Abstracts of Invited Speakers

L-01

What is Equivalence as it Applies to Drug Products

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A generic product that is Pharmaceutically Equivalent (PE) and Bioequivalent (BE) to the reference product is approved as a therapeutically equivalent (TE) and interchangeable product in US. For a product to be PE, it must have the same active ingredient in the same amount as the reference product in the same type of dosage form and should have comparative labeling as the brand name drug. This paradigm is difficult to apply to the biological and non-biological complex drugs. Because of this reason, a different, complicated and case-by-case approach is being applied (to determine PE and BE) for the approval of these complex dosage forms.

The equivalence approach and regulatory pathway currently used for approval of various types of dosage forms will be discussed. In the absence of complete characterization of the API, FDA requires nanomaterial containing drug products (including NBCDs) to be Q1 and Q2 with the reference listed drug and comparative in depth characterization of the dosage form to assure PE. BE requirements vary from product to product. For biological products extensive analytical characterization with animal studies are required followed by clinical and other PK/PD and immunological studies. Challenges involved in establishing equivalence between brand and generic dosage forms will be discussed.

Keywords: equivalence, pharmaceutical equivalence, bioequivalence, therapeutic equivalence, Non-Biological Complex Drugs (NBCD)

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L-02

Landscape of Complex Drugs

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The rise of bio- and nanotechnology has resulted in rapid development of complex drug products and their copy versions. One of the key questions for these products is how to grant market authorization, since their complexity provides challenges for current regulatory pathways.

It is difficult and sometimes impossible to fully characterize complex drugs, and minute variations in the manufacturing process can substantially change the composition of final products. Biologics exemplify one category of complex drugs. Another category is non-biological complex drugs (NBCDs). This class of products includes – but is not limited to – nanomedicines, such as liposomes and iron-carbohydrate complexes.

For biologics and their biosimilars, clear legislation and global guidance policies are in place to aid the development of high-quality products. For NBCDs, on the other hand, no specific legislation was written, and guidance documents for NBCDs and their copies differ both between products and across the globe. This complicates requests for approval and may result in different decisions taken for the same NBCD product.

One of the key questions remains how to assess equivalence or similarity of these complex products. Essential for regulatory guidance alignment, is the definition and understanding of the critical quality attributes (CQAs) —those product characteristics that essentially ensure similar product efficacy and safety in humans.

In this lecture, I will lay out the complex drug landscape, reflect upon outstanding challenges and discuss recent steps that have been taken by different stakeholders to provide science based frameworks for the evaluation of NBCDs and their similars.

Keywords: equivalence, non-biological complex drugs, guidances, similarity, critical quality attributes

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L-03

Scientific Challenges in Biosimilars and Non-Biological Complex Drug Similars

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Biotechnology and nanotechnology can improve efficiency and safety of drugs to treat health conditions not accessible so far. Less expensive copies of such drugs are needed to reduce health care costs and to give larger access. In contrast to small molecular drugs, biotech and nanotech products consist of large molecular moieties. Depending on production and origin, they are defined as biological or non-biological complex drugs (NBCDs). The non-homomolecular composition induces high structure complexity, limited stability and specific drug profiles. Nanomedicines (part of NBCDs), are engineered with a dimension in the nanoscale range

(1-100nm) or with dimension-related properties causing specific tissue/cell targeting or biodistribution. The assessment of the properties and the relevance for the profile - critical quality attributes (CQA) - is a scientific and regulatory challenge in these not fully characterized drugs and highly demanding when evaluating similars, not fully identical to a reference product aiming for therapeutic equivalence. The robustness and control of the synthetic and biotech manufacturing are crucial for the batch-to-batch consistency and the comparability of similars (CQA). These complex drugs are composed of a mixture of related high molecular structures eventually affecting nano size (range), modifications, structure, stability and immunogenicity. The evaluation of similars follows a stepwise and totality of evidence approach to prove sufficient pharmaceutical and bio-equivalence to a reference product indicating therapeutic comparability with interchange or switch between the products. Biosimilars have a world-wide accepted regulatory evaluation procedure, elaborated as a central procedure by EMA over more than 12 years starting upon authorization of the first biologics. Biosimilars in the EU show increasing market share, lower therapy costs and increase patient access. In contrast, the approval process for nanosimilars or nano-NBCD similars is not defined. Safety and efficacy issues occurred upon initial, national market authorization using the generic (sameness) approach, not valid for these not fully characterized complex drugs. For progress and to imply knowledgeable stakeholders, a platform for exchange and learning from the existing evidence is promising for an appropriate nanosimilar evaluation and authorization requested to answer the regulatory uncertainty in this growing innovation field.

Keywords: complex drugs, biosimilars, nanosimilars, non-biological complex drugs, regulatory, scientific challenges

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L-04

Challenges in Bringing Scientific Innovations into the Market

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Drug discovery and development is a very complicated, time consuming and expensive process in which a truly multidisciplinary approach including scientists, regulators, engineers and legal professionals, to name a few, can lead to success. The duration can be around 10-12 years in case of an original medicine, and the costs can raise up to 2 billion USD. While big pharma companies are prepared for the risks and obstacles of drug development, newer therapies and technologies, such as cell based products or 3D printed medicines, are often developed by unexperienced small companies or academic research groups. A report of the European Medicines Agency on innovative drug development approaches has highlighted the importance of improved communication between regulators and industry already in 2007. A close and intensive communication is even more necessary for success in case of innovative products where the regulation may not be fully established and agencies and companies should work together to define standards.

During the last decade there is more and more demand from various stakeholders of drug development for scientific and regulatory support by medicines agencies, which at the end can facilitate patients' access to good quality, safe and efficacious products as quickly as possible. This has led to the transition where authorities that previously acted as "gate keepers" are now in the role of "enablers". National competent authorities across the globe including FDA and the European Medicines Agency have various tools that aim to facilitate early access to medicines or support innovation. These include, for example, traditional scientific advice or the adaptive pathway, but also has elements that directs early developers, such as the EU Innovation Network, which tries to reach potential innovative products on a national level.

Apart from these tools the use of big data and real world evidence (RWE) will have a more prominent role in the future. Authorities are investigating ways for greater use of RWE and consult with various stakeholders to formalize standards and expected methods. Innovations like digital drugs, personalized treatments or the use of nanotechnology will have a great impact on our healthcare system in the future. For the benefit of patients, it is the regulator's task to follow emerging technologies and to provide the necessary support to facilitate the processes in which innovations can materialize.

Keywords: regulatory support tools, innovation, European Medicines Agency, early access, real world evidence

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L-05

Nanomedicines: Bioequivalence Decision Based on Biodistribution

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Since more than one decade the complexity of nanomedicines with regard to the relationships between their quality attributes and the biological activity, including target interaction, dose-response, biodistribution patterns, has been recognised and, for many of the existing products, has been the basis for their development. This complexity is often driven by the difficulties behind a thorough characterization and analytical measuring of nanoparticles and the drug-particle complexes, and the incomplete knowledge on how changes (even small) in attributes of the particles or drug-particle conjugates impacts on the bioactivity of the product, related to target access as well as target interaction. Of particular relevance for the activity of any nanoparticle-based medicine is the understanding on how some changes in the components attributes can impact on the pattern of tissue distribution, and the potential for changing the pattern of activity, efficacy and safety. These aspects have been considered by Regulators in Europe and abroad. Several guidance documents have been issued to help Companies and Researchers on developing innovative or follow on nanoparticle-based medicinal products. The thinking behind the produced documents and some practical examples will be discussed.

Keywords: nanomedicine, nanoparticle, liposome, bioequivalence, generics

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L-06

Nanomedicine Characterization: The Spectrum of Complexity

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Non-biological complex drugs (NBCD) are intricate formulations that constitute a range of physical and chemical properties, making characterization of these formulations challenging. The quality and performance of these complex drugs depends on their ingredients, but may also depend on the biophysical state of those ingredients once they interact with the body. It is not sufficient to characterize only the physicochemical and analytical properties of an NBCD, these properties must be linked with an in vivo biological response – to inform and define critical quality attributes (CQAs).

Having evaluated more than 400 different nanoformulations, the Nanotechnology Characterization Laboratory (NCL) has considerable insight into the nuances required for detailed physical, chemical, and biological characterization of NBCDs nanomedicines and in identifying CQAs for nanomedicine NBCDs. The NCL also looks at trends across nanoparticle platforms, parameters that are critical to nanoparticle biocompatibility, and develops assays for preclinical characterization of nanomedicines. The NCL has developed protocols that rigorously characterize nanoparticle physicochemical properties, as well as in vitro immunological and cytotoxic characteristics and PK and biodistribution profiles in animal models. These assays have undergone extensive in-house validation and are subjected to regular revision to ensure applicability to a variety of nanomaterials.

This talk will highlight the spectrum of characterization challenges for nanomedicines in NBCDs, and offer analytical and bioanalytical techniques that may prove helpful in establishing CQAs, and similarity of follow-on's.

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Keywords: nanomedicine, nanoparticle, critical quality attributes, characterization

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L-07

Nanomedicines in Clinical Practice, Critical Aspects to Consider

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Nanomedicines are important representatives of non-biological complex drugs (NBCDs). They are becoming increasingly available. Up to 23 nanomedicines are approved, and approximately 50 are in clinical development.

Iron sucrose similar have entered as first nanosimilars the European market by application of the generic approval pathway. Post launch significant clinical differences have been observed between the copies and the reference product. Many hospital pharmacists are unaware of the specific characteristics, the *in vivo* profile of nanomedicines and the potential clinical consequences. This lack of awareness causes an observed not appropriate substitution practice with products that are not therapeutically equivalent endangering safe and effective therapy. Furthermore, the lack of clear regulatory pathways and requirements is hindering the introduction of nanosimilars, which potentially could generate significant savings in healthcare spending.

Evaluation criteria for rational decision making for the inclusion of nanomedicines into the hospital formulary were discussed in a consensus round table with an international panel of experts and hospital pharmacists. Special emphasize was put on criteria for evaluation of substitutability and interchangeability. On top of previously published criteria for biosimilars, a set of seven criteria, that specifically apply to nanosimilars, were identified and incorporated into an evaluation tool. These include particle size and size distribution (1), particle surface characteristics (2), fraction of uncaptured bioactive moiety (3), stability on storage (4), bioactive moiety uptake (5) and distribution (6), and stability for ready-to-use preparations (7).

Such an evaluation tool can assist the responsible hospital pharmacist to have the necessary data and criteria applied for the nanomedicine evaluation.

Keywords: nanomedicines, healthcare cost, formulary evaluation, clinical practice, hospital pharmacy

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L-08

Complexity of Iron Carbohydrate Preparations and Their Evaluation

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The application of nanotechnology to the development of advanced therapeutics has brought about a number of so-called "nanomedicines". Such drugs, though showing high variability in terms of size, shape, materials used, etc. share complex structures, which cannot be characterized in their entirety. Being mostly of synthetic origin and their preparation often including a self-assembly step, such drugs are referred to as non-biological complex drugs (NBCDs) [1]. Examples include glatiramoids (Copaxone®) [2], liposomal formulations (Doxil®) [3], and nanoparticles such as iron-carbohydrate particles (Venofer®) [4, 5]. Their size and attributes at the molecular scale confer these systems certain properties that impact their interaction with their biological environment, and thus influence PK/PD and safety profiles.

In a recent statement [6], FDA commissioner Gottlieb called for an improved review process of generic drug applications (ANDAs). This must be viewed in relation to nanomedicines, as well, as several follow-on products have entered the market, including a glatiramer generic approved by FDA has now issued a guidance draft on products containing nanomaterials [7] as an answer to a request by the U.S. House of Representatives' Committee on Energy and Commerce in 2015 to the U.S. Government Accountability Office (GAO).

Even though the draft guidance is suggesting a list of properties of nanomedicines ("Nano-material Quality Attributes") to be tested, it lacks true guidance on individual (groups of) nano-medicines. It presumes levels of knowledge of product behavior that are typically not available, neither for NDA nor for generic, ANDA products.

Due to their inherent complex structure, nano-medicines are impossible to be fully characterized by physicochemical methods alone, *in vitro* as well as *in vivo* studies, leading up to verification in clinical trials, are required. Therefore, an effort is needed to discover these correlations between specific critical quality attributes (CQA) and their impact on biological activity, ideally to be able to predict in vivo behavior. This can only be realized through a multi-pronged analytical approach and correlation to clinical data.

This presentation will discuss our efforts to characterize iron carbohydrate products (originator and "nanosimilars") with the aim to contribute to the identification of relevant CQAs for this group of NBCDs.

Keywords: iron carbohydrate, non-biological complex drugs, critical quality attributes, nanomedicines

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L-09

Biosimilars, an Ever Evolving Landscape

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Biologics, or biotechnology derived medicinal products are revolutionising drug treatments for various life threatening diseases over the past decades. Currently, with patents expiring for some of the highest selling global drug products, biosimilars provide a huge opportunity to broaden patient access for such life altering medicines. Barriers to market entry of biosimilars have stayed high in comparison to small molecule generics, particularly with regards regulatory requirements, scope of development and required R&D and manufacturing infrastructure. Nevertheless, the past years have seen an explosion of biosimilar marketing authorisations and market launches around the world, with differing levels of resulting market access.

The presentation will aim to provide an overview of biosimilar development challenges, an overview of market opportunities and a perspective on the current biosimilar landscape.

Keywords: biosimilars, biopharmaceuticals, bioanalytics, regulatory approval, pharmacoeconomics

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L-10

Biosimilar Pathway: US Approach

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A **biosimilar product** is a biological product that is highly similar to the reference product not withstanding minor differences in clinically inactive components, and has no clinically meaningful differences in terms of safety, purity and potency (safety and effectiveness) from the reference product.

The goal of a biosimilar product is to demonstrate biosimilarity in safety and effectiveness between the Test (T) and the Reference (R) product. Analytical similarity data is the foundation of biosimilar product. Understanding the relationship between the quality attributes and the clinical safety and efficacy profile is important. FDA recommends a stepwise approach in developing evidence to support a demonstration of biosimilarity and includes analytical characterization, animal studies, clinical studies followed by PK/PD and immunogenicity studies as needed. FDA intends to use risk-based, totality-of-the-evidence approach to evaluate all available data and information.

Even though several biosimilar products are approved in US, only 4 have reached patients. To address this and other regulatory concerns including interchangeability requirements, FDA has announced Biosimilar Action Plan which seeks to achieve a balance between innovation and competition.

Keywords: biosimilar, regulatory pathway, totality of evidence, Biosimilar Action Plan

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L-11

Opportunities and Challenges in Developing Biosimilar Products

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Since the approval of the first biosimilar product in the European Union, biosimilar approvals intensified in the recent years, reaching 38 succesful registrations in the EU. Based on the experience in the Eurpean Union, biosimilar medicines have succesfully contributed to lowering the cost of biological therapies. According to analysts, the global biosimilar market could reach 25-35 billion USD by 2020. Certain countries established advanced local regulations to intensify biosimilar uptake, e.g. France has established the framework for automatic substitution of certain biosimilar products at the pharmacy level, if the product belongs to a predefined 'similar biologic group'.

The success of the biosimilar concept is substantiated by the above examples, however, from biosimilar development perspective, several old and new development and regulatory challenges has to be tackled yet. This paper will discuss these challenges, including the naming of biosimilar products, potential for using global reference products, waiwing of bridging studies etc. Authorities have to intensify international cooperation to ensure the most optimal use of resources by promoting mutual reliance, convergence of global standards for development and regulatory approvals. A new paradigm for the limited use of clinical studies in biosimilar development is also under consideration. Regulators are looking forward to innovative clinical trial designs to demonstrate biosimilarity and interchangeability, while reaserch experts call for more streamlined clinical studies in selected indications, especially in oncology.

Keywords: biosimilars, clinical development, reference products

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L-12

Biosimilar Pathway: EMA Approach – Pharmacist Perspectives

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At first, the early implementation of a biosimilar licensing regulation by the European Medicines Agency (EMA) should be emphasized again. Thereby a sound approval procedure of biosimilars was guaranteed. The competition of biosimilars should improve patient access to safe and effective **biological medicines** with proven quality. Of note, the development, approval and usage of biosimilars - especially monoclonal antibodies - is far more complex than the implementation of generics. This issue is also relevant in clinical practice and rules the evaluation and usage of biosimilars in hospital patients. In the hospital, formulary committees select the biosimilars to be added to the formulary and develop local guidelines for prescribing, substitution, switching and pharmacovigilance in an appropriate and pragmatic manner. Useful information can be found in the European Public Assessment Reports (EPAR) and various position statements of associations of healthcare professionals. According to the EMA-Guidelines the brand name and the international non-proprietary name are to be recorded when prescribing biosimilars. EMA representatives underline that the approval of a biosimilar does not mean interchangeability. Automatic substitution of biosimilars on the level of pharmacy is a national prerogative in the EU and not allowed in 9 of 28 member states. Of course, automatic substitution of bioidenticals is allowed. Switching from the reference product to a biosimilar or vice versa, switching between biosimilars, single switching and multiple switching are to be evaluated case by case for each type of product based on the available literature. Post-marketing pharmacovigilance is mandatory to determine the benefit–risk profile of biologics and biosimilars throughout their life cycle. Different post-marketing commitments for different biosimilars regarding the same reference product are to be regarded. Especially in the case of switching, patients should be monitored closely for adverse drug reactions and reporting should be encouraged by hospital pharmacists. Best practice approaches will be presented for the biosimilar antibody products of infliximab, rituximab, and trastuzumab.

Keywords: biosimilar, hospital formulary, prescribing, switch, pharmacovigilance

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L-13

The Interchangeability of Biotech Products: Issues to Consider Now and Further Into our 'OMICS Future"

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Fifty+ years of biotechnology have provided a plethora of innovative new biologic drugs. These large complex molecule drugs, created using proprietary and unique processes involving living cells, are chemically and physically different from the small molecule drugs that are part of the existing generic drug ecosystem. As these biologics have begun to come off patent, there has been increased interest in developing and marketing biosimilar versions, that have similar therapeutic properties, and which could potentially interchange with, and ultimately replace, the more expensive innovator drug. Since the European Medicines Agency approved the first biosimilar, Omnitrope , in 2006, they have approved over 40 biosimilars. In March 2015, the U.S. FDA approved its first biosimilar through the 351k pathway. Thus far, they have approved many fewer biosimilars, yet, it's estimated that $54 billion worth (in the U.S. market) of patents will expire before 2020.

Biosimilars will become an increasingly important part of the pharmaceutical ecosystem as they continue to face barriers to production and adoption. We know that the determination of biosimilarity is a complicated process, which involves assessment of the totality of the evidence for the close similarity of the two products. A biologic medicine typically has around 250 in-process tests during manufacturing, compared with around 50 tests for a chemical medicine. Biosimilars will continue to face barriers to adoption: the current questions of interchangeability, a typical lack of approval for all the reference biologic's indications, the need for biosimilar manufacturers to negotiate with payers, etc.

New developments in OMICS and the utilization of additional new biotechnologies will create new classes of drugs and therapeutic interventions that will challenge the science, the production, and the clinical use of interchangeable biosimilars. Genomics, proteomics, metabolomics, the microbiome, systems biology, synthetic biology, and many more biotechnologies have become the basis for today's new drug discovery and development science. These "OMIC" technologies and additional new biotechnologies will produce new drugs and therapeutic interventions that will have a defined patent life and new and unique challenges to biosimilar production and approval. In addition these new biotechnologies will create new challenges as well as new approaches to biosimilar production and biobetter medicines.

Keywords: biologics, biosimilars, OMICS, interchangeable, biobetters

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L-14

Controversies on the Switching and Substitution of Biological and Non-Biological Complex Drug Products

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The declaration of bioequivalence between two pharmaceutically equivalent small-molecule drug products generally indicates also their therapeutic equivalence. Therefore it is often assumed that the drug products may be substituted within patients even without the awareness of the prescriber. Comparisons of complex drug products, both biological and non-biological, are much less straightforward. These products cannot be the same but only similar, their equivalence cannot be determined but only their close similarity can be concluded. Consequently, the therapeutic equivalence of these products cannot be established. Various jurisdictions follow differing paths when they consider the interchangeability of complex products. EMA and Health Canada forward the judgment and decision to the constituent jurisdictions (provinces and member states). As a consequence, the regulatory expectation on interchangeability ranges very widely from requiring prescription by brand name to readily permitting substitution. In contrast, the United States has centralized legislation and regulatory expectations. Following the Biologics Price Competition and Innovation (BPCI) Act, the US-FDA recently published a draft guidance on the interchangeability of biological products. According to the BPCI Act and the draft guidance, in order to declare a product to be interchangeable, it must have been approved to be biosimilar to the reference formulation. It is stated further that once a product has been approved to be interchangeable with a reference product then it may be substituted without the intervention of the health care provider who prescribed the reference formulation. A difficult and controversial expectation is that the interchange-able product should produce the same clinical result as the reference product in any given patient. Thus, while Europe has several interchangeable products, the United States has none so far. Nevertheless, several states have enacted enabling legislations for interchangeability. The consequently arising legal disagreements and conflicts may sustain uncertainties for some time.

Keywords: similarity, interchangeability, complex drugs, BPCI Act, generic pathway, biosimilarity pathway

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L-15

Drug-Loaded Micro-and Nano Fibrous System as Enabling Formulations

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A significant proportion of new drug candidates possesses poor solubility and membrane permeability. Several promising techniques have been developed to overcome these disadvantageous properties, including different fiber formation methods. The electrospinning is one of the most commonly used spinning techniques for fiber formation induced by the high voltage applied to the drug-loaded polymeric solution. The characteristics of the solution and the spinning parameters can influence the quality of the obtained fibers. The fiber properties (high specific surface area, porosity, the possibility of controlling the crystalline-amorphous phase transitions of the loaded drugs) enable the solubility and thus the absorption enhancement.

In regenerative medicine, peculiar importance has been attributed to the structure of nanofibers because microarchitecture very similar to that of the extracellular matrix can be achieved. They are also capable of controlled drug delivery over time for local or systemic drug administration. The solubility of the polymer, the fiber diameter and the fiber structure are the primary parameters affecting drug release. In the case of small molecules, developments focus mostly on overcoming the unfavourable physicochemical feature of the active agents.

The presentation intends to provide a comprehensive overview of the application possibilities of fibrous systems, with a particular focus on the enabling formulations assuring the required drug concentration at the site of action and topical drug delivery systems with extracellular matrix mimicking effect. The physical and chemical stability of these systems has not yet been thoroughly investigated and thus poses a challenge in their development. Since the interactions between the fiber base, in most cases a polymer, and the loaded drug involve secondary bonds; thus their rearrangements modify the size and distribution of free volume holes as a function of storage time. The applied complex solid-state characterisation setup including positron annihilation lifetime spectroscopy represents a useful approach to track the destabilisation of either the polymeric carrier or the embedded drug which significantly modify the functionality-related characteristics of drug delivery systems. The presentation illustrates a comprehensive stability tracking including the free volume distribution changes of various fibrous delivery systems.

Keywords: drug-loaded fibrous mats, solubility enhancement, buccal sheets, topical delivery, stability tracking, solid state characterization

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L-16

Strategies in Developing a Transdermal Dosage Form

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Today, more than 74% of drugs are taken orally and are not found to be as effective as desired due to limitations such as bioavailability and patient compliance. Transdermal drug delivery system (TDDS) was developed to overcome such inherent limitations. TDDS consists of medicated adhesive patches of different sizes and shapes with one or more active pharmaceutical ingredients (APIs). They are applied on intact skin to deliver therapeutically effective amount of API into systemic circulation passing through skin barriers via diffusion. TDDS recently gained popularity in the pharmaceutical industry due to its ability to consistently deliver drugs at fixed rateover a long durationwith fewer systemic side-effects. Importantly, this dosage formimproves patient compliance since these patches can be removed with no pain and conveniently applied, anywhere in the body, as needed. TDDS is capable of transporting the drug or macromolecules painlessly through skin into the blood circulation. The skin is the largest, multilayered and most accessible organ in the human body by mass, with an area of between 1.5 and 2.0 m^2 in adults. Drugs can penetrate the skin via multiple pathways including intercellular, intracellular , hair follicles, sebaceous glands and sweat glands. Skin offers largest compliant interface for painless and controlled delivery over long duration. Because, TDDS avoids liver metabolism, theyincrease bioavailability of drugs, which improves drug efficacy and reducessystemic side-effects. TDDS are being used to treat various skin disorders, cardiovascular diseases such as angina pectoris, management of pain in arthritis and cancer, smoking cessation and neurological disorders such as Parkinson's disease. Recently, use of physical methods such as ultrasound, electrical, laser, radio and microneedle assisted approaches have greatly improved the efficiency of TDDS, and facilitated delivery of difficult to deliver APIs. We will cover most of the new active transport technologies involved in enhancing the transdermal permeation via effective drug delivery system can be discussed.

Keywords: transdermal drug delivery system, skin permeation, passive approaches, penetration enhancers, mechanical and physical approaches

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L-17

In Vitro Release and Q3 Measurements for Topical Drugs

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The skin represents a complex biological barrier, presenting multiple pathways for the penetration of active pharmaceutical ingredients from topical formulations. The dosage form contains the drug completely dissolved or dispersed in one or more phases of a semisolid matrix. The excipients play a critical role in the quality, efficacy and safety profiles. *In vitro* release tests are currently used for comparative assessments of gels, creams and ointments, mostly for screening the potential impact on the performance of controlled changes in composition or manufacturing process. Even though comparison of multisource drug product were previously discouraged as having no relevance, several draft guidance documents on bioequivalence were issued by US-Food and Drug Administration (US-FDA) which include an in vitro option. The recommended methodologies are highly dependent on the degree of complexity of the dosage form, varying from comparative physicochemical characterization and *in vitro* release to in depth, detailed analysis of particle size, morphology and crystal habit or systemic pharmacokinetics. Shah V.P. et al proposed a Topical drug Classification System (TCS), which considers the composition of the products and their microstructural similarity, based on comparative in vitro release testing. The Q1, Q2 and Q3 equivalence is to be used for the assignment into one of the four TCS classes. Biowaiver is proposed TCS class 1 (Q1, Q2 and Q3 similarity) and TCS class 3 (Q3 similarity), only after evaluation of the potential impact of Q1 and Q2 differences on the in vivo outcome. The Concept paper issued by the European Medicine Agency (EMA, 2014) enunciated the concept of extended pharmaceutical equivalence, corresponding to the aggregate weight of evidence of US-FDA. A draft guidance is expected for 2018 and will prospectively include in vitro release as one on the alternative methodologies. Additionally, EMA will consider the impact of methods of means of application, considered as critical for the arrangement of the matter at the site of administration. The presentation will include a short description of the current status of the validation of TCS and study cases illustrating the utility of correlated microstructural and in vitro assessments for demonstration of Q3 similarity.

Keywords: topical semisolid, *in vitro* release, microstructure, Q3 similarity

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L-18

Advances in the Analysis of Originator and Biosimilar Protein Therapeutics

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Protein biopharmaceuticals such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) are becoming a core aspect of the pharmaceutical industry. Together with a huge therapeutic potential, these molecules come with a structural complexity that drives state-of-the-art chromatography and mass spectrometry to its limits. Opposed to small-molecule drugs, protein therapeutics are large (mAbs have a MW of ca. 150 kDa) and heterogeneous (due to the biosynthetic process and subsequent manufacturing and storage). Despite the fact that only a single molecule is cloned, hundreds of possible variants differing in post-translational modifications (N-glycosylation, asparagine deamidation, aspartate isomerization, methionine oxidation, etc.), amino acid sequence, higher order structures, etc. may coexist, all contributing to the safety and efficacy of the product.

After a short overview of state-of-the-art LC and LC-MS methods applied nowadays to measure critical quality attributes (CQA) in originator and biosimilars, recent advances in LC and LC-MS will be highlighted. The versatility of hydrophilic interaction chromatography (HILIC), the benefits of instrument and column inertness, the possibilities of micropillar array columns (µPAC) and the many flavors of two-dimensional liquid chromatography (2D-LC) all in hyphenation to high-resolution mass spectrometry (MS), will be discussed and exemplified using protein therapeutics (mAbs and ADCs).

Keywords: protein therapeutics, originator, biosimilar , LC/LC-MS, recent advances

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L-19

Glycosylation Aspects of Biosimilarity

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Recent expiration of a number of protein therapeutics opened up the door for biosimilars, the next frontier in precision medicine. Biosimilars are biologic medical products, which are similar but not identical copies of already authorized biotherapeutics. The carbohydrate moieties on the polypeptide chains in most glycoprotein based therapeutic proteins play essential roles in such major mechanisms of actions as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), anti-inflammatory functions and serum clearance. In addition, alteration in glycosylation may influence the safety and efficacy of the product. Glycosylation, therefore, is considered as one of the critical product quality attributes (PQA) of glycoprotein biotherapeutics, and consequently for biosimilars. Thus, the carbohydrate components of such biopharmaceuticals (both innovator and biosimilar products) should be closely scrutinized during all stages of the manufacturing process. In this presentation a rapid sample preparation in conjunction with a high resolution capillary gel electrophoresis based separation process is introduced to compare and quantitatively assess the glycosylation aspect of biosimilarity (referred to as glycosimilarity) between the innovator and a biosimilar versions of several high profile biotherapeutics, based on their N-linked carbohydrate signatures. Differences in sialylated, core fucosylated, galactosylated and high mannose type glycans were all quantified and evaluated in respect to their possible effects on the mechanism of action and corresponding PQA importance. Finally, the term of glycosimilarity index is introduced, based on the averaged biosimilarity criterion.

Keywords: biopharmaceuticals, glycosylation, capillary electrophoresis, product quality attributes, glycosimilarity

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L-20

Is There a Possibility of Regulatory Harmonization?

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Various kinds of „regulatory harmonization" may exist. For this lecture, the practices of two regulatory agencies are taken harmonized if the submission of the same documentation, with only slight and predictable regional differences, results in the same regulatory decision.

The present work would like to point out that evolvement and harmonization of drug regulatory affairs follow certain patterns. Thus, on the basis a former successful regulatory harmonization story, the trend of a recent issue may be predicted.

Regulatory requirements and practices in the EU and in the USA are compared.

The previous example of harmonization to foresee trends is the in vitro biowaiver in case of generic medicines. The actual issue where the way and level of the regulatory harmonization is to be evaluated and predicted is the registration (marketing authorization) of biosimilar medicines. At present, EU is much ahead of USA in creating both the legal basis (2003 *versus* 2009) and approving the first (2006 *vs.* 2015) as well as the total number (around 10 *vs.* more than 40) of biosimilars. However, predicting it from the biowaiver story, a regulatory convergence between these two regions is expected.

Actually, there are fields with a high degree of harmonization such as the definitions of biosimilar drugs/medicinal products and the content of the relevant scientific guidelines.

Due to the different regulatory framework, there are factors that, on the short term, delay the harmonization, such as CDER and CBER (consequently NDA pathway for simple proteins until 2020) in the USA while the same CHMP assessment in the EU. There are also issues that are expected to hinder full harmonization for the long term. In the USA, two distinct applications should be filed to the FDA, one for biosimilarity and the other for interchangeability. In the EU, the latter is at the member states' discretion, although the EMA, without clearly stating it, suggests that biosimilars are interchangeable with the reference products. This is mirrored in the name of the active principle (a for-letter suffix identifying the biosimilar in the USA, while the same API name, based on the "regulatory sameness" principle, in the EU.) Moreover, both regions need the reference product authorized locally. (Exemptions possible if duly justified and supported by bridging studies.)

However, although EU and USA follow a somewhat different path to biosimilars, the target is the same and the regulatory convergence towards harmonization is expected.

Keywords: biosimilar, regulatory, harmonization, USA, EU

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L-21

Complexities Involved in Liposome Formulations

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Liposomes are non-biological complex drug products. Their structural features directly impact their pharmacokinetic and pharmacodynamic profile. FDA and EMA have drawn up guidance documents (FDA) and reflection papers (EMA), respectively, to assist generic manufacturers in receiving approval for generic versions of the innovator's product. When comparing these regulatory documents side-by-side, it is clear that differences of opinion exist. Zheng et al (2017) point that out in a table for doxorubicin-liposomes where the sameness of various parameters that potentially influence bioequivalence for the generic product compared to the innovator product (RLD) are listed. At some points in the list the FDA and EMA positions differ. Generally speaking, FDA's position is that these complex nanomedicines can be physico-chemically characterized to the point that (non)-clinical studies other than pharmacokinetic studies demonstrating equivalence in total exposure and exposure of non-encapsulated and encapsulated active are not necessary. On the other hand, EMA required non-clinical studies to be performed prior to bioequivalence testing. In the newest version of the EMA reflection paper (EMA 2018) the non-clinical study requirement is less stringently formulated. Further harmonization of these guidance documents will significantly help the generic manufacturers' community in streamlining their research programs.

Both FDA and EMA mention in their guidance/reflection documents Critical Quality Attributes (CQAs), which have a major impact on the in vivo pharmacokinetic and pharmacodynamics properties (e.g., EMA, 2013; FDA 2015; FDA 2017, FDA 2018a). For liposomes a number of CQAs are mentioned such as particle size, size distribution, release rate, zeta potential (Zheng et al., 2014). Undoubtedly, variation in the values of these physicochemical characteristics may and will affect safety and efficacy. But, so far, no information could be found in the public domain where the design space for such CQAs related to safety and efficacy was established. Launching an international effort to establish quantitative CQA – safety and efficacy relationships for liposomes drug products will increase access to these products. As an increasing number of generic nanomedicines will be developed, the insights obtained for generic liposomes will prove highly valuable.

Keywords: liposomes, FDA, EMA, critical quality attributes, generics

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L-22

Bioequivalence of Liposomal Parenterals: Nice-to-Know vs. Need-to-Know

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Conditions for assessing bioequivalence of liposomal parenterals have been discussed intensively during previous years. Specific attention was paid to liposomal doxorubicin due to Marketing Authorization Applications submitted for generic alternatives of the innovator product Caelix®, both in Europe and the USA.

Requirements for clinical BA/BE studies have, therefore, been reviewed by the "Global Bioequivalence Harmonization Initiative" at its 3rd international conference held in April 2018 in Amsterdam/The Netherlands. Two aspects were intensively discussed based on experimental data presented during the conference, i.e.

1. the question of the most appropriate analyte to be measured for BE conclusion – the active ingredient encapsulated in liposomes, the unencapsulated ("free") compound or the total drug – and
2. the problem of different doses to be administered – due to patient's advice in the labelling – and considered for intraindividual comparison.

Outcome of this discussion could be an excellent example for establishing appropriate regulatory requirements based on scientific evidence and the essential contribution of pharmaceutical scientists from academia and industry to science-driven regulations. However, the product-specific Guidance published by EMA in the meantime does not reflect all suggestions presented at the Amsterdam conference and, therefore, main conclusions of the Global Bioequivalence Harmonization Initiative will be summarized again for further debate.

Keywords: liposomes, doxorubicin, bioequivalence, encapsulated, unencapsulated

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L-23

Application of Nanotechnology to Targeted and Innovative Therapies

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Nanotechnology has been applied to pharmaceutical development since the last 50 years starting with the iron sucrose nanoparticles to treat iron deficiency. Since then, several nanomedicines have entered the market and currently more than 50 products that contain nanomaterials have already reached the patients. Consequently, this rise in the number and diversity of nanomedicines and the development of "nanosimilars" have led to the establishment of regulations for the marketing approval of original or follow-on nanomedicines by EMA and FDA. This presentation will discuss the application of nanotechnology to targeted and innovative therapies with two main examples that were carried out by our group. Global omics approach to the development of nanomedicines will be discussed through the development of polycationic nanoparticles that are able to extract cholesterol from cancer cell membrane inducing a mitochondrial apoptotic pathway. Nanoparticle-induced regulation of proteins in breast cancer cells were evaluated by proteomics and resulting metabolites were further analyzed through bioinformatics to underline the metabolic pathways induced by these nanoparticles. On the other hand, incorporation of nanoparticles to inkjet printing technology will also be discussed with the aim of developing a fixed dose combination bioadhesive system to treat human papilloma virus induced cervical cancer. The effect of nanoparticulate printing on the physicochemical properties of the bioadhesive gel was evaluated as well as drug release from inkjet printed nanoparticulate films. Ex vivo analysis was carried out to determine the drug retention on cervical tissue treated with inkjet printed film containing nanoparticulate drug.

In conclusion, the wide range of innovative applications of nanotechnology and flexibility of the products containing nanomaterials can be both a challenge and an advantage in terms of innovative drug development.

Keywords: nanoparticle, cancer, omics, inkjet printing, drug resistance

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L-24

Application of *In Vitro* Dissolution Studies to Evaluate Drug Interactions

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In vitro dissolution tests play an increasingly important role as a predictor of the *in vivo* performance of drug products. This greatly contributes to reducing the number of human studies required and to making drug development more cost-effective, emphasizing the possibility of exploring such advantages in other areas of the pharmaceutical industry.

In recent years, dissolution testing has become a promising *in vitro* screening system in the field of drug interactions. Due to recent advances in the development of dissolution methods and biorelevant dissolution media, it has become possible to predict the presence and the magnitude of drug and nutrition interactions (e.g. food components, specific supplemental nutritiens, nutrition status) *in vivo* by reflecting all the key aspects of the gastrointestinal tract. However the complex composition of such media provides an additional challange in the quantitative determination of the drug dissolved and requires application of advanced sample preparation and bioanalytical techniques.

The purpose of our lecture is to overview the role of dissolution studies in the forecasting and in the assessment of drug interactions, primarily focusing on nutrition interactions. Some case studies with particular emphasis on recent advances and progressive application of dissolution tests are also presented which may contribute to more in-depht understanding of the mechanism as well as the molecular background of drug interactions.

Keywords: dissolution studies, *in vitro-in vivo* correlation, drug interactions, biorelevant dissolution media, prediction, bioanalysis

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L-25

A Reaction-Limited *In Vivo* Dissolution Model for the Study of Drug Absorption: Implications for the Biopharmaceutic Classification of Drugs

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A gastrointestinal (GI) drug absorption model based on a reaction-limited model of dissolution will bepresented (1). Its impact on the biopharmaceutic classification of drugs is also considered. Estimates for thefraction of dose absorbed as a function of dose, solubility, reactio n-dissolution rate constant and thestoichiometry of drug-GI fluids reaction-dissolution were derived by numerical solution of the modelequations. The undissolved drug dose and the dissolution-reaction rate constant drive the dissolution rateand determine the extent of absorption when high-constant drug permeability throughout thegastrointestinal tract is assumed. Dose is an important element of drug-GI fluids reaction/dissolution whilesolubility exclusively acts as an upper limit for drug concentrations in the lumen. The 3D plots of fraction of dose absorbed as a function of dose and reaction-dissolution rate constant for highly soluble and low solubledrugs for different "stoichiometries" (0.7, 1.0, 2.0)of the drug-dissolution/reaction with the GI fluids revealedthat high extent of absorption was found assuming high drug-dissolution/reaction rate constant and high drugsolubility. The model equations were used to emulate *in vivo* supersaturation and precipitation phenomena.

The model developed provides the theoretical basis for the interpretation of the extent of drug's absorptionon the basis of the parameters associated with the drug-GI fluids reaction/dissolution. A new paradigmemerges for the biopharmaceutic classification of drugs, namely, a model independent biopharmaceuticclassification scheme of four drug categories based on either the fulfillment or not of the current dissolutioncriteria and the high or low % drug metabolism.

Keywords: reaction limited model, dissolution, solubility, supersaturation, BCS, BDDCS

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L-26

Bioequivalence Studies and Evaluations for DPI Drug Products: Regulatory Authorities Challenges

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Authorization and commercialization of the dry-powder inhalers (DPI) are on the increase during the recent years. The primary reason, for sure, is to meet the need for more effective drugs in the treatment of target indications. On the other hand, patent expiration of the innovator drugs also boosts the attention of the generic industry into this field. What is interesting when it comes to the authorization of the generic DPIs is the fact that how and on which criteria the bioequivalence (BE) studies, being one of the principal parameters for proving whether the generic drugs are therapeutic equivalents (TE) especially in terms of safety and efficacy of a product to be authorized, with a history of nearly 40-45 years, would be implemented have been determined based on the national and international guidelines.

In some countries, these DPIs related guidelines have been established based on the extensive studies; however, in most of the countries other than the said ones, there are no specific guidelines on DPIs established by the relevant authorities or the already existing guidelines are not satisfactory or the process goes ahead by making references to the current FDA or EMA guidelines. Another important point is that the desired level of harmonization has not yet been achieved between the existing guidelines. And this poses a significant problem in resolving the troubles encountered by the implementers during solution finding.

There are several factors affecting the failure to investigate TE of DPIs within the frame of the current BE guidelines. DPIs are formulation-device combinations, it is seen and has been proved that not only the formulation but also the device used has a significant impact on the BE of these products. The aim is to provide a local effect on the target indication in many of the marketed DPIs. This makes it easier to understand the importance of designing a device that would allow developing the most effective DPI considering the characteristics of the target organ. This makes it inevitable that, for proving the TE of generic DPIs to innovator DPIs, the other device parameters apart from the ones considered during the classical BE studies are investigated and to this end, the scientific and regulatory infrastructure of the guidelines are established and the required harmonization is achieved among the guidelines.

At this speech, the regulations published by the authorities of several countries (USA, EU, J, BR, IN, CN, TR) concerning the demonstration of the TE of DPIs shall be reviewed and the similarities, differences as well as requirements in this field shall be indicated.

Keywords: DPI, formulation-device combination, therapeutic equivalence, guideline, regulation

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L-27

Advances in Bioanalytical Mass Spectrometry in Support of Drug Therapies

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The past four decades have witnessed an extraordinary progress in mass spectrometry (MS) that enabled it to become not only the method of choice for bioanalytical support during the discovery, development and surveillance of small-molecule pharmaceuticals, but also has claimed its place in the analyses of biologicals such as therapeutic proteins. This paradigm shift originated in large part by the recognition of the principle to ionize molecules, both small and large, by desorption methods, out of which electrospray ionization has become a staple in today's instruments applied in both scientific and regulatory settings. Advances to accompany this included the increasing availability and consequent strive for routine uses of powerful techniques such as the Fourier transform mass spectrometry affording previously unprecedented mass resolution and mass accuracy, and various tandem MS (MS/MS and MS^n) approaches. In addition, surrogate methods coupled ("hyphenated") with the techniques such as chromatography and electrophoresis, as well as sample preparation have also been developed and commercialized rapidly to match advancing MS capabilities. Through selected examples, the presenter will review various MS-based approaches that impact current state-of-the-art and expected trends of scientific and regulatory advances to support therapies relying on biological and non-biological complex drugs alike. Despite the convergence of methodologies, unique requirements and specific challenges still remain in these separate areas of applications, which will require tailored solutions in many cases.

Keywords: mass spectrometry, electrospray ionization, hyphenated methods, tandem mass spectrometry, current approaches and future trends

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L-28

Micro-Flow Imaging as Analytical Tool in Pharmaceutical Development of Microparticulate Complex Drugs

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As the complexity of drugs is increasing, more advanced characterization is needed to achieve product understanding. There have been recent advances towards better characterization of biologics and biosimilars, particularly in the field of subvisible particles. One technique that became well established to characterize particles in the size range from 1 to 300 µm, is Micro-Flow Imaging or MFI. This technique combines digital microscopy with small sample volumes and high throughput.

MFI has found many applications, which are primarily focused on the detection of *undesired* microparticles in protein pharmaceuticals. In our research, we have evaluated the applicability of MFI to characterize *desired* microparticles that are used for drug delivery. In this presentation, Dr. Miranda van Beers will explain how MFI can be used to characterize drug-containing microparticles that are based on the biodegradable polymer poly(lactic-co-glycolic acid) or PLGA.

Size and agglomeration of PLGA microparticles are of critical importance for polymer degradation and drug release rates. Currently, the most common technique to evaluate the size distribution of PLGA microparticles is laser diffraction. However, this technique does not provide information on m icroparticle agglomeration. The presence of agglomerated microparticles may shorten the duration of action and hamper the injectability of the drug.

From our data we conclude that MFI is able to differentiate between 'fines', 'monospheres' and 'agglomerates' based on particle morphology and quantify the number and volume fraction of each particle population. This information can be applied in the formulation and process development of PLGA microparticles and to monitor the physical stability of the particles after reconstitution.

Thus, MFI can be a valuable tool to support development and manufacturing of microparticulate complex drugs and their follow-on versions. It helps to achieve the correct particle size and prevent agglomeration, in order to deliver drugs that are safe to the patient, suitable for injection, and show an efficacious drug release profile.

Keywords: flow imaging microscopy, laser diffraction, particle agglomeration, PLGA microparticles, drug release

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L-29

Advanced LC/MS Technologies for the Characterization and Monitoring of Complex Biotherapeutics

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Biotherapeutic agents are complex both due to their size and the microheterogeneity that they exhibit in their expression. Even 'pure' drug substance is often a varied array of proteoforms. The degree of complexity is then further multiplied by unwanted but inevitable modifications that occur during production and storage. Each modification has the potential to effect both safety and efficacy. Therefore, initially very large numbers of product quality attributes (PQAs) need to be identified and monitored for each biotherapeutic. Once the effects of these modifications are better known, they may be reduced to a shorter list of critical quality attributes (CQAs). These CQAs may then be monitored through early development though to manufacturing and lot release.

In order to fully characterize complex biotherapeutics no single analytical technique is comprehensive. An array of advanced liquid chromatography/mass spectrometry (LC/MS) techniques allow for very deep characterization. Demonstrated here are a number of cutting edge techniques that may be applied from early discovery all the way through to manufacturing.

Intact mass analysis allows for determination of structure without manipulating the sample enzymatically or chemically and provides direct measurement of a number of characteristics. The use of LC/MS under native conditions has greatly increased the utility of this application and is demonstrated for the analysis of very high MW species and highly complex antibodies.

The use of enzymes to cleave at the 'hinge region' in conjunction with high resolution accurate mass (HRAM) and advanced fragmentation techniques facilitates the sequencing of mAbs more directly without having to resort to a more traditional bottom up approach.

HRAM peptide mapping techniques provide full characterization at the peptide level and demonstrated here is the multi-attribute method (MAM) that allows HRAM peptide mapping to be used all the way from full characterization in Discovery to monitoring of critical quality attributes for lot release of drug substance.

Keywords: LC/MS, HRAM, Native MS, middle-down, MAM

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Poster Abstracts

P-01

A Method for the Prediction of Drug Content of Biodegradable Drug Carrier Nanoparticles Obtained by Nanoprecipitation

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Poly(lactic-co-glycolic acid) (PLGA) drug loaded nanoparticles (NPs) are applied successfully to increase the distribution and bioavailability of mainly hydrophobic drug molecules, although the generally low drug content of the NPs implies a limitation.

PLGA50 NPs were prepared by nanoprecipitation to encapsulate model drugs, a homologous series of alkyl-hydroxy-benzoate (parabens) from methyl- to octyl with gradually increasing hydrophobicity to address the influence of drug property on the encapsulation. The drug content and encapsulation efficiency were found to be substantially different for the various parabens. Size of the drug carrier NPs was obtained by dynamic light scattering and found to be in the range of 170-190 nm with a polydispersity of <0,1. Drug content and encapsulation efficiency were determined spectrophotometrically.

The analysis of the miscibility of PLGA50 matrix with parabens using their solubility parameters could not provide satisfactory explanation for the experimental findings. The treatment of the nanoencapsulation however, as a partitioning process involving PLGA50 and the aqueous phase led to a relationship between the drug content of the NPs and the characteristic properties of the drug molecule. According to that both the high hydrophobicity and high aqueous solubility of the drug favour the efficacy of its encapsulation. The actual and also the achievable maximum drug content of PLGA50 NPs can be predicted and was tested in several systems. The above approach opens the way for more reasonable design of nanoparticle drug carrier in addition to the possible explanation for the drug content achievable for a given compound.

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Keywords: nanoparticle, drug delivery system, biodegradable, biocompatible, PLGA

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P-02

Admet Evaluation of Phosphodiesterase Type 5 Inhibitors Found in Adulterated Dietary Supplements - In Silico Study

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The popularity and interest in phosphodiesterase type 5 (PDE-5) enzyme inhibitors for the treatment of erectile dysfunction (ED) has led to the increase in prevalence of illicit food supplements with these drugs. Structural analogues of the registered ED drugs sildenafil, tadalafil, and vardenafil were identified as the main adulterants. More than fifty analogues have been reported in the literature and it is expected that their use in seemingly harmless food supplements may cause serious adverse effects.

The aim of this in silico study was to evaluate ADMET properties of phosphodiesterase 5 inhibitors (PDE5Is) including sildenafil and its analogues and to predict their safety profile by ADMET PredictorTM (SimulationsPlus Inc., USA) in order to highlight their potential impact on health. Based on ADMET Predictor analysis the following risks with corresponding computed scores were revealed: ADMET_Risk 1.013-9.644, Absn_Risk 0.000-5.568, CYP_Risk 0.000-3.641, MUT_Risk 0.0-1.0, and TOX_Risk 0.000-1.086. All investigated molecules (n=11) are characterized with relatively high molecular weight (MW 439.518-1035.218) and with relatively low lipophilicity (MlogP -0.614-1.591). The main risk in physico-chemical part that reflect on overall absorption revealed water solubility (S+Sw 0.018-0.562). Among all predicted ADMET risk codes, the size of molecules, charge and permeability prevail over CYP3A4 clearance, hydrogen bond acceptors, rotatable bonds, as well as mouse (Xm) and rat (Xr) carcinogenicity. For mirodenafil and morfolinoacetidenafil, both, Xm and Xr were predicted, and for acetilenafil and hydroxyacetildenafil only Xm and for lodenagil carbonate only Xr was predicted. The cardiotoxicity (TOX_hERG) was predicted for aildenafil. For five investigated PDE5Is no TOX_Risk was computed (sildenafil, homosildenafil, hydroxyhomosildenafil, vardenafil, udenafil). However, the low TOX_MUT score 1 has been predicted for sildenafil, homosildenafil, hydroxyhomosildenafil, mirodenafil and aildenafil. All these drugs are extensively metabolized by CYP enzymes (CYP 2C8, 2D6 and 3A4) and all of them are CYP 3A4 inhibitors.

PDE5Is in clinicl use are safe drugs for ED treatment. However, the results of this study revealed that in addition to potential carcinogenicity of some investigated PDE5Is present in food supplements, their CYP 3A4 inhibition could contribute to drug-drug and drug-food interactions, and the most unfavourable safety profile was predicted for mirodenafil.

Keywords: phophodiesterase type 5 inhibitors (PDE5Is), sildenafil analogues, food supplement, safety, ADMET

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P-03

Analytical Challenge of Estimating the Effectiveness of Organic Fermented Food for Evaluation of the Gut-Liver Axis – A Clinical Study

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Background: Recent studies suggest that the gut-liver axis plays an important role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), so probiotics could be a therapeutic tool. Organic food seems to contain fewer pesticide residues and statistically more selected health-related compounds, but the health relevance for consumers is not clear yet. Comparing food from organic origin with so called conventional food seems not to be appropriate, because there is no biomarker that would signal the difference with good specificity and sensitivity.

Aim: The aim of this study was to investigate the indicators to evaluate the difference between fermented food from conventional and organic origin and their health effects in NAFLD.

Methods: We performed a prospective, cohort study consisting of 37 (age=51.73±11.82, male=21, female=16) patients with NAFLD at Semmelweis University. We divided the patients randomly into two groups. The patients consumed individually daily 300 g yoghurt from organic or conventional origin for 8 weeks. We collected 37 routine laboratory data, measured 4 cytokines, 3 markers of the redox-homeostasis and 14 body composition values before, at the end of 8-week and at 12. week after the yoghurt consumption. Patients were selected with shear wave elastography according to hepatic fibrosis stage.

Results: We found a mild elevation of vitamin D (from 20.25 to 26.66ng/ml) and LDL (from 64,7 to 68,08%) concentration at 12. week after the yoghurt consumption, but there was no statistically difference in the other 35 routine laboratory data. Adiponektin and leptin levels were elevated after the yoghurt consumption in the "conventional group". In contrast, we found significant decrease of adiponektin levels (from 12017.57 to 8833.5 pg/ml) in the "organic group" after the treatment. Only the adiponektin tendency was different in the two groups. The induced free radical content was also statistically lower after the yoghurt consumption. In the body composition measurements were no significant differences.

Conclusions: These data suggest that adiponektin could be a possible marker to evaluate the effectiveness of probiotic treatment in non-alcoholic fatty liver disease. Our work can serve as a basis for future studies investigating relationships between fermented food consumption and health outcomes.

Keywords: fermented food, organic, biomarker, adiponectin, leptin

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P-04

Bioanalytical Method Development and Validation for High Throughput Screening of Small Molecular Biomarkers

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Method development and validation for the quantitative determination of endogenous analytes are greatly hampered by the absence of analyte-free control matrix to prepare calibration and quality control (QC) samples. A vast number of endogenous compounds, such as amino acids, neurotransmitters and catabolic products have great significance in various clinical cases as biomarkers, therefore there is an increasing interest for novel validation approaches to overcome this challenge.

In this work we have developed and validated a sensitive, high-throughput high performace liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for simultaneous quantification of 8 potential migraine biomarkers (serotonin, kynurenine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan) in human blood plasma. Deuterated leucine for aliphatic amino acids and deuterated tryptophan for aromatic analytes were used as internal standards. Sample preparation was performed using protein precipitation. The analytes were separated under isocratic conditions and were detected in multiple reaction monitoring (MRM) mode. A "fit-for-purpose" validation approach was adopted by using artificial plasma as a surrogate matrix for the construction of calibration and QC samples. Parallelism experiment were carried out in order to demonstrate how well the validation samples track the response of the authentic analytes in human plasma. The assay was validated for determining the limit of quantification, linearity, inter- and intra-day accuracy, precision, matrix effect and matrix factor, dilution integrity and stability.

The method proved to be reliable and was succesfully applied for the determination of more than 850 human plasma samples. The results of method validation using a "fit-for-purpose" strategy were able to meet the requirements imposed by FDA.

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Keywords: HPLC, mass spectrometry, migraine, biomarker, bioanalytical validation

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P-05

Biodegradable Nanoparticles for Pulmonary Delivery of Apigenin

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Introduction: Pulmonary drug delivery is a non-invasive approach in the treatment of respiratory or systemic diseases. It allows direct targeting of the natural therapeutic agents such us apigenin. This flavonoid has remarkable antioxidant activity, moreover, is able to inhibit cell proliferation, migration and induce apoptosis in lung cancer cells (H1299, H460 and A549)[1]. The effectiveness of an inhaled bioactive component depends of the particle size, morphology and aerosol characteristics, as well as pulmonary physiology. Biodegradable nanoparticles offer improved drug solubility, increased local drug concentration and targeted site of action[2].

Materials and Methods: To prepare biodegradable nanoparticles, locust bean gum and chitosan biopolymers were used. High pressure homogenization was applied to encapsulate apigenin. The physical properties of the particles such as size, charge and drug loading efficiency were measured. The dissolution of Api was determined with Franz cell apparatus in simulated lung fluid. The samples were further lyophilized with carrier and residual water content was measured by Karl-Fischer titration. The morphology of dry powders was observed with scanning electron microscopy.

Results: The developed biocompatible nanoparticles had adequate size and apigenin could be effectively loaded with high pressure homogenization. The optimized freeze-drying conditions were suitable to produce particles with low residual moisture content and the particle size was maintaned following a rapid rehydration. The dissolution was enhanced compare to the raw apigenin.

Conlusions: The results indicate that the developed biodegradable 1:6 molar ratioHSA-LBG/Ch particles may have great potential for pulmonary delivery of bioactive agents such as flavonoid apigenin due to small particle size and porous structure.

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Keywords: Apigenin, flavonoid, pulmonary drug delivery, nanoparticles

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P-06

Bioequivalence and Generic Substitution – Croatian Regulatory Perspective and Impact in Practice

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Croatian regulatory body HALMED, approves medicinal products in Croatia with regard to their quality, safety and efficacy and assess bioequivalence of generic medicinal products as required by EU regulative. When a generic medicinal product is proven to be bioequivalent with the original such generic medicinal product can be replace the reference medicinal product (*generic substitution*). However, there are original medicinal products that can not be directly replaced with generics such as medicinal products with narrow therapeutic window and variable interindividual absorption, medicinal products with more then two active substances and medicinal products that are applied by medical devices which are used in various ways. These medicinal products are only replaceable in exceptional situations, and only based on a recommendation of a physician that will monitor the condition of the patient during the replacement therapy. In order to ensure a proper information on medicinal products that can be substituted in Croatia, HALMED is to issue a List of Substitutable Medicinal Products.The problem regarding the compilation of such list occured since basic principles for substitution for the medicinal products that have the same active substance are not specified within Croatian Medicinal product Act. In order to ensure the availability of the medicinal products to the patients, if the prescribed medicinal product is not available, the pharmacist is obliged to take measures to obtain the medicinal product within three days. Furthermore, if the pharmacy does not have the prescribed medicinal product due to its unavailability on the market, the pharmacists has the right to dispense the generic medicinal product, under the condition that the authorised person who prescribed the medicinal product did not note on the prescription that the medicinal product may not be substituted, and that the patient is in agreement with the substitution of the medicinal product. In this way supplie chain of medicinal products and availability of medicinal products to patients is intact in the interim period while all the legal requirements for issuance of List of Substitutable Medicinal Products are being fully adapted.

Keywords: Croatia, bioequivalence, generic substitution, regulatory

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P-07

Bioequivalence Testing with High Variability on Drug Product: Overview and New Alternative Statistical Method

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The evaluation of bioequivalence between a reference drug product and a tested drug product is generally done through the Average Bioequivalence (ABE) or the Population Bioequivalence (PBE) statistical methods. However, these two statistical methods present some limits that we propose to illustrate.

The first one is the effect of the within-product variability on the increase of the type II error. This phenomenon is well known for highly variable drug products (HV) with CV>30%. We bring out that this drawback doesn't appear only when CV>30%.

The second limit comes from the fact that current bioequivalence evaluation does not consider the batch-to-batch variability in study design or analysis. We bring out that the risk of a false bioequivalence conclusion can substantially be impacted by a highly between-batch variability.

In this framework of high within-product or high between-batch variabilities, we make an overview of the solutions found in the literature and we propose a new alternative statistical method first applied to parallel design studies. The main purpose of this study is to compare and evaluate the pertinence of the different methods, using both real data and power analysis of simulated data.

As part of the overview, we discuss the interest and the computing of the reference-scaled average bioequivalence (RSABE). Moreover, we propose a new statistical method, named BBRSABE (Between-Batch Reference-Scaled ABE). Conceptually, the new approach consists in a comparison of the difference between the means of the two drug products with the between-batch variability on the reference product. This alternative method generates an intuitive formulation and would give the ability to define easier the bioequivalence limits.

The different methods were compared on two *in vitro* bioequivalence studies for nasal spray products. Two reference products were analyzed with 20 batches on each product. The power analysis was conducted using Monte Carlo simulations based on real parameter distributions. We obtain similar results on both studies. Analyses of the simulated data revealed that the type II error rates for the different methods differed substantially according to the within-product variaiblity on the prarameter. These simulations indicate different and precise application contexts for each method and highlight the strengths of the new proposed method, especially the limitation of required samplings when the number of batches is statistically satisfying.

Keywords: high variable drug products, Between-Batch variability, Reference-Scaled Average Bioequivalence (RSABE), Between-Batch Reference-Scaled Average bioequivalence (BBRSABE), power analysis

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P-08

Biorelevant Physicochemical Properties and Cyclodextrin Complexation of Baicalin

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Introduction: Baicalin is a flavone glycoside, extracted from the root of *Scutellaria baicalensis Georgi*. It was shown, that the poorly water soluble, poorly permeable (BCS IV) compound has remarkable pharmacological effects including antioxidant, antimicrobial and antitumor actions. The low oral bioavailability (2.2% in animal modells) of this drug could be alleviated through cyclodextrin (CD) inclusion complexation. CDs are cyclic oligosaccharides composed of α-1,4- linked D-glucopyranose units possessing a hydrophilic exterior and hydrophobic cavity. The physicochemical profiling of baicalin (lipophilicity, acid-base parameters, solubility) is essential in order to develope CD-based carrier systems. The scope of the work was the biorelevant physicochemical profiling of baicalin in terms of protonation properties, lipophilicity, thermodynamic solubility and CD complexation analyzed by phase solubility, UV and NMR experiments.

Materials and methods: Baicalin (Actin Chem. Ltd., China); α-, β-, γ-, (2-hydroxypropyl)-β- (HP-β), random methylated β- (RAMEB), sulfobutylated β-CD (SB-β) (Cyclolab Ltd., Hungary). All other chemicals were of analytical grade. Distribution coefficient measurements were carried out by the stir-flask method, the determination of protonation constants was fullfiled by 1H NMR–pH titrations. The equilibrium solubility of baicalin in conventional and biorelevant media was determined by saturation shake-flask method, the stability constant of the inclusion complexes were calculated according to Higuchi-Connors. To elucidate the structure of the inclusion complexes between baicalin and CDs, 1H NMR and 2D ROESY experiments of free drug and host-guest complexes were undertaken.

Results: Biorelevant gastric fluid (BGF) has a considerable impact on the solubility of baicalin correlated to Ph.Eur.9. gastric fluid (CGF) (BGF: 33.21 μg/ml vs CGF: 11.64 μg/ml). RAMEB, SB-β and γ-CD significantly enhanced the poor aqueous solubility of baicalin and displayed A_L and B_S-type phase diagrams. 1H NMR confirmed the formation of inclusion complexes. Comparing the different CD complexes we found that γ-CD presented the strongest interaction.

Conclusion: Biorelevant physicochemical profiling of baicalin was carried out and the results can be used to determine the best formulation path. The study demonstrated that CDs are ideal carriers for baicalin. Baicalin and its γ-CD complex could serve for a promising Drug Delivery System with improved bioavailability.

Keywords: cyclodextrin complexation, solubility enhancement, physicochemical properties, 1H NMR, biorelevant media

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P-09

Chitosan-Xanthan Multilayer Nanofilms as a Potential Carrier in Buccal Drug Delivery

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Introduction: Amongst the vast variety of formulations for buccal drug delivery, polymer films have received the greatest recent attention. Multilayer films, which are generally prepared via layer-by-layer (LbL) assembly technique, represent an emerging and scarcely explored area of drug delivery. Typically, multilayer structures are based on the formation of polyelectrolyte complexes between oppositely charged molecules due to the formation of strong, but reversible electrostatic links. Drug molecules can be incorporated within the polymer layers, providing a drug-delivery depot. The aim of the current study was the preparation of polyectrolyte multilayer mucoadhesive nanofilms and evaluation of their suitability as a vehicle for buccal delivery of benzydamine (BZD).

Materials and methods: LbL deposition technique was applied for multilayer build-up. The deposition was done by alternative dip-coating of corona pretreated polylactic acid substrates into chitosan and xanthan solutions. For drug loading of the samples, BZD was dissolved in chitosan solution at 5% concentration. Chitosan deposition was followed by crosslinking with glutaraldehyde and sodium tripolyphosphate. The procedure was carried out until 8 layers were deposited. The films were evaluated for chitosan/xanthan interactions, surface pH, percentage moisture absorption and moisture loss, swelling behavior, content uniformity, drug release and mucoadhesion.

Results: FTIR-ATR spectra of the films proved the formation of a complex between the polymers. The percentage swelling confirmed big relevance for buccal administration foreshadowing high drug diffusion rate and preventing excessive polymer erosion. Drug release was biphasic with a significant burst effect. The films exhibited satisfactory mucoadhesion and high drug stability after one-year storage.

Conclusions: The developed polyelectrolyte multilayer films represent a feasible approach and could, therefore, be used as a promising buccal drug delivery system.

Keywords: LbL assembly, multilayer films, buccal drug delivery

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P-10

Classical *Versus* Experimental Design Optimization in DLLME-SFO for HPLC Analysis of Anti-inflammatory and Hormone Drugs

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In this work, a comparative study between the classical (univariate approach) and the modern (experimental design based on factorial factors) for the development of a dispersive liquid–liquid microextraction based on solidification of floating organic droplet (DLLME-SFO) for the simultaneous extraction of four nonsteroidal anti-inflammatory (NSAIDs; ketoprofen, naproxen, ibuprofen, diclofenac) and three hormone (17ß-estradiol, 17α-ethynylestradiol, estriol) drugs from wastewater samples followed by HPLC-UV analysis was performed.

The influence of the main extraction parameters (disperser and extraction solvent volumes, pH, sample ionic strength) was evaluated.

For the classical optimization, each parameter was studied by changing one single variable at a time and keeping the other constants while for the modern approach, a 24 experimental design based on the factorial factors was chosen. The extraction recoveries of each compound were considered as responses. To find the effects of factors and of interactions on DLLME and, also, the conditions that give the highest extraction recovery, the JMP14 statistical software package was used.

Before applying the experimental design, the maximum, minimum and average values of each variable were established. Based on 19 experiments, of which 16 (2^4) organized as factorial design and 3 as replicates of central point, it was possible to find the optimum DLLME-SFO conditions. A desirability function was used to perform the simultaneous optimization of all factors and the surface response plots for graphical view of effects of experimental variables and of their interactions.

Our results showed comparable conditions for DLLME-SFO for all compounds in both approaches having advantage for the experimental design which significantly reduces the experiment number and provides information about the influence of variables and of their interaction over the extraction recovery. Good DLLME-SFO-HPLC-UV results: enrichment factors, 131–150 for anti-inflammatories and 90–146 for hormones; linearity, R^2>0.999 in the concentration range of 3.25–100 µg/mL for anti-inflammatories and of 10–100 µg/mL for hormones; intra- and inter-day precision (RSD<7%); low limits of quantification; relative recoveries over 80%, except estriol (50%), both in synthetic and real wastewater samples.

The developed DLLME-SFO-HPLC-UV method was successfully applied to the analysis of wastewater samples collected from the effluents of a hospital.

Keywords: DLLME-SFO, HPLC, experimental design, NSAIDs, hormone drugs

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P-11

Determination of Binding Constant of Human Serum Albumin to Monoclonal Antibody using Capillary Electrophoresis and Isothermal Titration Calorimetry

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The specificity and the affinity of antibody-antigen interactions are important for understanding the biological activities of these molecules. Therefore, the determination of the binding constant is needed for the characterization of the molecular recognition and thus for the development of drugs like biotechnological engineered therapeutic antibodies. Several methods of capillary electrophoresis (CE) have proven the applicability for the determination of binding constants [1].

Capillary zone electrophoresis (CZE) and affinity capillary electrophoresis (ACE) were tested to characterize the binding interaction between monoclonal antibody (anti-HSA-mAb) and its protein antigen. The binding constants determined by isothermal titration calorimetry (ITC) were compared to the results obtained by CZE and ACE. The CZE revealed the formation of the anti-HSA-mAbHSA and anti-HSA-mAb{HSA}$_2$ complexes and the binding constants determined by plotting the amount of the bound anti-HSA-mAb as a function of the concentration of HSA. The ACE did not identify the formation of the complexes, the mobility of the single detected peak was the result of the weighted average of the mobilities of three species of the anti-HSA-mAb. The ACE provided information on the binding strength from the change in effective electrophoretic mobility of the anti-HSA-mAb. The equilibrium dissociation constant values obtained by CZE and ACE were found to be $2.26 10^{-6}$ M for anti-HSA-mAbHSA, $1.22 10^{-6}$ M for anti-HSA-mAb{HSA}$_2$ and $4.45 10^{-8}$ M for anti-HSA-mAbHSA, $1.08 10^{-7}$ M for anti-HSA-mAb{HSA}$_2$, respectively. The dissociation constant data obtained by ACE were in congruence with the values obtained by ITC ($2.74 10^{-8}$ M, $1.04 10^{-7}$ M). All three methods show that anti-HSA-mAb possesses two binding sites, which exhibit almost the same binding strength. Applying CZE and ITC, we could define the stoichiometry of the interaction (HSA : mAb = 2 : 1). The advantages of CE can be utilized in those fields where ITC has limitations (sample quantity, solvent, purity).

Keywords: monoclonal antibody, capillary electrophoresis, binding constant, antibody-antigen interaction, isothermal titration calorimetry

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P-12

Dissolution of Fampridine from HPMC Matrix Tablet Formulations Evaluated by QbD Approach

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Fampridine is used in today's therapy for the treatment of multiple sclerosis, being one of the novel approved medicines by major regulatory bodies. Our research focused on the evaluation of modified release, HPMC-based matrix tablets containing fampridine as active substance. The study is based on the use of experimental design, which represents one of the emerging challenges and opportunities in modern generic drug development and analysis. The optimal tablet composition and selected manufacturing technology were evaluated by a QbD approach, applying full factorial design, with the help of MODDE 12.1 software. Four different hypromellose types (K100LV, K4M, K15M, K100M), two direct compression grades of microcrystalline cellulose (102 and 112), homogenization and lubrication times were selected as factors. Beside in-vitro dissolution tests, accordingly to FDA recommendations (phosphate buffer, pH = 6.8, 900 mL, 50 rpm), the impact of the selected factors were analysed by classical in-process controls. Release kinetic results were evaluated based on similarity factor calculations in comparison to the originator product, Fampyra 10 mg prolonged-release tablets. As expected the dissolution behaviour was strongly influenced by the applied HPMC type, and the effect of lubrication time was also noticeable at the first dissolution time points; while the impact of other formulation factors and process parameters were negligible in the *in vitro* dissolution. Formulae including HPMC K100LV returned the highest F2 values and in-process control results fit into the proposed requirements. The results obtained of the optimizer batch, formulated and manufactured according to the prediction of the in-silico analysis, confirmed the appropriateness of the refined model.

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Keywords: fampridine, HPMC matrix tablets, design of experiments, modified release

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P-13

Encapsulation of Sulfamethazine by Beta-Cyclodextrin and Random Methylated Beta-Cyclodextrin

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Supramolecular host–guest complexes are a fast advancing research field in chemistry, where encapsulation of a guest molecule inside the macrocyclic cavity of a host occurs. Cyclodextrins (CDs) have magical supramolecular recognition ability (alpha-, beta-, and gamma-CDs, respectively), which can bind selectivity various guest molecules to form stable host-guest complex in various fields; this includes drug carrier, chromatography and separation sciences, pharmaceutics. Sulfonamides (SAs), a series of drugs, are one of the most widely administered groups of antibiotics in human medical and veterinary purpose. These drugs are preventive and therapeutic agents for certain infections caused by gram-positive and gram-negative microorganisms. During the last years, big efforts have done to prepare sensing tools for SAs by CDs, because the drug are presented with considerable concentrations in surface water and wastewater or in meat-producing animals. However, the detailed characterization of the related SA – CD complexes is still missing. It is known, that the cationic, neutral, or anionic species often have different properties such as water solubility or complexation ability. Therefore, in this works, to model the pH dependent complex formation of SAs with CDs, the interaction of sulfamethazine (SMZ; $pKa_{,1}$ = 2.07, $pKa_{,2}$ = 7.49), one representative member of the sulfonamide drug family, with beta-cyclodextrin and random methylated beta-cyclodextrin at different pH have been investigated using spectrophotometric methods. Results show formation of stable SMZ – CD complexes and reflect importance of the competition of the hydrogen bonding and electrostatic interactions. The complex geometry formed is affected by the orientation of the pH-dependent dipole moment of SMZ molecule and prolonged prior the SMZ molecule enter into the CD's cavity. Functionalization of the CD molecule does not affect considerably the complex stabilities, therefore the parent beta-cyclodextrin molecule looks the simplest and most effective inclusion host to design selective and sensitive tool for SA sensing.

Keywords: beta- cyclodextrins , sulfonamides, sulfamethazine, host–guest complexes

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P-14

Expectations and Limits of Colloidal Systems as Carriers for Peptide Drugs

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Patient-Centred Pharmaceutical Design is encouraged by the European Medicines Agency (EMA) to achieve the desired therapeutic outcome raising up the necessity to develop novel dosage forms and offer alternative delivery routes [1],[2].

Parallel with these health care issues, an increased interest can be seen toward peptides in pharmaceutical research and development. Formulation of peptide-containing dosage forms is a complex task. Due to these facts, the importance of colloidal systems as peptide drugs-carriers is increasing on the pharmaceutical market, and the focus of pharma R&D turns to these systems leading to an increased number of research activities in this field. Dosage form design and the development of the proper administration routes are also challenging tasks, taking also into consideration the regulatory requirements of the different authorities.

The risk based thinking and risk management has to cover the whole development process starting from the API selection, via production process, until the expectations related to the clinical use and performance [3].

Our research group shares experiences with polymeric nanoparticles and liposomes as great potentials, being able to incorporate and carry instable biologically active substances into promising deliverable treatment options [4],[5].

Keywords: Colloidal systems, peptids , polymeric NPs, Liposomes, risk assessment

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P-15

Formulation Development and Image Analysis of Propellant-free Foams

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Nowadays foams are becoming more popular in several areas of life, including pharmaceutics and cosmetics. They are colloid systems, where the gas phase is dispersed in a continuous liquid phase. Pharmaceutical foams, containing active ingredients, are mainly applied dermally, but vaginal, rectal, as well as solid foams are available too. Foam formulations own numerous advantages compared to conventional creams or ointments. Easier and more comfortable application even on large, sensitive or hirsute areas, good spreadability without oily residues contribute to their increased patient compliance. Given proper composition and formulation, good rate of drug transfer can be provided. Other than surface active agents, foams can contain solvents, co-solvents and depending on the container – propellant. Medicated foams are characterized by pharmacopoeial tests to determine relative foam density and duration of expansion for pressurized formulations.

The aim of the experiments is to develop a propellant-free foam formulation suitable for topical delivery by finding connection between composition and foam characteristics.

The examined foams are generated from distilled water and xanthan gum solution by using distinct surface active agents (Tween 80 and Labrasol) with a propellant-free pump device. Viscosity and relative foam density measurements are carried out for the characterization. Image analysis (Image J) of foams provides information on the structure, the individual cell shape and size.

Image analysis results suggest that the type and ratio of excipients influence the morphological parameters (eg. size distribution and variability) of the foam.

Conclusions: Foam characteristics are influenced by the composition, the type and amount of detergent. Altering the media, the amount and type of surface active ingredients have a significant effect on the shape and size distribution of foam cells, resulting in modified relative foam density.

Keywords: foam, surfactant, propellant-free foam, image analysis, topical drug delivery

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P-16

Freeze-Drying of Pegylated Liposomes Loaded with Rosemary Extract

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Liposome dispersions, as novel drug delivery systems, do not meet the required standards for long-term stability of pharmaceutical preparations, due to their nature for aggregation, fusion and tendency for drug leakage.

It has been reported that nanoliposomes (NLs) containing drug molecules can be lyophilized and reconstituted with significant drug retention and without any big changes in the mean size. However, both, freezing and drying can induce NLs structural and functional damage.

In order to investigate the effect of the liophylization process and the effect of type of lyoprotectant on the physicochemical characteristics of Rosemary extract loaded pegylated NLs, different formulations (mass ratio lechitin:cholesterol:LIPOID PE18:0/18:0-PEG 2000 = 8.7:1:1.7 and 9:1:0.17 for $F1_0$ and $F2_0$, respectively) were prepared by a modified lipid film hydration technique using maltose, lactose or saccharose as lyoprotectants (lipid:lioprotectant mass ratio = 1:1.25). Before the freeze-drying process (-40 °C, 0.052 mBar; FreeZone 2.5 Freeze Dry System – LABCONCO, USA), prepared PEG-NLs dispersions (*F1* and *F2)* were stored at -20 °C for 24h and at -80 °C for additional 2h. With the aim to achieve the physicochemical properties of the native NLs, reconstructed samples were homogenized on 6500 rpm for 5 min or on 24000 rpm for 5 min.

Reconstructed NLs were characterized in terms of particle size (d_{50}), particle size distribution (PDI) and zeta potential (ZP) (Zetasizer Nano-Series, Malvern Instr. Ltd., UK).

After reconstitution, high shear homogenization (24 000 rpm, 5 min) was identified as the most suitable method for NLs downsize. Conducted characterization measurements of reconstructed NLs indicated that *F1* containing maltose as lyoprotectant (d_{50}= 110.2 nm, PDI = 0.279, ZP = -20.7 mV) had most similar properties compared to the native $F1_0$ NLs dispersion (d_{50} = 107.2 nm, PDI = 0.271, ZP = -18.5 mV).

This study demonstrated that the process of freeze-drying and the choice of lyoprotectant significantly influence the physicochemical properties of NLs. Further studies on the optimization of lyoprotectant amount and improvment of the process efficiency by optimizing the primary and secondary drying time will follow.

Keywords: pegylated nanoliposomes, stability, liophylization, lioprotectant, reconstitution

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P-17

In Vitro Dissolution Studies of Solid Dispersions of Celecoxib Prepared by Using the Solvent-Evaporation Technique and Liquisolid Impregnation Technique

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Over 40% of active pharmaceutical ingredients (API) in development pipelines are poorly water-soluble drugs which limit formulation approaches, clinical application and marketability because of their low dissolution and bioavailability. The improvement of their saturation solubility and dissolution rate is very important and also challenging. Solid dispersion has been considered one of the major advancements in overcoming these issues.

We prepared two different types of solid dispersions of celecoxib with Syloid 244 FP. First solid dispersion type was made by using the solvent-evaporation technique (A) with celecoxib dissolved in acetone and second by using the liquisolid impregnation technique (B) with celecoxib dissolved in polyethylene glycol 400. We used different celecoxib contents: for the solvent-evaporation method 15%, 33%, 40% and 60%, and for the liquisolid impregnation method we used 15% and 33%. All in vitro dissolution experiments were carried out in phosphate buffer pH 6.8 with the addition of 0.5% sodium lauryl sulfate, which showed to be most discriminatory for our samples, after preliminary solubility investigations of celecoxib. All dissolution experiments were carried out under sink conditions.

Solid dispersions in varying concentrations of syloid and celecoxib were formulated using the solvent evaporation method. Comparing these solid dispersions, it is obvious that the celecoxib content plays an important role. The samples of solid dispersions formulated with greater amounts of celecoxib (over 33%) showed a slower release of celecoxib than the pure substance. This can be attributed to the decrease of surface area available for dissolution due to pore filling. It was shown for liquisolid impregnated syloid carriers with PEG400/Celecoxib containing higher concentrations of celecoxib (33%) slow down the release, compared to the low concentration sample (15%). This pattern, which was also seen in the solvent evaporation solid dispersion samples, is a clear sign of the hydrophobic influence

of celecoxib on the overall release rate, regardless of the used hydrophilic excipient PEG 400. Further studies on the optimization of these two techniques will follow.

Keywords: dissolution studies, solid dispersions, solvent-evaporation technique, liquisolid impregnation technique, celecoxib

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P-18

In Vitro Dissolution-Permeation Study to Characterize Itraconazole Formulations: The Effect of Formulation Additives, Food and Dose

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Introduction: Although dissolution tests provide a simple way of testing formulations, the *in vivo* predictive power of these tests are questionable. When an API is formulated to enhance its dissolution additives, such as surfactants and cyclodextrins have an effect not only on dissolution profile, but also on flux through the membrane. The aim of this study was to investigate the effect of formulation additives, food and dose on the *in vitro* dissolution-permeation profile of Itraconazole, and compare the results to *in vivo* data.

Materials and methods: 3 formulations of Itraconazole: Sporanox solution (100 mg), Sporanox capsule (100 mg) and Losanoc capsule (50 mg), were tested using MacroFLUX™. Receiver chamber integrated with permeation membrane (Double-Sink™ PAMPA, 3.69 cm^2) and fiber optic UV probe (Pion Inc) was inserted in the standard 250 mL vessel of USP 2 apparatus.

Results: For simulating the fasted state media change from simulated gastric fluid to fasted state simulated intestinal fluid was carried out after 30 minutes. In the case of Sporanox solution Itraconazole stayed fully dissolved in SGF (400 µg/mL), while changing the pH triggered its immediate precipitation. In the case of Sporanox capsule the API created a supersaturated solution and already started precipitating in SGF. Losanoc formulation only started releasing the API in a significant amount after media change was conducted. Although the dissolution and precipitation kinetics of the two capsules were quite different, the flux results obtained showed no significant difference (Sporanox: 0.235±0.033, Losanoc 0.0272±0.008). These flux results were found to be in agreement with *in vivo* C_{max} results.

For simulating the conditions after food intake in the gastrointestinal tract, fed state simulated intestinal fluid was used in the donor compartment. While in the case of capsules similar flux results were obtained (0.424±0.005 and 0.407±0.014), which predict well the similar *in vivo* C_{max} values.

Conclusion: The dissolution and flux results of three marketed Itraconazole formulations were compared in fasted and fed state to each other and to the *in vivo* study results published by the manufacturers. The *in vitro* test was found to

be sensitive enough to show differences between formulations caused by the use of different excipients and produce the same rank order the formulations in fasted and fed state as *in vivo* results do.

Keywords: dissolution, permeation, absorption, flux, itraconazole

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P-19

Investigating Primary Structure Changes of Lactase Enzyme Influenced by External Factors

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Lactase is the enzyme responsible for digesting the lactose in the intestines. Lactose is a disaccharide component of milk. More than 25% of the human population can be diagnosed with lactose intolerance. The treatment of this disease involves the administration of various lactase containing medication. Recently among the available products in the market there are very few drugs contains lactase compared to dietary supplements.

The enzyme proteins like lactase have different structures with different properties due to their diverse functions and variations. Some structures are stable even in the gastric fluid and remain active even at acidic conditions, however, the above mentioned dietary supplements do not co ntain these types of enzymes generally. The therapeutic activity can be decreased not only by the organism. These ingredients are sensitive to pH, moisture, temperature and even to the applied excipients during manufacturing, therefore they can lose their effectiveness before administration. High variety of degradation processes can be present in the background of loss of function producing a highly complex substance to analyse.

For analysing such complex mixtures highly effective separation and structure analysis methods are indispensable. The exact alterations in the primary structure are examined by HPLC-MS/MS and proteomic analysis. We optimized a bottom-up proteomic procedure for stability testing of lactase under stress conditions to unravel the relevant degradation pathways of it. This dataset will help us to develop better understanding of stability based on structural information providing higher quality medications to the patients.

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Keywords: lactase, enzyme, protein, stability, HPLC-MS/MS

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P-20

Investigation of the Endocytosis and its Cellular Effects of Beta-Cyclodextrin Derivatives on Intestinal Epithelial Cells

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Cyclodextrins are widely used excipients for increasing water solubility, delivery and bioavailability of lipophilic drugs. Using fluorescent cyclodextrin derivatives we showed previously, that cyclodextrins are able to enter Caco-2 intestinal cells by endocytosis, but the different fluorescent labelling have not been compared on the same cyclodextrin derivative. On the other hand the consequences of the cellular internalization of cyclodextrins have not been revealed yet.

Our aim was to compare the cellular internalization of fluorescein and rhodamine labeled hydroxypropyl (HPBCD) and randomly-methylated beta-cyclodextrins (RAMEB). Using fluorescent microscopy and flow cytometry we tested the endocytosis of the fluorescent cyclodextrins on Caco-2 cells. We also examined the effect of these cyclodextrins on Nf-kappa B pathway and autophagy on Caco-2 cells.

Both fluorescein and rhodamine labeled derivatives are able to enter the intestinal Caco-2 cells by endocytosis in a comparable manner. Cooling almost perfectly inhibited endocytosis, while the application of rottlerin inhibited significantly the uptake of cyclodextrins. We investigated the possible activation of nf-kappa B pathway, which is important in regulating cellular responses. Cyclodextrin pretreatment did not activate the translocation of the p65 subunit of nf-kappa B heterodimer into cell nuclei both in cell monolayers or undifferentiated cells. After HPBCD and RAMEB treatments the presence of autophagosomes is detectable on fluorsecent microscopic images, similar to control samples.

The type of fluorescent labelling does not influence the internalization of HPBCD and RAMEB cyclodextrin derivatives. FITC and rhodamine conjugates showed similar intracellular localization. The endocytosis of cyclodextrin does not activate nf-kappa B pathway, while the examination of autophagy induction requires quantitative analysis.

Keywords: cyclodextrin, endocytosis, epithelial cells, NF-kB, autophagy

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P-21

Investigation of the Interaction Between a Polyene Drug and Some Serum Albumin Using Fluorescence Polarization Technique

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Nowadays, the treatment of fungal infections is a serious challenge, since microorganisms are widely resistant to most of the conventionally applied medications. Amphotericin B (AmB) is one of the antifungal drugs produced by *Streptomyces nodosus*, which is commonly used in the treatment of chronic and systemic fungal infections. Serum albumins (SAs), lipoproteins and glycoproteins are known carrier molecules of several endogenous molecules, drugs, and xenobiotics, therefore, protein-drug interactions play an important role regarding tissue distribution and elimination of these bioactive molecules. AmB is known to highly bounds to these plasma proteins in monomeric form. However, the action of AmB in biological systems is very complex, due to the fact, that the amphiphilic structure of this molecule promotes its self-association and aggregation and the molecular organization of the drug highly influences its pharmacokinetic behavior in living body. Therefore, it is not surprisingly, that it is very important and also a very challenging exercise to discover the physical chemical background of the interaction between AmB and carrier proteins. Nevertheless, during the last years several groups investigated the binding process of AmB to SAs, several questions still remains opened related to the complex formation of these materials. In this work our aim was to get a deeper insight into the molecular mechanism of the interaction between amphotericin B and some selected serum albumins using fluorescence polarization technique. The study of these protein-drug interactions may provide knowledge of the thermodynamics features and the binding forces, which are related to the therapeutic efficiency of the drug.

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Keywords: antibiotics, serum albumins, fluorescence polarization, aggregation, protein-drug interaction

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P-22

Investigations of the Mechanism Behind the Rapid Absorption of Nano-Amorphous Abiraterone Acetate

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Objectives: Abiraterone acetate (AA, Zytiga®) is poorly water soluble which results in very low bioavailability in the fasted state, high inter-individual variability and significant positive food effect. The objective of the work was to develop a novel formulation which overcomes these unfavorable properties.

Methods: AA formulations produced by different methods exhibiting different particle size and crystalline structure were tested. In vitro solubility, dissolution and permeability measurements were performed in biorelevant media. Absorption modelling was performed to predict fraction dose absorbed. AA hydrolysis was assayed by the incubation of samples with porcine pancreatic cholesterol esterase. Beagle dog studies and a phase I clinical study were conducted to characterize in vivo pharmacokinetics and food effect.

Results: Absorption modelling based on biorelevant dissolution tests showed that the concurrent increase of solubility and dissolution rate is necessary for improved absorption. This could only be achieved by a nano-amorphous formulation produced by controlled precipitation. In dogs, nano-amorphous AA exhibited >10-times higher AUC and C_{max} in the fasted state when compared to Zytiga® and exhibited no food effect. The higher apparent solubility of AA resulted in its faster enyzmatic hydrolysis. Co-administration of an inhibitor of cholesterol esterase in dogs showed that improved absorption is dependent on the hydrolysis of AA. In healthy volunteers AUC following the administration of 200 mg of the novel formulation was 81% of the AUC for 1,000 mg Zytiga®. Inter-individual variability was low and the extensive positive food effect was not observed.

Conclusions: A 250 mg oral dose of the nano-amorphous formulation is expected to result in the same exposure as 1000 mg Zytiga® in the fasted state. The mechanism behind the improvement is the immediate transfer of AA to bile micelles in the intestine followed by rapid conversion of AA to abiraterone by cholesterol esterase.

Keywords: abiraterone acetate, nano-amorphous formulation, absorption, bioavailability, food-effect

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P-23

Liposomes for Mucosal Lipopeptide Vaccine Delivery

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Liposomes have been extensively studied in the vaccine delivery field as a carrier and an immune stimulating agent. Liposomes are usually formulated as nanoparticles, mimicking the properties of pathogens, and have the ability to induce humoral and cell-mediated immune responses[1]. Noticeably, a few liposomal vaccine formulations have already reached market.

We have investigated lipid core peptide (LCP)-based self-adjuvanting system as a preventive vaccine against Group A Streptococcus. The lead vaccine candidate contains GAS M-protein derived conserved B-cell epitope as well as T-helper. Both peptides upon conjugation into LCP system demonstrated ability to stimulate systemic immunity after subcutaneous administration in mice.

Oral and/or intranasal deliveries of drugs/vaccines are the most preferable routes of administration. In addition, induction of mucosal immunity by GAS vaccine is highly preferable as the first step of GAS infection is typically following mucosal colonization. To induce strong mucosal immune responses, we developed un/coated liposomal delivery systems for LCP-based vaccine[2]. These delivery systems induced higher uptake of the vaccine candidate by antigen presenting cells as well as significantly higher antibody titers (IgG and IgA) than LCP alone in mice upon immunization. Both oral and intranasal administrations have also shown ability to induce long lasting immunity. Thus, using liposomes and liposomes coated with polymers we have developed promising delivery system for simple oral and intranasal vaccination.

Keywords: liposomes, peptide vaccines, oral and intranasal delivery, Group A Streptoccocus

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P-24

Novel Biodegradable and Biocompatible Poly(aspartamide)-dopamine Conjugates

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Novel controlled drug delivery systems (CDDS) which hold the ability of targeting and controlled release of drug molecules became one of the most researched fields in the pharmaceutics in the last decades. Drug-polymer conjugates or prodrugs are novel class of CDDSs for effective drug administration. Drug molecules can be covalently conjugated to macromolecules in such a way that their release is primarily controlled by the rate of chemical or enzymatic cleavage of polymer-drug bonds. Furthermore these formulations can deliver drug molecules to a particular body compartment, leading to lower systemic drug level; and protect the active agent from early metabolism. With further nanoformulation these prosperous properties can be increased to reach better drug therapy.

As several enzymes can be found in the human body which cleaves amide bonds, poly(amino acids) are promising candidates to prepare polymer-drug conjugates. In this work poly(aspartamide) based conjugates were synthesized with different chemical constitutions with dopamine as conjugated drug. The conjugates were synthesized from poly(succinimide) by nucleophilic addition. The drug release from the different conjugates was investigated in the presence of different enzymes and the kinetics of release was described.

Nano-fibrous meshes were prepared with electrospinning and the fibers were characterized by scanning electron, two photon and atomic force microscopy. The effect of the formulation for the dissolution as well as drug release kinetics was investigated in different circumstances. The membrane permeability of the conjugates was determined by parallel artificial membrane permeability assay. The cytotoxicity of the conjugates was studied with human derived stem cells.

Results showed that prolonged release of dopamine can be obtained via the enzymatic cleavage of dopamine from the polymer backbone. By electrospinning we could obtain homogenous nanofibrous implants with a diameter of 250-700nm. Due to the nanoformulation the solubility of the conjugates as well as dopamine release significantly increased compared to the bulk conjugates. According to the cytotoxicity experiment the conjugates decreased the cytotoxic effect of the dopamine.

Keywords: poly(aspartamide), conjugate, dopamine, release kinetics, electrospinning

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P-25

Optimalization of the Critical Process Parameters of Albendazole Containing Nanosuspensions Produced by Wet Media-Milling

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Introduction: Pharmaceutical nanosuspension is defined as very fine, colloid, biphasic, dispersed, and solid drug particles in an aqueous vehicle, with mean size below 1 μm, without any matrix material, stabilized by surfactants and polymers [1]. The potential benefits of the Nanosuspension Technology for poorly soluble drug delivery are increased drug dissolution rate, increased bioavailability, low incidence of side effects by the excipients, increased physical stability to settling and decomposition, provision of passive targeting,…etc. [2–4].

There are two converse methods available for manufacturing nanosuspensions: the assembling 'bottom-up' from molecules and the 'top-down' disintegration approach from large particles [5,6].

Materials and methods: The main objective of this work was to optimize the process parameters of albendazole containing nanosuspension development by top down, surfactant assisted media milling method, utilizing a planetary ball mill. Investigations included ideal composition determinations, type and amount of milling medium, loading ratios, milling speed and process time evaluations. Laser diffraction and dynamic light scattering were applied for particle size distribution determinations.

Response surface methodology and desirability approach were utilized to select the most suitable process parameters. The effect of scale up has also been investigated, along with the physical stability of the optimized nanosuspension, thermodynamic solubility values and dissolution profiles of albendazole in various pH buffer solutions.

Results: Nanosuspension criteria (submicronic fraction size> 90%, D [4,3] <1.0 μm, minimize Span values) have been passed at milling speed range of 200-500 rpm, with process time interval of 30-60 minutes, when 66.66 v/v% d=0.3 mm zirconium- oxide beads were applied in 50 ml stainless steel container. Scaling up to 500 ml however resulted in microsuspension, even after prolonged operations. Reducing the size of the milling medium to d=0.1 mm, yielded close to nanosuspension form after 60 minutes of milling on 600 rpm speed in 50 ml milling container. Scaling up to 500 ml seemed to spare some energy and time, lowest mean diameter, polydispersity values (173.5 ± 0.9659 nm, 0.175 ±0.012) were registered at 300 rpm, 30 minutes.

Conclusions: Nanosuspension development was successful, both solubility values and dissolution rates have been improved due to the effect of surfactant and particle size reduction.

Keywords: wet media milling, parameter optimalization, nanosuspension, scale up, particle size distribution, solubility, dissolution rate

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P-26

Optimization and Characterization of Nanosuspension of Cilostazol Utilizing Wet Ball Milling Technique

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Background: Cilostazol (CLZ) inhibits platelet aggregation and is a direct arterial vasodilator. It is approved for a treatment of Ischemic Symptom related to peripheral arterial occlusive disease in Japan. It is approved for a treatment of Intermittent Claudication in USA and UK. Recently, it used for prevention of Recurrence of Cerebral Infarction. Cilostazol is one such molecule which belongs to Biopharmaceutical Classification System (BCS) Class II based on poor solubility and high permeability. Therefore, improving solubility would enhance the oral bioavailability. Particle size reduction has been a much smart approach that can be applied to nano-specific formulation for many years. The particle-size distribution of the solid particles in nanosuspensions is usually less than one micron . The potential benefits of the Nanosuspension are an increase in their surface area which proportionally increase the dissolution rate and the saturation solubility, subsequently improve the bioavailability of poorly water soluble drugs and may also decrease systemic side effects.

Aim: The aim of this study is to overcome the limitations associated with the CLZ (The clinical efficacy of CLZ has been limited because of its poor solubility and dissolution leading to incomplete absorption in the GIT). Therefore, the objectives of this project involves around resolving the two main problems, solubility and dissolution rate of Cilostazol (CLZ) through ball milling technique by optimizing the different milling parameters like the milling time, milling speed, emulsifier type and concentration and milling program (periodic 5:5 and 10:10)

Results: In order to minimize thermal stress, the long milling cycles followed by equally long cooldown programm have reached sufficient particle size reduction rates with relatively low loading temperature values. The nature and quantity of the surfactants affect on size, shape and purity of the final product. The increasing milling speed and process time have siginicicantly reduced the mean particle size of CLZ, the widths of distributions (PDI) and also have a positive impact on physical stability indicated by the tendency of Zeta-potential values.

Conclusion: The significant mechanical forces, prolonged opperation and nature and quantity of surfactant are needed to meet the requirements of nanosuspension dimensions.

Keywords: cilostazol, dissolution rate, bioavailability, thermal stress, zeta-potential

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P-27

Parenteral Nanomedicines in Clinical Practice: Are Iron Sucrose Colloidal Solutions Interchangeable?

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Iron sucrose (IS) is an intravenously administered colloidal solution indicated in the treatment of iron deficiency (anemia). In Europe - but not in the USA - the marketing authorization of follow-on versions of IS has been obtained according to the classical generic approval for low molecular weight, fully characterized drugs. However, several clinical and physicochemical studies have demonstrated the lack of comparability and therefore of interchangeability between these different IS drug products, suggesting that the generic approach is not suitable to show equivalence of these nanomedicines.

The objective of this study was the evaluation of size and size distribution of IS and several iron sucrose similars (ISSs) as a critical quality attribute, after dilution with saline in polypropylene infusion bags, mimicking the preparation for administration in the hospital setting. The dilution of the drugs was performed in accordance with the IS's summary of product characteristics (SmPC).

Aliquots of IS and ISSs were withdrawn from the polypropylene bags at regular intervals over the course of 72 hours. Nanoparticles size and size distribution were examined using dynamic light scattering in addition to visual inspection of the physical appearance of the colloidal solutions.

The recorded data for the diluted drugs analyzed were dramatically diverging, especially at early sampling times. Statistically significant differences ($p < 0.0001$) were found when comparing both Z-average values and polydispersity index of IS and ISSs. Moreover and unexpectedly, the visual inspection of the ready-to-use dilution showed different colors as well as changed viscosity of some ISSs in comparison with the IS. These results are in agreement with previous, non-clinical and clinical data on IS and ISSs, demonstrating their non-equivalence and therefore, absence of interchangeability of such complex drugs in clinical practice.

Keywords: NBCDs, clinical practice, iron sucrose, size, critical quality attributes

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P-28

Pharmacokinetic Disimilarity of the Teicoplanin Composition Influencing its Bioequivalence

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Antecedents: Teicoplanin consists of a combination of five similar subcomponents ($A2_i$) and different compositions in pharmacopoeial-compliant batches can result in wide pharmacokinetic (PK) variations. As a result, bioequivalence of generic teicoplanin cannot be directly accepted from its chemical composition[1] as is commonly assumed[2] for injectables. Narrower specifications could ensure that PK differences are sufficiently small as to waive the bioequivalence study required to demonstrate therapeutic equivalence of generics.

The subcomponents composition can predict *in silico*[3, 4] the expectable total area under the curve (AUC) as the extent of systemic exposure. Nevertheless, reference data are hardly found in the literature and additional sources are required to enhance the validity of this approach.

Total AUC results from the additive contribution of each A2 subcomponent assuming that:

1. all subcomponents are equiactive antimicrobials, and
2. PK differences between them are only due to their physicochemical properties

Methods: PK results of a recent bioequivalence study (26 volunteers) following EMA Bioequivalence Guideline, have been used to investigate the validity of these previous assumptions.

The corresponding parameters: AUC, Clearance (CL) and elimination rate (k) have been calculated[5] for the sponsor's data. AUC values were used to calculate the clearance as CL = D/AUC. Elimination rate was calculated as the linear terminal slope and a virtual Volume of distribution (Vd) was finally estimated as the slope between CL and k.

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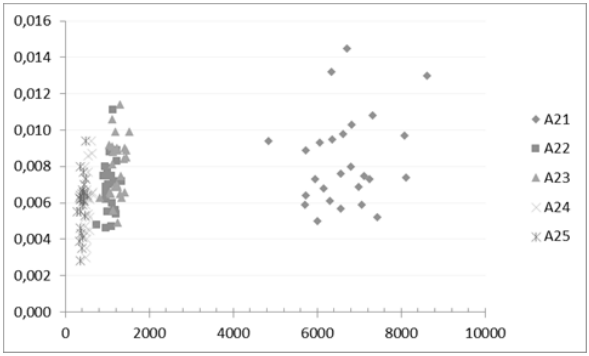
Polarity of the aliphatic radicals of each subcomponent was expressed as their theoretical partition coefficients (logP)[6]

Results: Interindividual variability of CL is remarkably higher for the more polar component A2-1 than for the others.

Determination coefficient (R^2) between CL and logP for all subcomponents equals to 0.5092. Excluding the value of A2-1, it raises up to 0.9187 ($p<0.05$)



A non-linearly correlated plot between CL (abscissa) (X) and k (ordinate) shows remarkable differences between A2-1 and the rest of subcomponents. Resulting Vd values suggest also a marked difference for A2-1 subcomponent which is about a 15% of the bulk.

Conclusion: A2-2 to A2-5 subcomponents are pharmacokinetically similar. Values obtained for A2-1 are clearly inconsistent. If confirmed with additional data, properties of these component cannot be considered additive to the whole drug product.

Keywords: teicoplanin, bioequivalence, variabilities

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P-29

Polymer Thin Coatings with Nanofibrillar Cellulose: Properties and Characterization

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New excipients in pharmaceutical industry have an important role for successful formulation of drug delivery systems and pharmaceutical dosage forms. Nanofibrillar cellulose (NFC) has several unique material properties for an excipient use in both immediate-release and controlled-release dosage forms. NFC is non-toxic, has a high surface area, optical transparency, and possesses excellent mechanical strength that could be used to improve the mechanical properties of solid dosage forms, especially coatings or protective material for wounds [1-3].

The aim of this study was to investigate the effects of NFC on the physicochemical properties of the moulded thin films. Special attention was paid to the capacity of the water imbibition /swelling and to the changes of mechanical strength.

For film moulding aqueous solutions of NFC (2.7-% aqueous dispersion, UPM Biofibrils AS 103, Finland) in different concentrations ranging from 0.27% to 0.54% were mixed in different proportions with polyethylene oxide (PEO, Sigma-Aldrich, Germany) 4% and 8% w/v, or polyvinyl alcohol (PVA, Mowiol®, Germany) 4% and 12% w/v water solutions. The moulded solutions were dried at 37˚C and then characterized by SEM (Zeiss EVO® 15 MA, Germany), optical microscope (CETI MagtexT), and texture analyzer (CT3 Ametek Brookfield, USA). The capacity of the water imbibition/ swelling was determined by weighing (APX-200, Denver instrument, Germany) and the thickness was measured by digital caliper (IRONSID).

The composite thin films of NFC and PEO or PVA differed by structure and transparency. The additives of different amount of NFC changed the PVA films less constricted and transparent, but the transparency of PEO films increased. Mechanical properties of moulded films with NFC differed from pure films, too. The additive of NFC increased thickness and decreased weight and mechanical strenght. The ability to swell increased when the amount of NFC in the films was increased.

In conclusion, the concentration of NFC greatly affects the formation and perfomance of the thin films.

Acknowledgements: *This work is part of the national research grant projects IUT34-18 and PUT 1088. Estonian Ministry of Education and Research is acknowledged for financial support.*

Keywords: nanofibrillar cellulose, film coating, PVA, PEO

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P-30

Prediction of Properties Associated with the Function of Nanofibrous Drug Delivery Systems Based on Their Microstructure

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The nanofibrous materials have shown a great interest due to their several potential medical and pharmaceutical applications. The hydrophilic polymer-based electrospun nanofibrous orally dissolving webs are promising candidates for rapid drug release, which is due to the high surface area to volume ratio of the fibers and the high amorphization efficacy of the fiber formation process. However the enhanced molecular mobility of these materials is responsible for their physical and/or chemical instability.

The primary aim of the project was to prepare poly(vinyl alcohol)-based, metoclopramide hydrochloride-loaded electrospun nanofibers using either polysorbate 80 or hydroxypropyl-β-cyclodextrin and tracked how the excipient influences the electrospinning process, the macro- and microstructures, mechanical behavior of the nanofibers and the drug release from the fibrous samples. The electrospun samples were subjected to several imaging techniques, and complex physicochemical characterization of the fibrous delivery systems was carried out which were enabled the better understanding the supramolecular interactions of multicomponent systems.

Scanning electron microscopy verified that clearly fibrous structures were obtained, without any beads and film-like areas. The mechano-manipulation of Atomic Force Microscopy revealed that the usage of polysorbate led to about two times stiffer, less plastic fibers than the addition of cyclodextrin. The cross-polarization build-up curves of ^{1}H-^{13}C nuclear magnetic resonance spectroscopy verified that cyclodextrin is an inner plasticizer, while polysorbate acts as an outer plasticizer and can migrate in the polymer matrix, which is due to its "liquid-like" behavior. Solid-state methods suggested that as a result of the fiber formation process the metoclopramide incorporated into the fibers in a purely amorphous state, but the usage of the examined additives enabled the development of a molecularly dispersed system of different homogeneities. The performed accelerated stability indicated a large stress tolerance capacity of the formulations.

Keywords: metoclopramide hydrochloride, electrospinning, nanofiber, inner and outer plasticizer, mechanical behaviour

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P-31

Prediction of the Safety Profile of Indole Derivatives as Src Tyrosine Kinase Inhibitors - Admet *In Silico* Study

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Src Family Tyrosine Kinase Inhibitors (SFKIs) have been designed as anti-cancer agents in past several years and several small molecules were approved by FDA for clinical studies. The discovery of novel small molecules with potential usefulness and design a potent, selective and less toxic anticancer agent is still a major challenge for medicinal chemistry researchers. Unwanted pharmacokinetic properties of drugs are major problems for drug development (Hisaka A, *et al.,* Pharmacol. Ther. 2010;125:230-248). In literature, it was shown that various substituted indolin-2-one derivatives have the ability to inhibit several SFKs (Zhang S, Yu D., Trends Pharmacol. Sci., 2012;33:122-128).

The aim of this *in silico* study was to evaluate 2-oxindole derivates (n=44) that were previously synthesized and tested on tyrosine kinase activity (Kilic-Kurt Z, Bakar F, Olgen S., Arch. Pharm. Chem. Life Sci., 2015;348:1–15; Kilic-Kurt Z, Onay-Besikci A, Olgen S. LDDD, 2013;10:713-718; Kılıc Z, Isgor YG, Olgen S. Arch. Pharm., 2009;342:333-343). Safety profiles of these compounds were predicted by using ADMET Predictor™ (SimulationsPlus Inc., USA). The outcome of ADMET analysis of investigated compounds revealed that predicted ADMET_Risk was in the range 0.0462-8.861, Absn_Risk 0.000-2.667, CYP_Risk 0.000-4.633, TOX_MUT_Risk 0.0-2.5, TOX_Risk 0.0-3.0. Among the most active investigated Src inhibitors the (*Z*)-1-ethyl-3-(3-(4-fluorobenzylidene)-2-oxoindolin-5-yl)urea (**10**) was revealed as the most safe molecule with the following predicted risk scores, *i.e.*, ADMET_Risk 1.132, Absn_Risk 1.117, CYP Risk 0.015, TOX_MUT_Risk 0 and TOX_Risk 0. Additional toxicity parameters were also predicted, *i.e.*, TOX_hERG (pIC_{50}=4.984 mol/L), rat (Xr) and mouse (Xm) carcinogenicities, as TD50 9.18 and 1231.227 mg/kg/day, respectively. The TOX_Risk score 2.757 was predicted for reference compound **imatinib**, while for **PP1** was 1. The main TOX codes that have been revealed for imatinib were cardiotoxicity (TOX_hERG Risk, pIC_{50}=5.802 mol/L), carcinogenicities Xr and Xm (TD50 4,998 and 26,699 mg/kg/day, respectively), as well as hepatotoxicity while for PP1 was Xm (TD50 5.873). The mutagenicity (TOX_MUT_Risks 0.0-2.5) has been mainly predicted in the group of tiourea-oxindole derivatives.

The results of this *in silico* study revealed that investigated oxindole derivatives with good in vitro activity and predicted IC_{50} values, physico-chemical and toxicity parameters are comparable to reference compounds **imatinib** and **PP1**.

Keywords: ADMET, *in silico* study, toxicity, indole derivatives, tyrosine kinase

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P-32

Preformulation and Formulation Studies of the Alcoholic Extract of *Punica Granatum* L., (Lythraceae) Exocarp as Antimicrobial Ointment

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The purpose of the study is to formulate an antimicrobial ointment from alcoholic extract of *Punica granatum* L., (Lythraceae) exocarp. An experimental research design was used. Results revealed there was a 32.31% yield of powdered extract from maceration. Physicochemical evaluation of extract present a dark brown powder, acidic (pH 3), bulk density (0.82), freely soluble in water, 95% alcohol, 0.1N HCl, 0.1N NaOH, 0.9% NaCl, phosphate buffers (pH 4,7 & 10) and petroleum ether and melted at 117.10°C. Chromatography confirmed the presence of terpenoids, alkaloids, anthraquinones and sapogenins. Extract was most active even at lowest concentration (25%) to *Pseudomonas aeruginosa* (31 mm), followed by *Aspergillus niger* (17 mm) and *Trichophyton mentagrophytes* (17 mm) ; *Staphylococcus aureus* (14 mm) and inactive to *Candida albicans* (9 mm) at all concentrations (100%, 75%, 50% and 25%). Minimum inhibitory concentration exhibited that *Trichophyton mentagrophytes* (MIC 0.000625g/mL) was the most sensitive to extract followed by *Pseudomonas aeruginosa* (MIC 0.00125g/mL), *Aspergillus niger* (MIC 0.0025g/mL), *Candida albicans* (MIC 0.005g/mL) and the least sensitive was *Staphylococcus aureus* (MIC 0.080g/mL). Extract was stable at different temperatures (4°C, 29°C & 40°C) after 3 months of exposure. Differential Scanning Calorimetry thermograph indicated that lyophilized extract was compatible with excipients namely polypropylene glycol 4000 (water soluble base), petrolatum (oil soluble base), methyl and propyl parabens (preservatives) and stearic acid (emulsifying agent). Formulated antimicrobial ointments (60% concentration) were prepared using mechanical incorporation. They were brown in color, odorless, non-gritty, neutral (pH 6.6), average spreadability (30.55 mm), average viscosity (64,000 cP) and average sensitivity test (0) for both water and oil based 60% formulations. It disclosed sensitivity to all test microorganisms; *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger*, *Candida albicans*, and *Tricophyton mentagrophytes*. Plastic ointment jar was used as packaging. Formulated ointments accelerated stability showed they were stable until the 6th month however on the 12th month it showed instability like solidification of ointment, bleeding and crystallization. It is conclusive that lyophilized extract of pomegranate can be formulated into an antimicrobial ointment however it needs further study in formulation it manifested instability on 12th month.

Keywords: preformulation, formulation studies, *Punica granatum* L., (Lythraceae)

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P-33

Quantitative Determination of ABC-Transporters by LC-MS/MS

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ATP-binding casette (ABC) transporters are very common in both prokaryotes and eukaryotes. ABC transporters often consist transmembrane domains and membrane-associated ATPase domains. ABC transporters transfer different substrates across the cell membrane using the energy of ATP hydrolysis. In human these are expressed in brain, small intestine, liver and kidney under physiological conditions. It is important to quantify these transporters in drug kinetics studies as these determine the drug concentrations in the cells. Overexpression of these transporter proteins in cancer cells may cause drug resistance during chemotherapy.

Our aims were to establish a suitable LC-MS/MS method for the absolute quantification of ABC transporters (MDR1, BCRP, MRP4, OATP1B1, OATP1B3, OCT2, OAT2) in various cell lines after development a simple sample preparation method.

CaCo2, KCR and HEK-293 cells were homogenised with ultrasonic homogenizer in RIPA buffer and with ProteoExtract® kit. Detergent removal spin columns and liquid-liquid extraction with ethyl acetate were tested to remove detergents from the samples. Proteins were digested with trypsin as a step of the 'filter-aided sample preparation' (FASP) method. Peptides were separated by nanoUPLC and analysed by coupled quadrupole-orbitrap hybrid mass spectrometer (Thermo Q-Exactive Plus). Two peptides with the least possible interferences, highest intensities and no post-translational modifications and their stable isotope-labeled derivatives were selected for quantitative determination of MDR1 protein. Using different sample preparation methods we were able to determine the absolute quantity of MDR1 in CaCo2, HEK293-MRP4, HEK293-OAT2, HEK293-OATP1B1, HEK293-OATP1B3, HEK293-OCT2 és KCR cell lines.

Regarding the other transporters we have already identified of those peptide pairs which will be appropriate for quantification of BCRP, MRP4, OATP1B1, OATP1B3, OCT2, OAT2 in different cell lines using their stable isotope-labeled counterparts.

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Keywords: ABC-transporters, MDR1, proteomics, LC-MS, cell lines

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P-34

Questions Concerning Equivalence of Vitamin D3 Therapies

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Cholecalciferol is available for treatment or prevention of Vitamin D deficiency. It is a well-known substance for which pharmacodynamic, pharmacokinetic properties are "text-book knowledge"; measured by a well characterized interim metabolite (25OHD) that has an individual based accumulation in various compartments and has an exceptionally long half-life in the body.

On the other hand, the case of cholecalciferol appears to be rather complex and remains one of the unanswered questions concerning bioequivalence. The absorption of vitamin D3 is controlled by a number of physiological processes and factors, the true absorption of this otherwise also endogenous substance could be difficult to determine in a bioavailability study. Increments in 25OHD per given microgram cholecalciferol varies in a broad range of 0.58-3.50 nmol/L in different studies. The in vitro dissolution data due to BCS class cannot be easily interpreted. The concerns to establish BE on daily or accumulated monthly doses have clinical impact and therefore it is also a regulatory issue. Moreover, the questions of substitution in between various doses applied or accumulated over the biological half-life period were examined in recent clinical trials, also in observational trials for real-life conditions that should provide input for operating guidelines in clinical practice. Clinical data shown that, if administered orally at level of daily dosing (1000-5000IU) absorption without an evident peak with a slow accumulation over 8-12 weeks expected. Higher single dose of >100,000IU shown a well-defined peak at 7 days and 3 months to restore pre-dose levels; however loading administrations of similar total doses provide a marked increase with a plateau.

Here we address some of the typical problems assessing the therapeutic equivalence of generic substitution. The concerns of supplementation therapy assessed by observational approach are based on various „real-life" conditions included age, lifestyle (UV-B exposure) and adherence of individuals, but also on level of depletion, accumulated dose and daily dose-equivalent given in a daily-, weekly-, monthly-schedule compared to bolus or loading dosing.

Keywords: vitamin D3, daily equivalence, substitution, clinical research, lifestyle

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P-35

Separation of Selected Group of Nucleobases and Nucleosides by Liquid Chromatography

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The aim of the present work was to study the separation and chromatographic behavior of mixture of 10 nucleobases and nucleosides (adenine, guanine, cytosine, thymine, uracil, adenosine, thymidine, uridine, xanthine and hypoxanthine) with the use of four kinds of stationary phases. Selected chromatographic columns (Waters XBridge Amide, Purospher Star RP-18, Zirchrom-Carb, Zorbax SB AQ) allowed to use different separation mechanism for analysis the mixture of analytes. Special attention was paid to the mobile phase composition (buffer type, pH, ionic strength), since it appears that this factor is very considerable in the case of chromatographic separation of nucleobases and nucleosides. We preferred usage of TRIS and TEAA (triethylammonium acetate) as buffer solutions, which are widely used in nucleic acids research. The achieved results show the potential of applied stationary phases with different selectivity using several buffer systems in liquid chromatography resolution of selected nucleic compounds.

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Keywords: HPLC, nucleobases, nucleosides, selectivity

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P-36

Stability of Ascorbic Acid in Hot Medicinal Drinks

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The stability of ascorbic acid (vitamin C) in different matrices is a well-studied topic, since it is known that different conditions (light, temperature, time) can cause the degradation of ascorbic acid.

The aim of this work was to study the stability of ascorbic acid in three types of vitamin C-containing hot drinks, available on the Romanian market (Coldrex®, Fervex®, and Gripovit®). The products – powders for oral solutions – indicated for the relief of cold and flu symptoms contain different amounts of ascorbic acid in a wide range (40-850 mg/sachet).

The solutions were prepared according to the product information: the content of a sachet was dissolved in 200 ml of hot (but not boiling) water and stirred well for two minutes. 100 µl samples were withdrawn periodically (0→120 min) and the amount of ascorbic acid was determined using an HPLC method (Merck HPLC system). The measurements were carried out using a pH=7.51 tetrabutylammonium sulphate buffer:methanol (88:12) as mobile phase at 1 ml/min flow rate and a Lichrocart (250-4) Lichrospher RP-18 (5 µm) column as stationary phase with detection wavelength of λ=264 nm. The injection volume was 100 µl. All experiments were performed in triplicate. The method was validated for parameters such as linearity, specificity, accu racy, LOD and LOQ.

The dissolution profiles for the powders for oral solution show a gradual increase, reaching 104.55±4.8%, 99.42±0.5% and 101.39±1.2% at about 40-60 minutes, for Coldrex®, Gripovit® and Fervex® hot drink, respectively. The dissolution/degradation profiles show that in the case of Coldrex® drink, at the beginning and the end of the experiment (0 and 120 min) 83.56±0.3% and 92.32±3.5% of the total amount of the declared ascorbic acid amount is dissolved/present in the solution. A higher degradation can be observed in the case of Gripovit®, when after 120 minutes only 57.82±4.8% vitamin C is present. In the Fervex® sample degradation of the ascorbic acid is not so ponderous, but the dissolution of vitamin C is slow.

The high variability between these products can be explained by the formualtion of the powders for oral solution. Despite the fact that the powders were dissolved in hot water, degradation of the vitamin C is slow and occurs in a lesser e xtent due to the low pH of the obtained solutions.

Keywords: ascorbic acid, stability, medicinal drinks

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P-37

Stability of Semisolid Extemporaneous Preparations of Cocoa Butter

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Pharmacy practice includes traditional and extemporaneous products that are well-tolerated by patients, for which shelf-life time cannot be accurately provided. In such cases, the product may not meet the desired requirements even within the expiry date. This problem not only gives rise to uncertainty among patients and pharmacists but is also of quality concern [1, 2]. Moreover, by carrying out certain examinations, even in the small-scale production, an appropriately stable pharmaceutical composition can be prepared.

The aim of this study is focused on the reproduction of a routinely used individual preparation, its physicochemical, accelerated and real-time stability testing to predict the rate of change at a proposed storage temperature.

Five variations of the chosen ointment were freshly prepared and subjected to accelerated stability testing at 40˚C; 75±5% relative humidity and 25˚C; 40±5% relative humidity. The preparations were monitored, and few units of the reference material were taken at 1, 3 and 6 month intervals. During the stability testing process the following experiments and tests were conducted according to the Hungarian and European Pharmacopoeias: Dropping point and freezing point measurements, extensometric test, microscopic examination, pH measurements of the aqueous phase, rheometric, dissolution and diffusion tests.

The study revealed that the choice of an optimal method of preparation results in a more stable pharmaceutical product than the original preparation. Even similar production methods resulted in ointments with significantly different physicochemical parameters. Based on the study, we can recommend a good manufacturing practice, expiry date, packaging material and storage conditions regarding the chosen formulation. These results confirmed that the physical and chemical stability of the ointments were achieved with the appropriate choice of the preparing conditions.

Keywords: stability testing, cocoa butter, extemporaneous semisolid preparation, dissolution test, rheology

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P-38

Structural Evaluation of Immunoglobulin G Glycans

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Glycosylation is a critical quality attribute for biopharmaceuticals. The latest technological advances facilitate the study of not just the oligosaccharide composition of a protein, but also localization of particular monosaccharides. We have developed a straightforward approach to distinguish core and antenna fucosylation in glycopeptides and applied this technique on antibodies which are N-glycosylated at the constant heavy 2 domain.

A complex purification procedure, including dialysis and IgG-specific immunochromatography was established to yield sufficient amount of IgG for glycopeptide analysis. Prior to glycomic analysis, samples were digested with Lys-C/Trypsin. For mass spectrometric analysis we used a nanoLC coupled to a Bruker Maxis II Q-TOF. Low collision energy CID spectra were evaluated to determine position of fucose.

The sample preparation method produced adequate amount (50 µg) of IgG for subsequent analysis. Glycosylation pattern of IgG subclasses were determined. The analysis revealed 20 different oligosaccharide structures from all the three major types of N-glycan classes. Optimization of collision energy resulted in spectra where only single step fragmentation processes occur, making the method well suited for analytical work. Monitoring the change in the proportion of core and antenna fucosylation at the same glycosylation site is also feasible. Detailed characterization of glycan structures contributes to improve quality control over antibody production and facilitates refined biomarker research.

Keywords: mass spectrometry, glycosylation, fragmentation, CID, fucosylation

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Taste Masking of Enalapril Maleate by Spray - Drying Technique

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Many active pharmaceutical ingredients have unpleasant or bitter taste, which is often a prerequisite for low patients' compliance. In order to improve the dosage forms organoleptic properties, various taste masking techniques have been developed. Microencapsulation is a reliable method for the masking of APIs' bitter taste. The purpose of this study was to obtain enalapril-loaded microspheres with high taste-masking efficiency. Microspheres were prepared by spray-drying technique using Eudragit EPO® as a polymer material. Seven models were obtained with varied drug-polymer ratios. Drug-polymer solutions were prepared in 0.1 M HCL and a certain amount of talc was added as a thickening agent. The suspensions were spray-dried under the following conditions: inlet temperature 65°C, outlet temperature 30°C, aspiration 100% and pump rate 10 %. The obtained powders were further characterized in terms of size, shape, production yield, moisture content, drug loading, encapsulation efficiency and drug release. The spray-dried models were analyzed spectrophotometrically for drug content in simulated gastric fluid (SGF, pH 1,2) and drug release study was carried out in simulated salivary fluid (SSF, pH 6,8). The obtained microspheres were white in colour and spherical in shape.The production yields varied between 51,29 ÷ 85,41 %, drug loadings were between 7,75 ÷ 24,69 %, drug encapsulation efficiency was in the range 58,52 ÷ 95.68 %, moisture contents varied between 7,12÷ 10,32 %, particle size ranged between 5,00 ÷ 17,47 μm. X-ray diffraction patterns and differential scanning calorimetry thermographs revealed transformation of the crystalline enalapril maleate into amorphous state. *In vitro* and *in vivo* taste evaluation studies determined the formulations with the highest taste-masking achieved. Models M6, M7 and M10 showed minimal or no detection of API in SSF. The results proved the taste masking abilities of the spray-drying method using pH-dependent polymer.

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Keywords: taste masking, spray-drying, microspheres, Eudragit EPO®, Enalapril maleate

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The Effect of the Secondary Interaction on the Fiber Formation Process and Stress Tolerance Capacity of Electrospun Delivery System

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The papaverine hydrochloride has a non-specific direct relaxant effect on smooth muscles. One of the therapeutic indications of the orally administered drug is the cerebral ischemia, but some unfavorable properties confine its clinical applicability. A nanofibrous buccal formulation can improve the oral bioavailability of the drug with the enhanced solubility and circumvention of the intensive first pass metabolism.

This study aimed to prepare papaverine hydrochloride-loaded, hydroxypropyl cellulose - poly(vinyl alcohol) composite-based buccal nanofibrous sheets. As the viscoelasticity and the polymeric chain entanglement density influence, the success of the fiber formation, the optimum composition of mucoadhesive polymers was determined with the combination of rheological and molar reflectance measurements of the initial aqueous gels, and with the morphological characterization of the prepared electrospun samples. In the case of the best composition system, an accelerated stability test was carried out to monitoring the physical ageing of the incorporated drug and the polymeric carrier. The stress indicated morphological changes and micro- and macrostructural alterations were detected using scanning electron microscopy, positron annihilation lifetime spectroscopy and Fourier transform infrared spectroscopy.

A correlation was found between the micro- and macrostructural properties of the gels and their electrospinnability: gels of the lowest elasticity and slightest intermolecular interactions resulted in the best fiber characteristics of the electrospun samples. During the stability test, two-step ageing process of the drug carrier and a partial phase transition of the papaverine hydrochloride were observed. The results pointed out that the formed weak secondary interactions were beneficial for the fiber formation, but the weak stress tolerance capacity can be attributed to this also.

Keywords: papaverine hydrochloride, electrospun nanofiber, dynamic moduli, molar reflectance, accelerated stability

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Validation of the Hansen Solubility Parameters as Co-Crystal Formation Prediction Tool

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Introduction: Co-crystals can improve the solubility of active pharmaceutical ingredients (APIs). Despite advances in co-crystal screening, selection of co-formers likely to form co-crystals as well as improve the API properties remains challenging. Arising from the definition of co-crystals which are homogeneous crystalline mixtures of the API and co-former, their miscibility can be used in the prediction of co-crystal formation. Hansen solubility parameters (HSPs) has previously been used as a tool to predict miscibility of co-formers using only the chemical structure. HSPs is an approach where the liquids total cohesion energy (δ) is split into contributions from hydrogen bonding, atomic dispersion and polar interactions. Co-formers with solubility parameters closer to the API are more likely to be miscible and to form co-crystals. The aim of this study is to validate the use of HSPs as co-crystal formation prediction tool, analyse its limitations and examine the $\Delta\delta$ inclusion cut-off value previously set at $<7M_P^{0.5}$.

Methods: A total of 111 reported co-formers of carbamazepine, theophylline and caffeine, of different preparation methods and conditions were used as a training set. The list included drug-drug co-crystals. Calculations were performed using HSPiP software. Regression correlations were used to best understand the parameters and 16 descriptors were statistically evaluated. Finally, the parameters were tested on 44 reported piroxicam co-formers.

Results and conclusion: Our results are in agreement with the reported cut-off value of $\Delta\delta$ for co-crystal formation prediction. This approach had shown high sensitivity (86.5%), but low specificity (50%). The use of a slightly different method ($\Delta\delta_t$) represents a better representation the 3-D solubility differences, and has showed a higher specificity (63.6%), but a rather low sensitivity (59.6%). Both approaches have statistically shown a difference between successful and failed pairs (p-values were 0.001 and <0.001 respectively). Further studies are needed to explain limitations of this method.

Keywords: Hansen solubility parameters (HSPs), prediction, pharmaceutical co-crystals, miscibility, screening

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Biocompatible Hybrid Hydrogels for Medical Use and Determination of Residual Monomers in Them

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Copolymer hydrogels, which are spatially crosslinked hydrophilic polymers, due to their high biocompatibility and a wide range of improved operating parameters, widely used now in medicine and science-intensive technologies. Commonly hydrogels are network polymer materials with high hydrophilicity (due to the presence of ionogenic functional groups) and insolubility in water and other solvents (due to the presence of 3-dimensional spatial network of polymer chains). Increased biocompatibility of hydrogels is a consequence of their high hydrophilicity and similarity in structure to human body tissues. The main reagents which was used for the synthesis of hybrid hydrogels, are first of all monomers of different nature such as hydrophilic acrylamide, hydrophobic acrylonitrile, ionogenic acrylic acid and thermosensitive N-isopropylacrylamide. By means of copolymerization of monomers with various nature it becomes possible to purposefully vary polymer matrix properties in a wide range.

In particular, on their basis produced various sensors and transducers, separating membranes, implants for plastic and reconstructive surgery, transdermal therapeutic systems for address sustained release of incorporated drugs, biomedical electrodes, etc. The main condition for the successful application of hydrogels in medical practice is the absence of residual toxic monomers in their composition. In this regard, an urgent task is to develop techniques that allow reliable determination of residual amounts of monomers in a biocompatible hydrogels.

We have developed a HPLC method for the joint determination of acrylamide, acrylonitrile, N-isopropylacrylamide in the copolymer hydrogels, synthesized on the basis of these monomers. Extraction of monomers from the hydrogels was carried out by 90% methanol. Separation of substances realized on reversed-phase column, eluted with aqueous methanolic solution.

This technique was used to optimize the cleaning process of new biocompatible hydrogels for medical supplies from the trace amounts of unreacted monomers.

Keywords: biocompatible hydrogels, acrylamide, acrylonitrile, acrylic acid, N-isopropylacrylamide, HPLC

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