a qualified epidemic stage with more than one billion overweight and at least 400 million clinically obese patients.
**Aims:** In search for new treatments to improve the management of the diseases related to obesity, Asian herbal medicines provide prospects with high therapeutic potential, but empirical knowledge has to be scientifically validated [1].
**Methods:** Using a novel hypothesis-driven screening approach [2], the root of *Pueraria montana* var. *lobata* (PL) was identified as a promising candidate. Since starting analysis of natural products in *in vitro* cell culture systems can lead to false negative results, the efficacy of the aqueous extract was directly tested in an *in vivo* model of obesity. Mice were fed with a high fat diet containing 60% of fat and with 2 g/kg/day of the extract for 14 days. The effects on body weight and metabolic parameters were measured. Chromatographic profiling on UHPLC-PDA-ELSD-HRMS were performed to characterize this aqueous extract.
**Results:** Interestingly, we could show that treatment with PL led to a significant weight loss combined with an improvement in glycemic control. Moreover, an induction of inguinal brown adipocytes was observed which could explain the metabolic phenotype. Analysis by UHPLC-PDA-ELSD-HRMS confirmed that the extract contained several isoflavones [3]. Standardization of the extract was performed and quantitative analysis revealed that the level of puerarin, daidzin and genistin reached 5.15% (0.10% SD), 2.16% (0.04% SD) and 1.03% (0.02% SD) respectively (m/m). Since puerarin is the main isoflavone of the extract, its effect on the induction of brown adipocyte formation and function was studied. The assays demonstrated that puerarin in cell culture did not regulate the formation or activity of brown adipocytes.
**Conclusions:** The results suggest that either systemic modifications of puerarin or another constituent is responsible for the observed effects. Further pharmacological experiments are necessary to identify the active ingredients of PL which are responsible for its *in vivo* impact on obesity.

**Keywords:** Obesity, brown adipose tissue, Traditional Chinese Medicine, UHPLC-PDA-ELSD-HRMS, *Pueraria*.

**References:**
[1] Pan SY et al. Evid Based Complement Alternat Med 2013; 2013: 627375.
[2] Friedemann T et al. Science 2015; 350 (6262): S69-S71.
[3] Zhang Z, Lam T-N, Zuo Z. J Clin Pharmacol 2013; 53: 787-811.

**P-36**

## Is Use of Antiepileptic Drugs Associated With an Increased Cataract Risk? A Case-Control Analysis

**C. Becker[1], S. S. Jick[2], C. R. Meier[1,2,3]**

*[1] Basel Pharmacoepidemiology Unit, Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, 4003 Basel, Switzerland*
*[2] Boston Collaborative Drug Surveillance Program, Boston University School of Public Health, 02421 Lexington, Massachusetts, USA*
*[3] Hospital Pharmacy, University Hospital Basel, 4056 Basel, Switzerland*

**Introduction:** Several antiepileptic (AE) drugs such as phenytoin, valproate and carbamazepine have been associated with cataract in previous case reports. One case-control study reported an increased risk of cataract surgery in epileptic patients exposed to clonazepam (OR 1.5, 95% CI 1.1-2.1) or carbamazepine (OR 1.4, 95% CI 1.05-1.8) [1]. The results were based on a small number of patients and need to be confirmed in additional population-based studies.
**Aims:** To explore the association between use of AE drugs and the risk of cataract.
**Methods:** We conducted a case-control analysis within the UK-based Clinical Practice Research Datalink (CPRD). Cases (≥ 40 years) had either an incident cataract diagnosis or a recorded cataract extraction (i.e., the index date, i.d.). Individuals with a history of cancer, alcoholism, HIV, or traumatic or secondary cataract as well as blind persons were excluded. Cases and controls were matched 1:1 on age, sex, calendar time, general practice, and number of years of history in the CPRD prior to the i.d. We assessed the number of prescriptions for antiepileptic drugs before the index date and conducted conditional logistic regression to derive Odds ratios (ORs) with 95% confidence intervals (CI). Drug exposure was assessed as concomitant as well as mutually exclusive use. The contribution of various potential confounders (co-morbid conditions or exposure to other drugs previously associated with cataract development) was evaluated in univariate models, and final results were adjusted for BMI, smoking, hypertension, diabetes, glaucoma, and oral steroids. We did the following sensitivity analyses: a) (to account for the non-acute onset of cataract) we shifted the i.d. backwards for two years b) we excluded individuals with a glaucoma diagnosis prior to the cataract.
**Results:** A total of 206'931 cataract cases and the same number of matched controls were identified. Long-term use (≥ 25 prescriptions) of the following AE drugs was associated with a statistical significantly increased risk of cataract: gabapentin (adj. OR 1.28, 95% CI 1.10-1.49) and pregabaline (adj. OR 1.47, 95% CI 1.14-1.89). Results were not materially changed in the analysis restricted to patients without glaucoma. In the analysis with the shifted i.d., long-term clonazepam (adj. OR 1.50, 95% CI 1.13-1.98) and valproate exposure were also associated with an increased cataract risk (adj. OR 1.32, 95% CI 1.13-1.55).
**Conclusions:** According to our study, several AE drugs seem to be associated with cataract development.

**Keywords:** Pharmacoepidemiology, antiepileptic drugs, cataract, case-control study.

**Reference:**
[1] Hanhart J et al. Curr Eye Res 2010; 35: 487-491.

**P-37**

## LST-3TM12 – a Functional Transporter or a Genetic Relict?

**V. Malagnino[1], J. Hussner[1], A. Stolzenburg[2], I. Seibert[1], H. E. Meyer zu Schwabedissen[1]**

*[1] Biopharmacy, Department Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
*[2] Institute of Pharmacology, Ernst Moritz Arndt University, D-17489 Greifswald, Germany*

**Introduction:** One major family of drug transporters is the protein superfamily of Solute Carriers (SLCs), where the family of Organic Anion Transporting Polypeptides (OATPs, formerly SLC21A) plays a significant role in pharmacokinetics. Especially members of the OATP1B subfamily, OATP1B1 and OATP1B3, have repeatedly been reported to contribute to the hepatic handling of their substrate drugs. However, between the gene locus encoding for OATP1B3 (*SLCO1B3*) and OATP1B1 (*SLCO1B1)* another sequence has been identified, which is annotated as SLCO1B7. This sequence exhibits high similarity to OATP1B1 and OATP1B3 but is considered as a pseudogene. However, in 2003 a mRNA sequence was published by Mizutamari and Abe (GenBank: AY257470.1) that displays segments identical to SLCO1B7 and that was annotated as LST-3TM12.
**Aims:** The aim of this study is to find out whether SLCO1B7 is transcribed into mRNA and if the chimeric mRNA sequence LST-3TM12 is its transcription product. Furthermore we are investigating the expression of the putative protein in human tissue and cell lines. The long term goal is to characterize the protein function with transient and stabile transfected cell lines.
**Methods:** An *in silico* comparison of LST-3TM12, SLCO1B7, and SLCO1B3 has been conducted. The quantification of mRNA expression of SLCO1B7/LST-3TM12 in different human tissue samples and human cell lines was performed by real-time PCR. For the identification of the putative protein product Western blot analysis of human liver homogenate and immunohistochemistry with liver slices was employed. A set of plasmids coding for SLCO1B7, SLCO1B3, and LST-3TM12 was created.
**Results:** Based on our *in silico* analysis of LST-3TM12 we assume that LST-3TM12 is a fusion gene of SLCO1B3 and SLCO1B7. We demonstrated the expression of SLCO1B7/LST-3TM12 mRNA in human liver and the human liver cell line Huh-7. Western Blot analysis revealed expression of the the putative SLCO1B7/LST-3TM12 protein in human liver. In addition immunohistochemical staining of human liver suggested localization of the protein in the intracellular compartments. A set of native and tagged gene sequences of SLCO1B7, SLCO1B3, and LST-3TM12 is ready for functional studies.
**Conclusions:** Although SLCO1B7 is a pseudogene it is transcribed into mRNA. Based on our *in silico* analysis we think that SLCO1B3 and SLCO1B7 share the same promotor. The protein product is mainly expressed in human liver tissue. The exact localization of the protein in hepatocytes with identification of the cellular organelles has to be determined in further studies. Clearly, the protein is accumulated in intracellular granular structures. Further studies with transfected cell lines will elucidate the functional characteristics of the protein product.

**Keywords:** SLCO1B3, SLCO1B7, LST-3TM12, solute carriers, organic anion transporting polypeptides.

**P-38**

## Functionalized PEG-*b*-PCL Nanoparticles to Target Human Brain Capillary Endothelial Cells *In Vitro*

**P. Grossen[1], G. Québatte[1], D. Witzigmann[1], C. Baschong-Preschianotto[2], L.-H. Dieu[1], J. Huwyler[1]**

*[1] Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
*[2] Biozentrum, University of Basel, 4056 Basel, Switzerland*

**Introduction:** The blood-brain barrier (BBB) prevents entry of many xenobiotics into the brain. This leads to difficulties in the treatment of CNS related diseases [1]. Besides chemical optimization of the API and invasive delivery techniques, targeting of receptors expressed on the BBB microvasculature is a promising strategy to deliver drugs to the CNS [2]. Biodegradable polymeric micelles, so-called nanoparticles (NPs), are extensively studied as targeted drug delivery systems [3]. While antibody drug conjugates can deliver their cargo specifically to a distinct tissue or cell type, NPs exceed their drug payload by three to four orders of magnitude.
**Aims:** In this study, NPs consisting of biodegradable and FDA-approved PEG-*b*-PCL were used to implement a drug targeting strategy. NPs were characterized and cellular interactions with a model of the human BBB and human liver derived cells were studied *in vitro*.
**Methods:** NPs were analyzed using dynamic and static light scattering (DLS, SLS), transmission electron microscopy (TEM), fluorescence correlation spectroscopy (FCS), specific volume measure-ments, and pyrene encapsulation. Particle toxicity was assessed using the MTT assay. *In vitro* cellular uptake was analyzed using flow cytometry, confocal laser scanning microscopy (CLSM), and TEM.
**Results:** NPs had a hydrodynamic diameter of 80 nm, a homogenous size distribution (PDI 0.078) and a slightly negative zeta-potential (-4.521 mV). The critical aggregation concentration (CAC) was low ($2.8 \times 10^{-7}$ M). The aggregation number, determined by specific volume measurements, was between ≈ 2200 and ≈ 10 000. Toxicity of PEG-*b*-PCL NPs on human BBB derived hCMEC/D3 cells and human liver cells (HepG2) was analyzed. Even at high concentrations, only a slight decrease in cell viability was observed. To target brain capillary endothelial cells, NPs were successfully modified with an anti-insulin receptor antibody (83-14 mAb). On average, 5 molecules of 83-14 mAb were covalently coupled to one NP. hCMEC/D3 cells were incubated with 83-14 mAb-modified PEG-*b*-PCL NPs *in vitro* and the cellular uptake was analyzed. Targeted PEG-*b*-PCL NPs showed an increased uptake as compared to their non-modified counterparts. Cellular uptake increased with time and could be competitively inhibited. In addition, uptake of PEG-*b*-PCL gold-nanohybrids by hCMEC/D3 cells grown on transwell filters was analyzed by TEM. First internalized NPs were observed after 15 min of incubation. After 60 min, PEG-*b*-PCL NPs were found to accumulate in multi-vesicular bodies (MVBs).
**Conclusions:** PEG-*b*-PCL NPs were successfully modified with 83-14 mAb. Functionalized NPs showed an increased uptake by cells of the target tissue *in vitro* without toxic effects. These *in vitro* results will need to be confirmed by *in vivo* pharmacokinetic and tissue distribution studies.

**Keywords:** Blood-brain barrier, PEG-*b*-PCL nanoparticles, human BBB model, liver cells, toxicity.

**References:**
[1] Begley DJ. Pharmacol Ther 2004;104: 29-45.
[2] Pardridge WW. Pharm Res 2007; 24: 1733-1744.
[3] Chen W et al. J Controlled Release 2014; 190: 398-414.

**P-39**

## $GABA_A$ Receptor Modulating Piperine Analogs - *In vitro* Metabolic Stability, Protein Binding, CYP450 Reaction Phenotyping And Metabolite Identification

**V. Zabela[1], T. Hettich[2], G. Schlotterbeck[2], L. Wimmer[3], M.D. Mihovilovic[3], F. Guillet[4], B. Bouaita[5], B. Shevchenko[5], M. Hamburger[1], M. Oufir[1]**

[1] *Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
[2] *Institute for Chemistry and Bioanalytics, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, 4132 Muttenz, Switzerland*
[3] *Institute of Applied Synthetic Chemistry, TU Wien, A-1060 Vienna, Austria*
[4] *Eurosafe, 35760 Saint Grégoire, France*
[5] *Biopredic International, 35760 Saint Grégoire, France*

**Introduction:** During a screening of natural products for γ-aminobutyric acid type A ($GABA_A$) receptor activity, piperine was identified as a positive allosteric modulator targeting a benzodiazepine-independent binding site [1]. However, piperine is also an activator of TRPV1 receptors (transient receptor potential vanilloid type 1) involved in pain signaling and thermoregulation. The structure of piperine was systematically modified to dissect $GABA_A$ and TRPV1 activating properties, and a library of piperine analogs was prepared. The most potent and efficacious analogs were identified from *in vitro* and *in vivo* studies [2].
**Aim:** We here investigated the metabolism of piperine and selected analogs (SCT-29, LAU 397, and LAU 399) to guide further structural optimization of analogs.
**Methods:** Metabolic stability of compounds was tested in the presence of pooled human liver microsomes. Intrinsic clearance was calculated using the substrate depletion approach. Metabolites were analyzed by UHPLC-QTOF-MS, and with the aid of metabolite identification software Mass-MetaSite. Unbound fraction in whole blood was determined by rapid equilibrium dialysis. CYP450 reaction phenotyping studies were carried out with Silensomes™.
**Results:** Piperine was the metabolically most stable compound. The principal routes of oxidative metabolism were found to be aliphatic hydroxylation, and N- and O-dealkylation. Piperine was exclusively metabolized by CYP1A2. CYP2C9 contributed significantly in the oxidative metabolism of all analogs. Extensive binding to blood constituents was observed for all compounds.
**Conclusions:** Analogs were rapidly metabolized and showed strong binding to blood constituents due to increased lipophilicity. Therefore, lipophilicity should be reduced to lower metabolic liability and extensive binding of analogs.

**Keywords:** $GABA_A$ receptor, metabolic stability, metabolite identification, protein binding, CYP450 reaction phenotyping.

**References:**
[1] Zaugg J et al. J Nat Prod 2010; 73: 185-191.
[2] Wimmer L et al. Org Biomol Chem 2015; 13: 990-994.

**P-40**

## Multifractal Analysis and Dispersion Imaging of Pharmaceutical Hot-Melt Extrudates

**C. Adler[1,2], A. Teleki[3], M. Kuentz[1]**

*[1] University of Applied Sciences and Arts Northwestern Switzerland, Institute of Pharmaceutical Technology, 4132 Muttenz, Switzerland*
*[2] University of Basel, Institute of Pharmaceutical Technology, 4056 Basel, Switzerland*
*[3] DSM Nutritional Products Ltd., R&D Center Formulation & Application, 4002 Basel, Switzerland*

**Introduction:** Fractal and multifractal analyses have become powerful tools to describe complex physical objects that have irregular shapes or fragmented structures. Fractals are characterized by their self-similarity and multifractals can be viewed as a superposition of homogeneous monofractal objects [1]. Fractals have been used in many fields using image analysis, e.g. geoscience, food sciences, pharmaceutical technology. It is usually based on analyzing binary pictures by a box counting method to assign a mathematical dimension that can be linked to material properties. To the best of our knowledge multifractals have so far not been used in pharmaceutics.
**Aims:** To apply multifractal analysis to better understand the structural properties of hot-melt extrudates containing different types of silica based adsorbents. The purpose was to better understand the influence of the adsorbent concentration, the type of adsorbent or the screw speed on the microstructure. In a second part, the erosion and dispersion behavior of extrudates was assessed by optical imaging since it can be critical for oral dosage form performance.
**Methods:** Vinylpyrrolidone-vinylacetate copolymer (Kollidon VA 64) was used as polymeric carrier, Labrafac PG was selected as plasticizer, and hydrophilic colloidal silica (Aerosil 300), granulated form of colloidal silica (Aeroperl 300), aluminum magnesium silicate (Neusilin US2) and calcium silicate (Florite R) were employed as adsorbents. PVPVA/LabrafacPG/adsorbent premixes were manually fed into the extruder hopper. HME was performed at 150°C, and 150, 250 and 350 rpm screw speed. Extrudates were pelletized and silicon distribution in the pellets was analyzed by scanning electron microscopy/ energy-dispersive X-Ray spectroscopy. Collected pictures were converted into binary pictures and the box counting method was used for multifractal analysis. Aqueous dispersion and erosion of the pellets was assessed by dynamic optical imaging.
**Results:** Multifractal analysis showed that the adsorbent concentration as well as the screw speed had an effect on the pellets microstructure. Interpretation of the generalized fractal dimensions indicated that increasing the adsorbent concentration or the screw speed led to higher space coverage, higher degree of disorder and lower clustering level of the silica-based material. It was also found that the type of adsorbent influenced the generalized fractal dimensions of the pellets. The use of granulated adsorbents resulted in higher heterogeneity and clustering level compared to fumed silica materials. Finally, self-dispersion analysis displayed that pellets containing hydrophilic excipients swelled and eroded, whereas samples containing hydrophobic adsorbents only swelled without self-dispersing.
**Conclusions:** Multifractal analysis provided a tool to gain better understanding of the microstructure caused by adsorbent concentration, screw speed and type of adsorbent. The comparison of the generalized fractal dimensions provided information of coverage, homogeneity and cluster level. All this information is of great interest in pharmaceutical product development to tailor drug delivery systems of desired attributes. However, no significant correlations between self-dispersion and multifractal analysis results could be evidenced, these two study parts were complementary. Thus, self-dispersion analysis suggested that erosion and swelling behavior can be modified by changing the nature or concentration of the adsorbent. We found that erosion could be inhibited by incorporating a hydrophobic silica material.

**Keywords:** Multifractal, hot-melt extrusion, adsorbent, scanning electron microscopy, dispersion.

**Reference:**
[1] Lopes R, Betrouni N. Med Image Anal 2009; 13: 634-649.

**P-41**

## Targeting Tumor Cells with Anisamide-Functionalized Colloids

**A. Dasargyri, C. Kümin, P. Hervella, A. Christiansen, S. Proulx, M. Detmar, J.-C. Leroux**

*Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, ETH Zurich, 8093 Zurich, Switzerland*

**Introduction:** Anisamide, or 4-methoxybenzamide, is a small organic molecule that was suggested as a tumor-targeting moiety for colloidal systems and has been found to enhance endocytosis of particulate systems by tumor cells *in vitro* as well as *in vivo*. With its structure inspired by larger benzamides formerly shown to bind to Sigma-1 receptor ($\sigma_1$R), the cell target with which anisamide was hypothesized to interact was $\sigma_1$R. This interaction, however, has never really been characterized.
**Aims:** The purpose of this work was to design and develop different anisamide-decorated systems and test their uptake by tumor cells. At a next step, the goal was to investigate the putative interaction of carrier-tethered anisamide with its proposed target, $\sigma_1$R.
**Methods:** Three different anisamide-functionalized systems were prepared: poly(styrene) particles of 1 μm diameter, liposomes of 200 nm diameter and a soluble star-PEG of 20 nm theoretical diameter. The poly(styrene) particles, bearing a fluorescent dye, were functionalized by reaction of the surface amine groups of the starting commercially-available particles with a synthesized anisamide-PEG-COOH. For liposome targeting, a phospholipid-PEG-anisamide conjugate was prepared and incorporated, together with a fluorescent dye, in the liposomal formulation. Finally, a 8-arm star-PEG-$NH_2$ was derivatized with anisamide and a fluorescent dye. The fluorescence of the 3 systems served for the tracking of their *in vitro* uptake by B16-F10 murine melanoma cells, which were used as a model cancer cell line. The anisamide-tethered poly(styrene) particles were also utilized for the investigation of the interaction of the targeting ligand with $\sigma_1$R. Their uptake by B16-F10 cells was tested in the presence of a $\sigma_1$R agonist for competitive binding, as well as by cells with down-regulated $\sigma_1$R expression after siRNA transfection. Ultimately, the subcellular localization of $\sigma_1$R in B16-F10 cells was assessed by immunostaining.
**Results:** The 3 systems presented divergent cell uptake results. While the large anisamide-decorated poly(styrene) particles showed a pronounced uptake compared to their PEGylated non-functionalized counterparts, this difference was less evident for the smaller liposomes and even abolished in the case of the soluble star-PEG. Concerning the participation of $\sigma_1$R in the uptake of the anisamide-decorated poly(styrene) particles, the presence of a $\sigma_1$R agonist did not affect at all the extent of uptake. No difference in uptake was also observed when $\sigma_1$R expression was reduced to less than 10% following $\sigma_1$R siRNA treatment of the cells. Finally, $\sigma_1$R immunostaining on B16-F10 revealed intracellular localization of the receptor in these cells.
**Conclusions:** The difference in cell internalization between the 3 tested anisamide-targeted systems suggests that their uptake might be related to their physicochemical properties rather than to the presence of the targeting moiety. The lack of $\sigma_1$R agonist competition in the uptake of the anisamide-targeted particles, and the absence of influence of $\sigma_1$R expression on the extent of uptake both challenge the presumed interaction of anisamide with $\sigma_1$R. In line with this, the intracellular localization of $\sigma_1$R in the tested cells precludes the binding of carrier-attached anisamide with this receptor. Taken together, these data point out the limitations of anisamide's tumor-cell targeting properties when it is attached on the surface of colloidal systems.

**Keywords:** Anisamide, sigma-1 receptor, receptor-mediated endocytosis, drug targeting.

**P-42**

## Case-Control Study Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

**N. Frey[1,2], M. Bodmer[3], A. Bircher[4], S. S. Jick[5], C. R. Meier[1,2,5], J. Spoendlin[1,2]**

[1] *Basel Pharmacoepidemiology Unit, Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
[2] *Hospital Pharmacy, University Hospital Basel, 4056 Basel, Switzerland*
[3] *Internal Medicine, Cantonal Hospital Zug, 6340 Baar, Switzerland*
[4] *Allergology University Hospital Basel, 4056 Basel, Switzerland*
[5] *Boston Collaborative Drug Surveillance Program, Boston University, Lexington MS 02420, United States*

**Introduction:** Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions. Previous hospital-based case-control studies identified allopurinol, aromatic antiepileptics, sulphonamide antibiotics (mostly cotrimoxazole, i.e. sulfamethoxazole/ trimethoprim), oxicam analgesics, and nevirapine as the main culprit drugs. Other classes of antibiotics and other specific drugs have been associated with insufficient evidence.
**Aims:** To provide further evidence on established and unknown culprit drugs of SJS/TEN.
**Methods:** We conducted a matched (1:4) case-control study using the UK-based Clinical Practice Research Datalink. Cases were 488 patients with a validated incident diagnosis of SJS/TEN between 1995 and 2014 (validation presented separately). In conditional logistic regression analyses (multivariable if ≥ 3 exposed cases/controls), we calculated odds ratios (OR) for incident SJS/TEN in association with first-time prescription of various drugs ≤ 84 days before SJS/TEN onset. For all drugs with potential for confounding by indication we performed the same analyses excluding the last 14 days before SJS/TEN onset.
**Results:** We observed strongly increased ORs for most previously identified main culprit drugs (i.e. carbamazepine, lamotrigine, phenytoin, allopurinol [insufficient numbers for oxicam analgesics and nevirapine]), but also for aminopenicillins, quinolone antibiotics, and cephalosporins. The OR for SJS/TEN were strongly increased in association with trimethoprim only use (OR 9.35, 95% CI 3.62-24.18), but not for other sulphonamide antibiotics. We further observed previously unreported associations for COX-2 inhibitors (crude OR 24.17, 95% CI 2.91-200.77), omeprazole (OR 4.25, 95% CI 1.34-13.49), and lansoprazole (OR 5.85, 95% CI 1.39-24.64).
**Conclusions:** The observed associations between SJS/TEN and previously identified culprit drugs support the findings of previous studies and corroborate the validity of this SJS/TEN study population (first large SJS/TEN study population from a longitudinal database). Our results further suggest that trimethoprim rather than sulphonamide antibiotics in general may be a trigger for SJS/TEN. The observed associations of SJS/TEN with COX-2 inhibitors, omeprazole, and lansoprazole remain to be confirmed in further research.

**Keywords:** Stevens-Johnson syndrome, toxic epidermal necrolysis, culprit drugs, epidemiology.

**P-43**

## Investigation of Permeation Through Model Membranes in Single Vesicle Traps by Fluorescence Correlation Spectroscopy

**S. Bachler, N. Naredi-Rainer, P. S. Dittrich**

*Bioanalytics Group, Department of Biosystems Science and Engineering, ETH Zurich, 8093 Zurich, Switzerland*

**Introduction:** In this work, we present a two-layered microfluidic device that allows trapping, treatment, and analysis of up to 60 individual giant unilamellar vesicles (GUVs) or cells. The trapped objects can be exposed to chemical treatments like penetrating peptides, drugs, lysis buffers, antibodies, or staining dyes with precisely controlled durations while being constantly monitored with microscopic or spectroscopic methods.
**Aims:** The permeability of model GUV membranes as a function of lipid composition, such as the percentages of charged or curvature-inducing lipids, is investigated.
**Methods:** We use peptides that either partition into or penetrate across the membrane. Short polypeptides, in particular the HIV-1 trans-acting activator of transcription (TAT) domain and the nona-arginine (Arg-9) peptide possess the ability to cross natural cells as well as artificial membranes, enabling also cargo transport into the cells. Permeation of the fluorescently labeled peptides into GUVs is then characterized with fluorescence correlation spectroscopy (FCS), which provides information on the intra- and extra-vesicular concentrations.
**Results:** The results indicate that the composition of the membrane (anionic lipids and negative curvature inducing lipids in combination with cholesterol and neutral lipids) influence the membrane permeability of the tested cell-penetrating peptides (CPPs).
**Conclusions:** With its single-molecule sensitivity, FCS, in combination with the microfluidic trap arrays, constitutes a valuable platform for drug or toxin screening.

**Keywords:** Permeation, cell-penetrating peptides, giant unilamellar vesicles, fluorescence correlation spectroscopy.

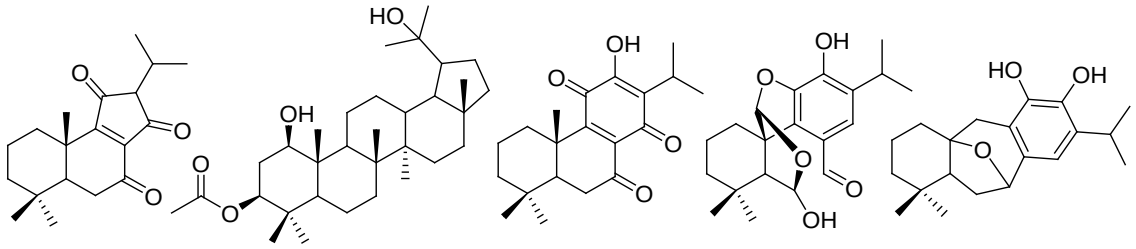
**P-44**

## Chemical Constituents From the Root of *Salvia leriifolia*

**M. M. Farimani[1] H. Moradi[1] M. de Mieri[2] M. Kaiser[3] M. Hamburger[2] S. N. Ebrahimi[1]**

*[1] Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran*
*[2] Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
*[3] Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, 4051 Basel, Switzerland*

**Introduction:** *Salvia* species are important medicinal and culinary plants [1]. *Salvia* is the largest genus in the family of Lamiaceae, and 61 species are found in Iran, 17 of which are endemic. *Salvia leriifolia is an* endemic species occuring in Khorasan and Semnan provinces. Cytotoxic, antibacterial, anticonvulsant, anti-ischemic, anti-inflammatory, and hypoglycemic properties have been reported for extracts of *S. leriifolia* [2]. Despite the numerous studies on pharmacological properties the phytochemistry of the species has been poorly studied up to now.
**Aims:** In a project directed on discovery novel bioactive metabolites from Iranian endemic plant from Lamiaceae, we investigated an *n*-hexane extract of *Salvia leriifolia* roots.
**Methods:** Phytochemical investigation of the extract by a combination of normal phase column chromatography and preparative thin layer chromatography afforded five terpenoids. Their structures were established on the basis of extensive spectroscopic data, including $^{1}$H-NMR, $^{13}$C-NMR, $^{1}$H-$^{1}$H COSY, HMQC, HMBC, and DEPT experiments.
**Results and Conclusions:** Compounds were identified as one new nor-abietane diterpenoid (1), one new lupane triterpenoid (2), as well as three known abietane and icetexane diterpenoids (3-5). Compound 1 features an unprecedented 6/6/5 membered carbon scaffold. *C*ompound 1 showed *in vitro* anti-protozoal activity against *Trypanosoma brucei rhodensiense, Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum,* with $IC_{50}$ values of 0.99, 4.62, 0.99, and 3.64 µM, respectively.




1 2 3 4 5

**Keywords:** *Salvia leriifolia*, Lamiaceae, *nor*-diterpene, triterpene.

**References:**
[1] Moridi Farimani M et al. Planta Med 2015; 81: 1290-1295.
[2] Loizzo MR et al. J Med Food 2010; 13: 62-69.

**P-45**

## Role of HNF4α in Proliferation of Renal Carcinoma Cells

**K. Prestin, J. Heinzer, F. Wiss, Y. Fischer, H. E. Meyer zu Schwabedissen**

*Department of Pharmaceutical Sciences, Biopharmacy, University of Basel, 4056 Basel, Switzerland*

**Introduction:** Mammalian nuclear receptors are transcription factors regulating expression of various target genes involved in drug metabolism, transport, cellular signaling pathways, and cancerous diseases. One member of this protein superfamily is the hepatocyte nuclear factor 4 alpha (HNF4α) which has not only been shown to be expressed in human liver, but also in human kidney, intestine and pancreatic beta-islet cells. Previous findings show that HNF4α is downregulated in human hepatocellular carcinoma and suggest that this nuclear receptor functions as a tumor suppressor which modulates activity of genes associated with cell cycle and apoptosis [1,2].
**Aims:** The aim of our study was to test whether HNF4α also plays a role in other tumor entities such as renal cell carcinoma, and whether activity of this nuclear receptor can be modulated by pharmacological compounds.
**Methods:** Protein and mRNA expression in human kidney samples and cells in culture were characterized by quantitative real-time RTPCR and Western blot analysis. Adenoviral transfer of HNF4α was conducted to modulate expression of this nuclear receptor in human renal carcinoma cells. The impact of heterologous transfer of HNF4α and treatment with previously identified HNF4α-ligands on proliferation and viability of kidney cells was assessed by capillary-based cell counting, bromodeoxyuridine enzyme linked immunosorbent assay (BrdU-ELISA), and monitoring of resazurin turnover. Hematoxylin/eosin staining was performed to assess cell morphology.
**Results:** HNF4α expression was significantly lower in tumor samples of human renal cell carcinoma comparing non-malignant transformed tissue suggesting that mechanisms of reduced HNF4α expression may also play a role in this tumor entity. After validation of adenoviral-transfer of HNF4α, the impact of heterologous expression of HNF4α on cell proliferation and cell viability was analyzed. Cell counting and BrdU-ELISA revealed a significant lower proliferation rate of renal carcinoma cells in presence of HNF4α whereas no effect on cell morphology was observed, even if metabolic activity was slightly reduced. The antiproliferative effect of the nuclear receptor was even more pronounced in presence of BCR312 (2-nitro-7-methoxynaptho[2,1-b]furan) which has previously been identified as an HNF4α ligand [3]. Even though alverine citrate (*N*-ethyl-3,3′-diphenyldipropylamine) and fatty acids including myristic acid (tetradecanoic acid) have been identified as activators of this nuclear receptor before, these compounds did not modulate HNF4α activity in renal carcinoma cells.
**Conclusions**: Taken together, our findings confirm that HNF4α is downregulated in renal cell carcinoma and that reexpression of this factor in renal carcinoma cells diminishes cellular proliferation *in vitro* supporting its regulatory role in tumor progression. Future studies are warranted to specify the mechanism responsible for diminished proliferation of renal carcinoma cells in presence of HNF4α.

**Keywords**: Hepatocyte nuclear factor 4 alpha, HNF4α, renal cell carcinoma, kidney, proliferation.

**References:**
[1] Lazarevich NL et al. Hepatology 2004; 39: 1038-1047.
[2] Ning BF et al. Cancer Res 2010; 70: 7640-7651.
[3] Le Guével R et al. Bioorg Med Chem 2009; 17: 7021-30.

**P-46**

## Species Specific Differences in Structure and Function of the Organic Anion Transporting Polypeptide 2B1

**J. Hussner, S. Nageleisen, A. Foletti, I. Seibert, H. E. Meyer zu Schwabedissen**

*University of Basel, Department of Pharmaceutical Sciences, Biopharmacy, 4056, Basel, Switzerland*

**Introduction:** Membrane transporters are determinants of transcellular transport of endogenous and exogenous compounds, thereby influencing physiology and drug response. One family of transporters that have been studied for their implication in pharmacokinetics is the family of organic anion transporting polypeptides (OATPs), which also includes the member OATP2B1. This transporter is known to govern the uptake of compounds like estrone 3-sulfate and statins. It is widely accepted that OATP2B1 is expressed in the small intestine and the liver, thus contributing to absorption and elimination of its substrates. In order to determine the role of drug transporters in pharmacokinetics, *in vivo* animal models are often employed. However, little is known about the species differences of human and rodent OATP2B1.
**Aims:** The aim of this project was to compare expression and function of the human and rodent orthologue of OATP2B1. Therefore, we established a Madin-Darby Canine Kidney (MDCK) cell line overexpressing rat Oatp2b1 (MDCK-Oatp2b1) and compared the characteristics of these cells with those of a previously reported cell line expressing the human orthologue (MDCK-OATP2B1) [1].
**Methods:** The cell line MDCK-Oatp2b1 was generated by stable transfection of MDCKII cells with a eukaryotic expression plasmid. Presence of Oatp2b1 protein was determined by Western blot analysis and immunofluorescence. Transport activity was quantified in uptake studies using radiolabeled estrone 3-sulfate ($E_1S$), a known substrate of human OATP2B1. Due to the fact that the human transporter has been described to exhibit two binding sites for $E_1S$ [2], we tested whether the rat transporter shows similar characteristics. Therefore, the accumulation of 0.005 or 50 µM $E_1S$ was determined in presence of binding site specific inhibitors/substrates previously reported for the human orthologue including testosterone, bromosulfophthalein, and atorvastatin. In addition to functional experiments, the expression of Oatp2b1 was assessed in various tissues by quantitative real-time PCR.
**Results:** Even though Western blot analysis showed a lower protein expression of Oatp2b1 compared to the cell line transfected with the sequence encoding for the human transporter, immunofluorescent staining revealed basolateral membrane localization for both proteins. The difference in protein expression level appeared to be associated with the significantly lower $V_{max}$ for $E_1S$ in MDCK-Oatp2b1 (86.04 ± 10.67 fmol/min/µg protein) when compared to MDCK-OATP2B1 cells (220.80 ± 29.05 fmol/min/µg protein). However, there was a comparable affinity for $E_1S$ for both transporters (MDCK-Oatp2b1 $K_m$ = 35.75 ± 11.59 µM; MDCK-OATP2B1 $K_m$ = 36.76 ± 12.51 µM). Uptake studies using high and low concentrations of $E_1S$ indicated that Oatp2b1 does not exhibit the two binding sites (low affinity and high affinity) for $E_1S$. Although we observed uptake in presence of high concentrations of $E_1S$, there was no inhibition using testosterone that is known to inhibit the low affinity site for $E_1S$ in the human transporter. Quantitative real-time PCR revealed a strong expression of Oatp2b1 in rat liver and a moderate expression in the intestine.
**Conclusions:** Our data showed species differences in the activity of the drug transporter. It is the aim of future studies to provide further understanding of this difference by identification of the structural basis of the multiple binding sites.

**Keywords:** OATP2B1/Oatp2b1, estrone-3 sulfate, multiple binding sites.

**References:**
[1] Grube M et al. Mol Pharmacol 2006; 70: 1735-1741.
[2] Shirasaka Y et al. Drug Metab Pharmacokinet 2012; 27: 360-364.

**P-47**

## *De novo* Metabolite Production Through Co-Cultivation of Different Fungal Species on Solid Media

**A. Azzollini[1], J.-L. Wolfender[1], K. Gindro[2]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] Agroscope, Institute for Plant Production Sciences IPS, Mycology and Biotechnology, 1260 Nyon, Switzerland*

**Introduction:** In the field of natural products research, finding sources of novel bioactive compounds is of primary importance. In this respect, microorganisms have provided a large number of biologically active molecules, but due to continuous re-isolation of known secondary metabolites this source is losing attractiveness. To discover original microbial natural products, new strategies that switch on silent genes appear as an interesting alternative to yield more structurally diverse secondary metabolites.
**Aims:** Recently the use of fungal co-cultures for the induction of new bioactive compounds has emerged as a promising field in drug discovery [1]. In this study, a standardized co-cultivation methodology compatible with high throughput analytical procedures [2] was applied to reveal the induction of original bioactive fungal secondary metabolites.
**Methods:** In this work, a method based on the use of 12-well-plate miniaturized Petri dishes (2 cm diameter) and compatible with UHPLC-HRMS metabolomics has been applied to screen for metabolite induction in co-cultures of 4 fungal species (*Epicoccum* sp., *Eutypa* sp., *Fusarium* sp., *Aspergillus* sp.) grown on solid media [3]. A miniaturized multi-well procedure was used as it allows growing and analyzing with high-throughput a large number of samples (single- and co-cultures) necessary for statistical studies. PCA (Principal Component Analysis) was performed in order to explore the data through an unsupervised approach and reveal metabolome variation among single culture and co-cultures.
**Results:** The results of the screening showed that these fungal species produced new compounds when co-cultivated with another fungus. For example, *de novo* induced metabolites (detected in the co-culture but not in the single cultures) were revealed in the interaction of *Fusarium* sp. vs *Aspergillus* sp. as well as in the interaction of *Eutypa* sp. vs *Epicoccum* sp. and some fungal secondary metabolites, like O-methylmellein, were dereplicated.
**Conclusions:** This miniaturized strategy provided a satisfactory reproducibility, moreover it is generic and can be applied to other types of microorganisms that can grow on solid media such as those that are part of the microbiome. This study demonstrates the consistent induction of new metabolites through fungal co-cultures.

**Keywords:** Fungal co-culture, *de novo* induction, high-throughput strategy, MS-based metabolomics.

**References:**

[4] Bertrand S et al. Biotechnol Adv 2014; 32: 1180–1204.
[5] Glauser G et al. J Agric Food Chem 2009; 57: 1127–1134.
[6] Bertrand S et al. Mol BioSyst 2014; 10: 2289–2298.

**P-48**

## Low-Cost Analytical Device for Detecting Counterfeits and Sub-Standards in Emerging Countries

**J. Schappler[1], E. A. Diop[1], E. Reginato[1], C. Rohrbasser[2], P. Bonnabry[1], S. Rudaz[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1205 Geneva , Switzerland*
*[2] Pharmelp (http://pharmelp.ch/), Fribourg, Switzerland*

**Introduction:** The fight against counterfeit medicines is complex and different levels of action are necessary. Among them, the quality control (QC) of imported batches can be achieved, although this strategy is often difficult to apply in developing countries due to (i) the lack of suitable analytical equipment, (ii) the high cost of analytical instruments, maintenance and consumables, and (iii) the low availability of reference substances and consumables.
**Aims:** A strategy based on multiple injections was validated for the simultaneous identification and quantitation of active principles and was successfully applied to drugs selected from the WHO list of essential medicines.
**Methods:** In this context, the use of capillary electrophoresis (CE) appears of utmost interest since (i) the separation is achieved in a capillary of reduced dimension (total volume of 1 µL) filled with an aqueous buffered solution, (ii) no organic solvent is needed, and (iii) injection volumes are in the nanoliter range, which enables simple, reliable, and cost-effective drug QC [1]. The University of Geneva collaborated with the University of Applied Sciences of Fribourg and the Geneva Pharmacy Hospitals to build a low-cost CE device and help transitional countries to fight against counterfeit medicines. The instrument was successfully implemented in emerging countries, including Mali, Cambodia, Senegal, Democratic Republic of Congo, and more recently Rwanda and Madagascar. In order to analyze a high number of compounds and benefit from the device with basic chemistry knowledge, simple and generic methods were developed.
**Results:** This last approach was illustrated by the application on two important active principles (AP), namely metronidazole and amoxicillin. After method validation, this strategy was used for the quality control as well as for the detection of counterfeits in samples coming from Tanzania, thanks to collaboration with *Pharmaciens sans Frontières* (PSF) [2].
**Conclusions**: This analytical tool and method validation with quantitative analytical robustness and accuracy is a reliable help for the QC of most medicines available in developing countries.

**Keywords:** Capillary electrophoresis, low cost analytical chemistry, counterfeit medicines, Pharmelp.

**References:**
[1] Westenberger BJ et al. Int J Pharm 2005; 306: 56-70.
[2] Schappler J et al. Spectra Analyse 2014; 298: 63-73

**P-49**

## Large Scale Preparative Isolation of Dimeric Flavonoids from *Arrabidea brachypoda* Root Extract

**C. Salgado[1], N. C. Aquino[2], C. Q. da Rocha[3], W. Vilegas[3], E. Ferreira Queiroz[1], J.-L. Wolfender[1], O. Jordan[1], E. Allémann[1]**

*[1] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] Department of Organic and Inorganic Chemistry, Federal University of Ceará, CEP 60020-18, Fortaleza CE, Brazil*
*[3] Department of Organic Chemistry, Chemistry Institute, Paulista State University "Júlio de Mesquita Filho", CEP 14800-060, Araraquara SP, Brazil*

**Introduction:** *Arrabidea brachypoda* is a plant used in traditional Brazilian medicine. Indigenous to the Brazilian Cerrado, its roots are commonly used for the treatment of kidney stones, arthritis and pain in general [1]. Recently, from the aqueous ethanol root extract of *Arrabidea brachypoda*, three unusual dimeric flavonoids (Fig. 1: **1**-**3**) have been isolated and characterized. These compounds have exhibited significant *in vitro* and *in vivo* activity against *Trypanosoma cruzi*, the parasite responsible for Chagas disease [2]. In light of the evidenced array of different activities, it would be of interest to evaluate these compounds' pharmacological role in the treatment of diseases considered chronic, those involving pain and inflammation as major symptoms. In order to do so, further exploring and developing complementary *in vitro* and *in vivo* studies are compulsory. Thus, so as to isolate a sufficient amount of the active compounds, preparative high speed counter current chromatography (HSCCC) coupled to UV detection has been investigated.
**Aims:** The aim of this work was to effectively isolate in a large scale know dimeric flavonoids of *Arrabidea brachypoda* from an aqueous ethanolic root extract.
**Methods:** The conditions of the HSCCC-UV separation were determined by HPLC-UV analysis. The better coefficient of partition (*Kp*) of the targeted compounds was obtained using a mixture of MeOH:EtOAc:Hexane:$H_2O$ (6:5:6:5 v/v). The separation was performed using a small coil of 260 mL where 5mg/mL of sample were injected at a 5mL/min flow rate and 800 rpm.
**Results:** Grams of high pure dimeric flavonoids (**2** and **3**) have been obtained in one step from the crude aqueous ethanol extract.

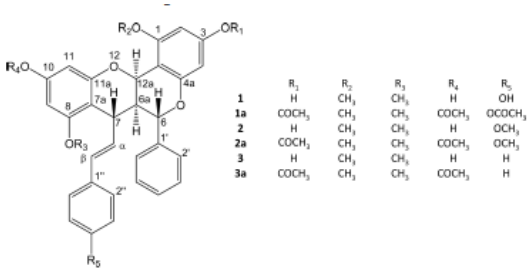



**Fig. 1**: General structure of the dimeric flavonoids found in *Arrabidea brachypoda* root extract (adapted from ref. [2])
**Conclusions:** It has been demonstrated that HSCCC-UV can be considered as an effective technique to isolate dimeric flavonoids from *Arrabidea brachypoda* root extract in a large scale setup. When compared to classical preparative chromatography, this method has the absence of solid stationary phase as advantage. Moreover, by applying this technology and given the fact that the separation is accomplished by using a mix of immiscible solvents, the main limitations related to the column over loading and back pressure are overcome. The fact that HSCCC-UV allows for a one step large scale isolation of these flavonoids from the crude extract, represents a key factor in further exploring the potential pharmacological roles of the *Arrabidea brachypoda* roots.
**Keywords:** *Arrabidea brachypoda*, dimeric flavonoids, high speed counter current chromatography.
**References:**
[1] da Rocha CQ et al. J Ethnopharm 2011; 133: 396-401.
[2] da Rocha CQ et al. J Nat Prod 2014; 77: 1345-50.

**P-50**

## Blood Glucose Levels Prior to New-Onset Diabetes Mellitus Manifestation and the Risk of Experiencing Pancreatic Cancer: A Case-Control Study

**A. M. Müller[1,2], C. Schneider[1,2], S. S. Jick[3], C. R. Meier[1,2,3]**

*[1] Basel Pharmacoepidemiology Unit, Division of Clinical Pharmacy and Epidemiology, Department of Pharmaceutical Sciences, University of Basel, 4031 Basel, Switzerland*
*[2] Hospital Pharmacy, University Hospital Basel, 4031 Basel, Switzerland*
*[3] Boston Collaborative Drug Surveillance Program, Boston University School of Public Health, Lexington, MA, United States*

**Introduction:** New-onset diabetes mellitus represents one of the few clinical symptoms that pancreatic cancer patients may show as early as 2 years prior to the cancer diagnosis. Distinctive criteria are required to differentiate these symptomatic pancreatic cancer patients from the majority of non-cancer patients with incident diabetes mellitus type II.
**Aims:** To compare the blood glucose pattern preceding the manifestation of new-onset diabetes mellitus between pancreatic cancer and non-cancer patients.
**Methods:** We conducted a matched (1:10) case-control study using data from the UK-based 'Clinical Practice Research Datalink'. Cases, aged 30 to 89 years, had a first-time diagnosis of pancreatic cancer and an incident diabetes mellitus within 2 years prior to the cancer diagnosis (defined as new-onset period). Controls were matched on various variables including age, sex, and diabetes duration. We categorized blood glucose levels according to 5-time intervals prior to the new-onset period (1, 2, 3, 4 years, and > 4 years), each divided up into quartiles. Applying conditional logistic regression, we calculated odds ratios for the association between blood glucose levels and the risk of pancreatic cancer.
**Results:** We identified 613 cases and 5774 controls. Within one year before the new-onset period blood glucose levels > 6.2 mmol/L (highest quartile) were associated with a 2.6-fold reduced pancreatic cancer risk [odds ratio 0.39, 95% confidence interval (CI) 0.26-0.58] compared with blood glucose levels ≤ 5.1 mmol/L (lowest quartile). Corresponding odds ratios for the time intervals 2 and 3 years were 0.32 (95% CI 0.21-0.49) and 0.43 (95% CI 0.27-0.70), respectively. Overall, pancreatic cancer risk remained reduced for increasing blood glucose levels across all time intervals.
**Conclusions:** Consistent with previous analyses based on median blood glucose levels, pre-diabetic conditions appeared to be less frequent in pancreatic cancer patients than in non-cancer patients with new-onset diabetes mellitus. Blood glucose levels preceding new-onset diabetes mellitus could additionally be considered to identify symptomatic pancreatic cancer patients within the population of new-onset diabetics.

**Keywords:** Pancreatic cancer, diabetes, new-onset, blood glucose.

**P-51**

## Solid Dispersions by Hot-Melt Extrusion: A Mathematical Model to Link Between Process Parameters and Product Properties

**A. Schittny, J. Huwyler, M. Puchkov**

*Divison of Pharmaceutical Technology, University of Basel, 4056 Basel, Switzerland*

**Introduction:** Oral drug delivery in form of amorphous solid dispersion (ASD) can increase bioavailability of low-soluble active pharmaceutical ingredients (API). Hot-melt extrusion has become a recognized, solvent free, and continuous method to produce ASD of API in polymers. The mean residence time is the characteristic time that materials spend in the hot-melt extruder and is a commonly used process property in process engineering. We propose that control of the mean residence time through modeling, in combination with a model for reaction time to form ASD, will allow for a rational process design in hot-melt extrusion.
**Aims:** The present study aims to create and validate a mathematical model to predict the mean residence time of an API-polymer formulation during hot-melt extrusion process. A second study is planned to model and validate the reaction time needed to form an ASD. The overall aim is to create a mechanistic model of hot-melt extrusion for rational process and formulation design.
**Methods:** The mean residence time model was computed in Wolfram Mathematica for a co-rotating twin screw extruder. It is mainly based on the following properties: The linear correlation between the mean residence time multiplied by a screw speed and a screw speed divided by a throughput [1], the geometry studies of fully wiped twin screw extruders [2], and the pressure decay characteristics along the screws [3]. The validation of the model is ongoing. Mean residence times were measured by tracer pulse experiments.
**Results:** First validation experiments showed promising results for some parts of the model. However, a detailed validation of individual parts of the proposed model will be necessary to identify the model validity range.
**Conclusions:** Hot-melt extrusion is a very promising method to increase bioavailability. A mechanistic model for rational process design has been established and has been partially validated. Further steps include improvement and refinement of the model.

**Keywords**: Hot-melt extrusion, amorphous solid dispersion, process modeling, mean residence time.

**References:**

[1] Gasner GE, Bigio D, Marks C. Polym Eng Sci 1999; 39: 286-298.
[2] Booy ML. Poym Eng Sci 1978; 18: 973-984.
[3] Potente H, Ansahl J, Wittemeier R. Int Polym Proc 1990; 5: 208-216.

**P-52**

## Targeted Drug Delivery System Triggered by MMP-9 for Treatment of Glioblastoma Multiforme

**D. Ehrsam, F. Porta, J. Huwyler, H. E. Meyer zu Schwabedissen**

*Biopharmacy, Department Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*

**Introduction**: Glioblastoma multiforme is one of the most aggressive malignant brain tumors, which is associated with a poor prognosis. Typically, patients have a life expectancy of around 15 months after diagnosis due to limited response and toxicity of the currently available treatment regimens. An extensive analysis of renowned databases showed a significant difference in matrix-metalloproteinase 9 (MMP-9) expression between cancerous and non-cancerous cells. MMP9 is an enzyme responsible for collagen degradation in connection with cell migration and proliferation. Thus, MMP-9 being a marker of enhanced malignancy potential of a tumor could be used for prodrug activation.
**Aims:** It is the aim of this study to confirm overexpression and increased activity of MMP-9 in cancer cells and investigate its possible application as a trigger for nanoparticles.
**Methods: D**atabase searches (GEO-database, Human Protein Atlas), allow to get a broader picture of MMP-9 expression and its activity. The data gathered from these databases is statistically analyzed. Furthermore, to identify cancer cells with high MMP-9 levels. different cell lines were characterized by zymography, Western blot, ELISA and real time PCR. Additionally tissue samples of different tumor types were stained immunohistochemically.
**Results:** Statistical analysis of database information suggests that in particular advanced tumors, like glioblastoma grade IV, exhibit high levels of MMP-9. This is supported by data received from real time PCR.
**Conclusions:** First results confirm the possibility to use MMP-9 as a trigger in prodrug activation.

**Keywords:** Matrix-metalloproteinase 9, MMP-9, glioblastoma multiforme, prodrug activation.

**P-53**

## HPLC-Based Activity Profiling for $GABA_A$ Receptor Modulators from *Searsia pyroides* Leaves Using a Validated Zebrafish Larvae Locomotor Assay

**F. Moradi-Afrapoli[1], S. N. Ebrahimi[1], H. van der Merwe[2], M. Smiesko[3], M. Hamburger[1]**

[1] *Division of Pharmaceutical Biology, University of Basel, 4056 Basel, Switzerland*
[2] *Department of Chemistry, University of the Free State, Bloemfontein, South Africa*
[3] *Division of Molecular Modeling, University of Basel, 4056 Basel, Switzerland*

**Introduction:** Gamma-aminobutyric acid type A ($GABA_A$) receptors are the key inhibitory neurotransmitter receptors in the central nervous system (CNS). They are target for numerous clinically important drugs used to treat anxiety, insomnia and epilepsy. We previously identified a series of allosteric $GABA_A$ receptor agonists with the aid of HPLC-based activity profiling, whereby activity was tracked with an electrophysiological assay in *Xenopus* oocytes.
**Aims:** To accelerate the discovery process, we developed and validated an in-house approach with zebrafish larvae. The validated protocol was then applied for HPLC-based activity profiling of traditional South African medicinal plants which were selected based on the *in vitro* screening data from *Xenopus* oocyte model.
**Methods:** Zebrafish larvae were incubated with the test samples in a 96-well format. Larval convulsions were provoked by the pro-convulsant $GABA_A$ receptor antagonist penthylenetetrazol (PTZ). $GABA_A$ receptor agonistic extracts and compounds were identified through a decrease in larvae locomotor activity. A dichloromethane (DCM) extract from *Searsia pyroides* which showed active in the bioassay was submitted to HPLC-based activity profiling. The extract was separated with analytical HPLC, and 3-min micro-fractions were collected. The micro-fractions were tested in the bioassay. The active time-window was localized in the corresponding HPLC chromatogram. Active constituents were purified in preparative scale and were identified by HRMS and microprobe 1D/2D NMR data.
**Results:** The zebrafish larvae locomotor activity model was validated for discovery of $GABA_A$ receptor modulators, and successfully translated into HPLC-based activity profiling. The protocol was applied for activity assessment of a selection of South African medicinal plants which were previously shown to be active in the *Xenopus* oocyte model. A dichloromethane extract from *S. pyroides* showed significant activity in both oocyte and zebrafish larvae models. HPLC-based activity profiling of the extract revealed lowering of larval locomotor activity by fraction 6 (Fig. 1, left). Compounds **1** and **2** (Fig. 1, right) were identified as the active constituents of the extract.

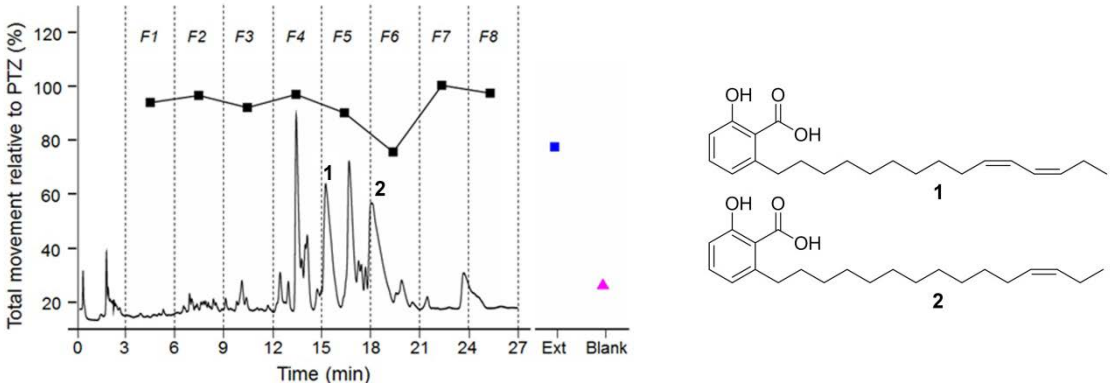



**Fig. 1.** HPLC-based activity profiling of a DCM extract from *Searsia pyroides* leaves.

**Conclusions:** A systematic validation of the zebrafish larvae locomotor activity model was done for discovery of GABAergic natural products. Bioactive constituents of *S. pyroides* were identified with the aid of a validated protocol. Anacardic acid derivatives are reported here for the first time as positive $GABA_A$ receptor modulators.

**Keywords:** $GABA_A$ receptor, zebrafish larvae, penthylenetetrazole, HPLC-based activity profiling, *Searsia pyroides.*

**P-54**

## Synthetic Exendin-4 Analogues for Imaging Pancreatic Beta Cells

**K. Janikowska[1], A. Babič[1], V. Chellakudam[1], L. Vinet[2]; S. Lamprianou[2], E. Allèmann[1], P. Meda[2], N. Lange[1]**

*[1] School of Pharmaceutical sciences, Pharmaceutical Technology, University of Geneva - University of Lausanne, 1205 Geneva, Switzerland*
*[2] University of Geneva, Department of Cellular Physiology and Metabolism, 1205 Geneva, Switzerland*

**Introduction:** Diabetes is a major public health problem that is approaching epidemic proportions worldwide. One of the main reasons in diabetes pathogenesis is the death and dysfunction of pancreatic beta cells. Therefore, the development of a highly specific and non-invasive imaging method for the visualization of pancreatic beta cells is needed. Glucagon-like peptide-1 receptor (GLP1-R) is highly expressed on the surface of beta cells and was selected as target. Exendin-4 (Ex-4) is a 39-amino-acid peptide with 50% amino acid homology to GLP1 peptide, the natural ligand of GLP1-R.
**Aims:** The objective of our study was to improve binding to GLP1-R through tethering multiple copies of Ex-4 to a peptidic scaffold. We hypothesized that multimerization increases avidity and circumvents the unwanted accumulation of imaging probes in the kidneys. The cyclopeptidic delivery vehicles allow preparing multimodal imaging agents and modulation of pharmacokinetic properties through side-specific PEGylation. Here we report the synthesis, characterization, and *in vitro and vivo* imaging properties of near-infrared fluorescent Exendin-4 (Ex-4) analogues.
**Methods:** A series of Ex-4 analogues has been synthesized. Up to 4 Ex-4C$^{40}$ peptides were attached to the cyclopeptidic scaffold via PEG linkers with different chain lengths (0.4 to 10 kDa). For fluorescence imaging Cy5 was coupled to the bottom of the RAFT. Cell imaging experiments were performed to visualize internalization of the probes. Assays were carried out in CHL/hGLP1-R cells that ectopically express hGLP1-R and were compared to the GLP1-R negative native CHL cell line to determine the binding affinity ($IC_{50}$) and the specificity to the GLP1-R of synthesized compounds. Selected compounds were tested *in vivo* on non-glycemic mice. Four nmol of Ex-4 analogues were injected intravenously. Mice were sacrificed after 6 h and blood, pancreas, kidneys, and liver were harvested. Pharmacokinetic (PK) studies of Ex-4 analogues in mice plasma were performed by the direct fluorescence measurements.
**Results:** All compounds showed high binding affinities in nanomolar range to CHL/hGLP1-R cells. No binding was observed in GLP1-R negative CHL cell line or other human control cell lines (HeLa, Panc-1). Competition binding assays confirmed the specificity of synthesized compounds to GLP1-R. *In vivo* studies showed highly specific uptake in the pancreatic beta cells. Furthermore, decreased uptake in the kidney compared to the free Ex-4 was shown. Our PK studies show complete elimination of the compounds from the plasma after 24 h post injection.
**Conclusions:** The synthesis, the characterization, and the *in vitro* and *in vivo* properties of new imaging probes, based on Ex-4 analogues, were reported. Multimerized compounds were labeled with near infrared dye for optical fluorescence imaging. In the future the molecule will be made suitable for *in situ* non-invasive imaging methods, such as MRI, PET or SPECT. The final goal of the project will be the creation of probes allowing bimodal imaging.

**Keywords:** Optical imaging, diabetes mellitus, beta cells, Exendin-4, GLP1-R.

**P-55**

## PDZK1 Is A Target Gene of the Thyroid Hormone Triiodothyronine

**C. Ferreira, K. Prestin, J. Hussner, I. Seibert, H. E. Meyer zu Schwabedissen**

*Biopharmacy, Department Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*

**Introduction:** Genome wide association studies (GWAS) revealed single nucleotide polymorphisms (SNP) within the 5` untranslated region (5`UTR) of the scaffolding protein PDZK1 to be associated with serum uric acid levels (rs1967017, rs1471633, and rs12129861). PDZK1 was introduced as post-translational modulator of membrane proteins including drug and uric acid transporters. Changes in protein activity due to genetic variations or transcriptional regulation have repeatedly been reported to translate into interindividual differences in pharmacokinetics.
**Aims:** The aim of the herein reported study was to further analyze the 5`UTR of PDZK1 by including the genetic variants previously reported to influence renal physiology in our considerations.
**Methods:** Expression of PDZK1 in cells and tissue samples was analyzed by quantitative real-time PCR and Western blot analysis. Cell based reporter gene assays were performed transfecting HepG2 cells with different variants of the 5` UTR of PDZK1 subcloned into the pGl3-vector. Potential binding sites were calculated *in silico* using the open access software NUBIScan. Chromatin immunoprecipitation (ChIP) was performed to verify binding of the nuclear receptor thyroid hormone receptor β (THRβ, NR1A2).
**Results:** Analyzing human kidney tissue, we observed a trend for lower mRNA expression of PDZK1 in samples being homozygous for the minor alleles of the SNPs located in the promoter of PDZK1. A screening with different nuclear receptors NR1A2, NR1I2, and NR1H4 demonstrated that the promoter of PDZK1 is a target of THRβ. The most likely binding site for THRβ was an *in silico* identified DR1-motif that includes the polymorphism rs1967017. The nucleotide exchange influenced transactivation of the scaffolding protein. Treatment of Caco2 cells with the THRβ ligand triiodothyronine ($T_3$) increased the mRNA and protein expression of PDZK1.
**Conclusions:** Our study revealed regulation of PDZK1 by thyroid hormones through activation of the nuclear receptor THRβ. Furthermore, our data provide a mechanistic basis for the association of the polymorphisms in the 5´-UTR of PDZK1, in particular the rs1967017 which is associated with uric acid levels in individuals as previously shown in GWAS. Thus, our data link thyroid hormone status with serum uric acid levels.

**Keywords:** PDZK1, thyroid hormone, regulation, scaffolding protein, nuclear receptor.

**P-56**

## In-Hospital Quality Management Project Reduces Incidence of a Common Adverse Event, Dyspnea, of the MRI Contrast Agent Gadoxetate

**S. Matoori[1], J. M. Fröhlich[1], D.-M. Koh[2], A. Gutzeit[1-4]**

*[1] Clinical Research Group, Hirslandenklinik St. Anna, 6006 Lucerne, Switzerland*
*[2] Department of Radiology, Royal Marsden Hospital, London, SW36JJ, UK*
*[3] Department of Radiology, Kantonsspital Winterthur, 8401 Winterthur, Switzerland*
*[4] Department of Radiology, Paracelsus Medical University, 5020 Salzburg, Austria*

**Introduction:** Gadoxetate-enhanced $T_1$-weighted MRI in the arterial phase is a widely used technique in the detection and assessment of focal liver lesions (e.g**.,** hepatocellular carcinoma). Dyspnea was reported to be an adverse reaction of the magnetic resonance imaging (MRI) contrast agent gadoxetate (Primovist®) [1]. Breathing artifacts can severely deteriorate the image quality of arterial phase gadoxetate-enhanced liver MRI, and may compromise the diagnostic value of the examination [1].
**Aims:** An in-hospital quality management project pointed to the usefulness of an improved breathing command which can be applied by the technicians during the scan. The aim of the study was to investigate whether the implementation of a modified breathing command by the technicians during gadoxetate-enhanced liver MRI reduces respiratory motion artefacts compared to the traditional breathing command.
**Methods:** The arterial-phase gadoxetate-enhanced liver MR images of 30 patients acquired following the traditional breathing command scheme and the 30 patients after training the technicians to implement a modified breathing command were included. A subgroup of 8 patients underwent scans with both breathing commands. Images obtained using the traditional and modified breathing command were rated based on the respiratory artefact-based image quality scores from 1 (optimal) to 5 (non-diagnostic) and compared.
**Results:** A highly significant improvement in the arterial phase image quality scores in patients following the modified breathing command were observed compared with the traditional command ($P < 0.001$). The percentage of patients with severe and extensive breathing artefacts in the arterial phase decreased from 33.3% to 6.7% after introducing the modified breathing command ($P = 0.021$). In the subgroup that underwent MRI following both breathing commands, arterial phase image quality improved significantly ($P = 0.008$) when the modified breathing command was applied.
**Conclusions:** In the context of our departmental quality management system, we introduced a modified breathing command scheme to reduce the incidence of dyspnea in arterial phase gadoxetate-enhanced liver MRI. This quality management project significantly improved the arterial phase $T_1$-weighted MR image quality scores and reduced severe and extensive respiratory motion artefacts on gadoxetate-enhanced liver MRI.

**Keywords:** Gadoxetate, MRI, dyspnea, liver, contrast agent.

**Reference:**

[1] Davenport MS et al. Radiology 2013; 266:452-461.

**P-57**

## Discovery of New C20 Polyunsaturated Fatty Acids and Endocannabinoid-Like Molecules in *Caenorhabditis elegans*

**S. Guha, S. Calarco, S. G. Otanez, J. Gertsch**

*Institute of Biochemistry and Molecular Medicine, University of Bern, 3012 Bern, Switzerland*

**Introduction:** *Caenorhabditis elegans* has emerged as a powerful model organism to study and dissect the components involved in lipid synthesis and metabolism. Many ω-3 and ω-6 polyunsaturated fatty acids (PUFA) have been identified to date and implicated to have key roles in membrane development and diverse signaling pathways not only in *C. elegans* but also in higher organisms. Many mutants and transgenics have been generated to study and understand fatty acid (FA) synthesis and metabolism in *C. elegans.* In this study we use a combination of genetics, biochemistry and analytics to identify, analyze and determine the roles of newly identified and known FAs and their metabolites.
**Aims:** Carrying out genetic manipulations in simple model organisms like *C. elegans* to understand synthesis and fate of FAs and their metabolites.
**Methods:** The *C. elegans* wild type N2 and *fat-3* mutant which lacks Δ-6 desaturase activity and fails to produce many C20 PUFAs were used to detect and identify FA profiles using LC-MS/MS. New molecules derived from novel FAs were synthesized and their roles were determined. A systematic RNAi knockdown of the genes responsible for synthesis of FAs and their metabolites was carried out in combination with analytical methods.
**Results:** In this study we report the presence of a new ω-3 PUFA (juniperonic acid, JuA) in the *C. elegans fat-3* mutant. Interestingly, JuA is a known fatty acid in Gymnosperms in the plant kingdom. Here, we describe the biosynthetic pathway that leads to its generation in *C. elegans*. Additionally, we report new endocannabinoid-like molecules derived from ω-3 arachidonic acid (AA-ω3) and JuA. We attempt to delineate the biological roles of these newly identified compounds and they will be studied in mammalian systems as to date nothing is known.
**Conclusions:** This study provides insights into how genetic manipulations can lead to altered metabolic states and how rare lipids of plant origin are generated in Animalia.

**Keywords:** Juniperonic acid, ω-3 arachidonic acid, *C. elegans*, LC-MS/MS, Δ-6 desaturase.

**P-58**

## NF-κB Inhibition of Patulin Isolated from *Penicillium vulpinum* via an IKK Independent Mechanism

**A. Monteillier[1], P.-M. Allard[1], K. Gindro[2], J.-L. Wolfender[1], M. Cuendet[1]**

*[1] School of pharmaceutical sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland*
*[2] Mycology and Biotechnology group, Institute for Plant Production Sciences IPS, Agroscope, 1260 Nyon, Switzerland*

**Introduction:** Nuclear factor kappa B (NF-κB) is a transcription factor regulating several cellular processes such as inflammation, cell transformation, survival, proliferation, invasion, angiogenesis, and metastasis. Its overexpression in several types of cancers, either constitutive or induced by chemotherapy, inhibits apoptosis and leads to chemoresistance, making it a very interesting target for the control of cancer. Therefore, an important effort has been made to find potent inhibitors from both synthetic and natural sources, and some of them made it to the clinic [1]. Among all natural sources, fungi represent a highly interesting field to explore with only a few NF-κB inhibitors described to this day [2].
**Aims:** The aim of this work was to find naturally occurring NF-κB inhibitors by investigating the metabolites of *Penicillium vulpinum*, a fungus that grows naturally on dung, and to investigate their mechanism of action.
**Methods:** *P. vulpinum* was cultured in Potato Dextrose Broth (PDB) and was extracted with ethyl acetate. Bioassay-guided fractionation was performed thanks to an NF-κB activity assay using a HEK293 cell line transfected with an NF-κB-driven luciferase reporter gene. Mechanism of action of the active compound was investigated in the human lung adenocarcinoma cells A549 using immunocytochemistry and western blotting. Inhibition of cell proliferation was investigated with the sulforhodamine B colorimetric assay.
**Results:** Bioassay-guided fractionation of the ethyl acetate extract of *P. vulpinum* led to the isolation of patulin, which inhibited TNF-α induced NF-κB activation with an $IC_{50}$ of 0.25 µM. This is in the same range of activity than the control parthenolide ($IC_{50}$ = 0.47 µM). This inhibition was further confirmed in A549 cells where patulin aborted TNF-α induced p65 nuclear translocation, without involving IKK inhibition. Finally, patulin exhibited antiproliferative activity on A549 cells with an $IC_{50}$ of 1.4 µM.
**Conclusions:** Patulin is a well-known mycotoxin produced by *Penicillium* species and considered as a contaminant in apples and apple-derived products. Beside the numerous articles regarding the food safety concern it represents, recent work reported that patulin also possessed potent anticancer activity through apoptosis induction in several cancer cell lines and one *in vivo* model of melanoma cells bearing mice [3]. To the best of our knowledge, the present work represents the first report of the NF-κB inhibitory activity of patulin and provides insights on its mechanism of action. This activity could be partly responsible for the anticancer effect of patulin since NF-κB activation represents a way for cancer cells to escape apoptosis.

**Keywords:** Patulin, NF-κB, *Penicillium vulpinum.*

**References:**
[1] Ghantous A et al. Drug Discov Today 2013; 18: 894-905.
[2] Gilmore TD et al. Oncogene 2006; 25: 6887-6899.
[3] Boussabbeh M et al. Tumour Biol 2015; 5: 6285-6295.

**P-59**

## Prescription of Half Tablets and the Particular Case of Split Quetiapine in Northern Switzerland

**S. Allemann[1], D. Bornand[2], B. Hug[3], K. E. Hersberger[1], I. Arnet[1]**

*[1] Pharmaceutical Care Research Group, Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland*
*[2] Hospital Pharmacy, University Hospital Basel, 4031 Basel, Switzerland*
*[3] Internal Medicine, University Hospital Basel, 4031 Basel, Switzerland*

**Introduction:** Although useful for individualization of dose, splitting tablets poses risks, in particular when score lines are missing. Albeit without score line, the antipsychotic drug quetiapine was 2011 the most often prescribed split tablet in discharge prescriptions at the University Hospital in Basel with 671 beds.
**Aims:** We aimed at investigating prescription of split tablets in general and of quetiapine in particular.
**Methods**: We analysed orders of community pharmacies in Northern Switzerland for fragmented tablets from Medifilm AG, the leader company in Switzerland in the repackaging of medication into unit-of-use soft pouch blisters. Data were analysed using the statistical Software R.
**Results:** In 2012, out of 4'785'593 tablets packed in unit-of-use soft pouch blisters, 406'956 (8.5%) were fragments of tablets that had been ordered by 29 community pharmacies for 1'321 patients residing in 53 retirement homes. The patients were in average 81.5 ± 14.7 years old (median 86; range 7-105) and obtained in average 1.8 fragments (median 1; range 1-8) with 577 (44%) patients receiving ≥ 2 fragments of tablets. The fragments were predominantly halves (87.6%) and quarters (11.1%), and marginally thirds, two-thirds and three-quarters (1.3%). They concerned 132 different active substances, and 50% of them were psycholeptics or psychoanaleptics. The most often split tablets were preparations with pipamperone (15.8%), levodopa/decarboxylase inhibitors (10.2%), and quetiapine (6.5%). The highest proportion of split quetiapine tablets was ordered in Basel (10'273; 39%).
**Conclusions:** Prescription of fragmented tablets is a common practice in Northern Switzerland and half tablets represent a substantial part of the pharmacotherapy. Above-average off-label prescription of half quetiapine is a local specificity of the region of Basel. In ambulatory care, prescription of split tablets poses an additional challenge for patients' self-management with an unknown impact on adherence.

**Keywords:** Tablet splitting, scored tablets, score lines, subdivision of tablets, prescription error, quetiapine, pharmaceutical care.