XV Italian-Hungarian Symposium on Spectrochemistry
Pharmacological Research and Analytical Approaches

June 12 – 16, 2016, Pisa, Italy

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XV Italian–Hungarian Symposium on Spectrochemistry
Pharmacological Research and Analytical Approaches

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Scope

The fifteenth edition of the Italian-Hungarian Symposium on Spectrochemistry is held in Pisa (Italy), 12 – 16 June, 2016, under the auspices of the Italian Society for Applied Pharmacological Science. As a part of the bilateral governmental programme for scientific and technical co-operation between Italy and Hungary, this series of biennial events are a well-established tradition which provides scientists from both countries with a permanent forum to discuss the outcome of their studies, primarily in the field of human health and environmental protection.

The impressive advancement made by pharmacology in recent decades has had undeniable benefits for the society as a whole, primarily because of the widespread availability of pharmaceuticals in the medical practice to effectively treat most pathologies.

The XV Italian-Hungarian Symposium on Spectrochemistry deals with the most recent advances in this field as well as with the role played to this end by innovative analytical technique as in many instances analytical chemistry strongly support pharmacological research. This aspect permeates every aspect of the conference. The backbone of the Symposium consists of approximately fifty oral presentations. In their turn, poster presentations – over thirty in total - are displayed for the entire duration of the event.

Last, but not least, a number of oral presentations are given by prominent scientists from countries other than Italy or Hungary so as to further stimulate the mutual exchange of views and gain information on other possible approaches adopted to promote research, stimulate innovation and establish new analytical approaches in the field of pharmacology.

Previous Symposia

I. Italian-Hungarian Symposium on Spectrochemistry: Environmental Protection and Spectrochemistry, 1983, Rome, Italy
II. Hungarian-Italian Symposium on Spectrochemistry: Natural Materials and Spectral Analysis, 1985, Budapest, Hungary
III. Italian-Hungarian Symposium on Spectrochemistry: Biomedical Research and Spectrochemistry, 1987, Ispra, Italy
V. Italian-Hungarian Symposium on Spectrochemistry: Quality Control and Assurance in Life Sciences, 1991, Pisa, Italy
VI. Hungarian-Italian Symposium on Spectrochemistry: Advances in Environmental Sciences, 1993, Lillafüred, Hungary
VII. Italian-Hungarian Symposium on Spectrochemistry: Innovative Methodologies for Health and Environmental Protection, 1995, Rome, Italy
VIII. Hungarian-Italian Symposium on Spectrochemistry: Analytical Techniques in Environmental Chemistry, 1997, Debrecen, Hungary
IX. Italian-Hungarian Symposium on Spectrochemistry: Urban Health: a Challenge for the Third Millennium, 1999, Siena, Italy
X. Hungarian-Italian Symposium on Spectrochemistry: Trace Substances in the Biosphere, 2001, Eger, Hungary
XI. Italian-Hungarian Symposium on Spectrochemistry: New Challenges in Human Health Protection: Anthropic and Remote Areas, 2003, Venice, Italy
XII. Hungarian-Italian Symposium on Spectrochemistry: Environmental Pollution and Human Health, 2005, Pécs, Hungary
XIII. Italian-Hungarian Symposium on Spectrochemistry: Environmental Contamination and Food Safety, 2008, Bologna, Italy
XIV. Hungarian-Italian Symposium on Spectrochemistry: Analytical Techniques and Preservation of Natural Resources, 2011, Sümeg, Hungary
Photo Gallery
The Way We Were

In Memoriam Professor Károly Zimmer
1928-1991

for his friends (spectroscopists all over the world):
Zimmer Karcsi

60th Birthday, Herlany, 1988
IHSS – Environmental Protection and Spectrochemistry, 1983, Rome
II IHSS – Natural Materials and Spectral Analysis, 1985, Budapest
[No photos available]

III IHSS – Biomedical Research and Spectrochemistry, 1987, Ispra
Photo Gallery - The Way We Were
IV IHSS – Spectrochemical Monitoring and Testing of Chemicals, 1989, Veszprém [No photos available]

V. IHSS – Quality Control and Assurance in Life Sciences, 1991, Pisa
VI IHSS – Advances in Environmental Sciences, 1993, Lillafüred
[No photos available]

VII IHSS – Innovative Methodologies for Health and Environmental Protection, 1995, Rome
VIII IHSS – Analytical Techniques in Environmental Chemistry, 1997, Debrecen [No photos available]

IX IHSS– Urban Health: a Challenge for the Third Millennium, 1999, Siena
[No photos available]

X IHSS – Trace Substances in the Biosphere, 2001, Eger
XI IHSS – New challenges in human health protection: anthropic and remote areas, 2003, Venice [No photos available]

XII IHSS – Environmental Pollution and Human Health, 2005, Pécs
XIII IHSS, Environmental Contamination and Food Safety, Bologna, 2008
XIV IHSS – Analytical Techniques and Preservation of Natural Resources, 2011, Sümeg
Oral Session 1

New Trends
in Pharmacological Research

Chairpersons
Marco Romano
Beáta Sperlágh
Until the mid ‘90s of the last century, talking about drug discovery and development was essentially referred to the process of discovery and development of small-molecule chemical entities. All the language, common procedures and technicalities were inherent to this setting; concepts such as drug design, hit and lead compounds and high-throughput screening were referring to the typical mode of investigating and selecting small-molecule candidates through pre-clinical development.

At that time, the arrival of the first biotech drugs has markedly changed the landscape, requiring a completely new approach to pre-clinical development. Issues related to drug-receptor interaction or to the selection from a huge number of candidates were obviously simplified to a minimum compared to the small-molecule setting. Conversely, the pharmaceutical development of biotech drugs showed far higher complexity and the overall high complexity of industrial production initially represented a major issue to justify the high costs of biotech drugs. Furthermore, the technical impossibility to make “generics” of biotech drugs was another strong reason driving pharmaceutical companies toward the development of biotech drugs.

Thus, in the beginning the early development phases of small-molecules and biotech drugs really stood as two distant planets. However, nowadays such initial distances have been progressively decreasing. From the biotech site, the technique of phage-display library scanning and similar approaches made the selection of biotech lead compounds resembling more closely what happens with small molecules; also the complexities and high costs of pharmaceutical production have progressively reduced their impact. On the other hand, the impressive progress of basic knowledge on the human kinome and other relevant fields of molecular and cellular biology made it possible today to have small-molecule drugs targeting the same pathologies once specifically targeted by biotech drugs, MOABS in particular.

Another factor (albeit not related to drug development) with an important role in reducing distances between small-molecule and biotech drugs has been the novel
approach to estimate the value of drugs and therefore their prices. Since nowadays the major drive fixing the value (and costs) of new drugs is the added value for human health, any difference related to the costs of production has been greatly reduced and we have today several high-cost small molecules along with their sibling biotech drugs. Last but not least, biotech drugs can be “copied” as well and there are now biosimilar drugs, although the pathway to develop copies of biotech drugs remains steeper compared to that leading to the equivalents of small-molecule drugs.
Finding the sweet spot – the role of nature and nurture in medicinal chemistry

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Given its position at the heart of small-molecule drug discovery, medicinal chemistry has an important role in tackling the well-known productivity challenges in pharmaceutical research and development. In recent years, extensive analyses of successful and failure in discovery compounds and drug candidates have improved our understanding of the role of physical-chemical properties (molecular weight, logP etc.) in drug attrition. These studies have also clarified the difficulties in finding the “sweet spot” in medicinal chemistry programs.

Challenges for identifying improved drug candidates within the “sweet spot” indicates that this goal can be achieved through a combination of identifying chem-

ical starting points with appropriate “nature” and then “nurturing” them carefully during lead optimization. Analysing medicinal chemistry programs we concluded that the use of ligand efficiency indices\(^2\) and considerations on binding thermodynamics\(^3\) would help selecting good quality starting points and could also contribute optimizing them to compounds with improved physical-chemical profile. In this presentation we show how the assessment of ligand efficiency and binding thermodynamics could support medicinal chemistry efforts and suggest optimization guidelines for drug discovery teams. These suggestions might help revisiting present medicinal chemistry practices with the aim of promoting more effective use of what is already known and wider appreciation of the risks of pursuing sub-optimal compounds.


Major Depression (MD) is the most common psychiatric disorder with 10-15% lifetime prevalence in developed countries. The incidence of depression is continuously increasing and is expected to be the second major cause of disability by 2020 (WHO, 2001). Schizophrenia is the second most common psychiatric disorder affecting about 1% of the population worldwide. Although symptomatic treatment exists for both of them, depression and schizophrenia are still devastating conditions that represent a huge economic and social burden. Moreover, a relatively high proportion of patients do not respond to existing medications, thus urging the discovery and validation of new potential therapeutic targets. According to current knowledge, psychiatric disorders are caused by complex interactions between genes, developmental and environmental factors involving a multiplicity of neurotransmitters and signaling pathways. Gene polymorphism studies have revealed that non-synonymous Single Nucleotide Polymorphisms (SNPs) in the human P2X7 gene (P2RX7) are associated with Bipolar Disorder (BP) and Major Depressive Disorder (MDD). These mutations, together with other SNPs might underlie or convey the effects of environmental factors to susceptibility to mood disorders. P2X7 receptors (P2rx7) belong to the ionotropic P2X receptors that are sensitive to ATP and other purine and pyrimidine nucleotides. Our studies revealed that the genetic knock-down or pharmacological antagonism lead to reduced depressive-like behavior, attenuated response in mania-models and alterations in stress reactivity. A potential mechanism of P2rx7 activation on mood related behavior is increased glutamate release, activation of extrasynaptic NMDA receptors and subsequent enduring changes in neuroplasticity. Moreover, dysregulation of monoaminergic transmission and HPA axis reactivity could also contribute to the observed changes in behavior.

In addition to mood disorders, P2rx7s might also participate in the pathological process leading to schizophrenia. Recently we examined how animal behavior and gene expression are altered in the absence of P2rx7s in a phencyclidine (PCP)-in-
duced rodent schizophrenia model. In wild-type mice PCP induced hyperlocomo-
tion, stereotype behavior, ataxia and decreased social interactions, mimicking posi-
tive and negative symptoms of schizophrenia. In mice, genetically deficient in P2X7
receptors, the social interactions were increased, whereas the PCP-induced hyperlo-
comotion and stereotype behavior were alleviated. P2X7 receptor antagonists large-
ly replicated the effect of gene deficiency on PCP-induced behavioral changes and
counteracted PCP-induced social withdrawal.

In conclusion, these results underscore the significance of understanding
gene-environment interactions in the identification of new drug targets and point
to the therapeutic potential of P2rx7 in psychiatric disorders.
New approaches in cancer therapy

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Cancer is a disease characterized by a deviation in the mechanisms that control cell proliferation and differentiation. Neoplastic cells show features of apparent “immaturity”, express surface antigens normally present during the fetal age, develop qualitative and/or quantitative chromosomal abnormalities, excessively proliferate and can invade several body tissues. The ideal antineoplastic agent should be able to destroy cancer cells only, without damaging normal tissues. However, most of the anticancer drugs currently available are not specific and, consequently, they not only kill cancer cells, but can also indiscriminately attack healthy cells, resulting in severe side-effects that reduce their clinical efficacy. The toxicity of conventional chemotherapeutic drugs, as well as the development of multidrug resistance, led to the search of new effective treatments based on the cancer biology. In the last two decades, the characterization of the molecular networks controlling cell proliferation and differentiation, as well as neoplastic transformation and progression, allowed the formulation of drugs specifically affecting those networks and opened the door to the “targeted” therapy. The aim of this therapy is to interfere with specific molecular targets involved in tumor growth and progression, which, although present in normal tissues, are vastly overexpressed or mutated in cancer cells.

Targeted therapies may involve direct or indirect approaches. The first approach alters specific cell signaling pathways using monoclonal antibodies or small molecule inhibitors, including antisense oligonucleotides, directed against receptors, growth factors, protein kinases and phosphatases, oncogenic transcription factors, molecules related to apoptosis and/or cell survival, angiogenesis and invasiveness, adaptor proteins, protein degradation and chaperoning targets, as well as chromatin remodeling factors. The indirect approaches deliver non-specific cytotoxic agents (i.e., conventional anticancer drugs, toxins, cytokines or radionuclides that can be conjugated to monoclonal antibodies or to small peptide ligands or included in nanocarriers) to molecular targets mainly expressed on the neoplastic cell surface. In this way, for example, antibody-drug conjugates combine the targeting properties of monoclonal antibodies with the cytotoxic effects of the above mentioned drugs, leading to a selective accumulation of the latter in the neoplastic cells, and decreas-
ing peripheral toxicity. In conclusion, the great number of potential novel and specific molecular targets offer a key opportunity for solving the problem of cancer chemotherapy and may improve the possibility of effectively treating many cancers that are currently intractable.
Why do we still not have cardioprotective drugs? Need for unbiased “omics” approach and co-morbidity models to find valid targets

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Ischemic heart disease is the leading cause of mortality worldwide. Therefore, identification of valid drug targets for cardioprotection is of great importance. On the other hand, we still do not have cardioprotective drugs on the market. The discovery of ischemic preconditioning (three decades ago), post conditioning and remote conditioning triggering endogenous cardioprotective mechanisms that render the heart more resistant to lethal ischemic-reperfusion injury gave much hope to identify cardioprotective drug targets. However, it seems that major cardiovascular co-morbidities such as hyperlipidemia, diabetes and their co-medications interfere with most of the known cardioprotective mechanisms. Ischemia reperfusion injury and cardioprotection by conditioning have been shown to affect global myocardial gene expression profile at the transcript level. Moreover, fine tuning regulators of mRNA expression, micro RNAs, also contribute to cardioprotective gene expression response of the heart. Cardiovascular co-morbidities have been also shown to affect global cardiac gene expression profile. Further understanding and comprehensive analysis of the cardioprotective gene expression fingerprint at the transcript and protein level in normal, protected and comorbid conditions may lead to the identification of novel molecular targets for cardioprotection.
To increase soundness of *in vitro* pharmacological preclinical research: focus on the appropriate cellular system, high-content analysis and regulatory considerations

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*In vitro* tests based on cell cultures are a key component in the drug discovery pipeline for both efficacy and safety studies, determining a high component of the attrition rate of new molecules. Thus, the appropriateness of the cell system, the choice of the experimental endpoints and the analytical tests must be carefully considered. Moreover, the arrival of new actors (e.g., biological drugs and biomaterials) and new technologies (high-throughput analysis) is further challenging the scenario.

In this presentation we will discuss these topics focusing on *in vitro* tests based on neural cells by comparing different cell types (neural cell lines, primary cultures, stem cells-derived cells), analytical methods (viability assays based on biochemical – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Lactate Dehydrogenase (LDH) – and imaging - mitochondrial dyes, nuclear morphology-) and technologies (conventional vs. cell-based high content analysis).

Results from two different sets of experiments are presented. In the first set, we analyzed cell death in the Oxygen and Glucose Deprivation (OGD) model, comparing cell system (pure neuronal, -98 % neurons; 2 % astrocytes- vs. mixed neuron-astrocytes, -50 % neurons; 50 % astrocytes- culture) and viability tests (biochemical-MTT, LDH- vs. high-content morphology). We found that the neurotoxic effect of the OGD is observed in pure neuronal, but not in mixed neuron-astrocytes culture. We then compared the statistic power of biochemical and high-content mor-
phological techniques to evaluate neuroprotection exerted by two classes of drugs, e.g., Poly(ADP-Ribose) Polyerase-1 (PARP) (Thieno[2,3-c]Isoquinolin-5-one, TIQ-A) and pan-caspase (ZVAD-FMK) inhibitors. In the second set of experiments, we used in vitro neural system to test efficacy and toxicity of biomaterial under study for the development of novel drug delivery solutions for regenerative medicine of the nervous tissue. The biological and molecular effects of Poly(L-Lactic Acid) (PLLA) scaffolds having different surface geometry (conventional 2D flat surface, random or aligned fibers as semi-3D structure) and chemical functionalization [laminin or Extracellular Matrix (ECM) extract] were studied using three neural systems, i.e., the neural cell line SH-SY5Y, primary cortical neurons and neural stem cells. The end-points were defined for efficacy (i.e., neural differentiation and neurite elongation) and for safety (i.e., cell death/survival) using high-content analysis. We demonstrated that: i) the characterization of biological properties of biomaterials is profoundly influenced by the test system used; ii) the definition of the in vitro safety profile of biomaterials for neural repair is also influenced by the test system; iii) cell-based high-content screening may well be successfully used to characterize both the efficacy and safety of novel biomaterials.
Angiogenin protein (Ang), member of the ribonuclease family, is a physiological constituent of the human plasma. However, Ang is also a pathological marker that has been found strongly overexpressed in patients affected by different types of cancers. Ang is a potent angiogenesis stimulator principally due to its interaction with endothelial cells and it acts as crucial pro-factor to induce a wide range of cellular responses eventually prompting blood vessels formation.

Copper(II) is a well-known essential cofactor in angiogenesis and serum Cu levels are raised in a wide variety of human cancers, correlating with the tumour malignancy. During angiogenesis, cellular Cu translocates in the extracellular space, therefore the metal binding to extracellular proteins involved in angiogenesis, including Ang, is a possible pathway through which Cu takes part in the signalling process. The activity of Ang is strongly influenced by the presence of Cu(II) ions, even though previous reports indicate that Cu and Ang stimulate angiogenesis by different mechanisms and pathways. It is to emphasize that the data reported till now have been obtained by using the recombinant form of Ang (r-Ang). Such a recombinant form contains a methionine as first residue, at variance with the wild-type isoform (wt-Ang), which has a glutamic residue, with amino group spontaneously cyclizing in the pyro-glutamate form.

Herein, the Cu(II) binding to wt-Ang and r-Ang proteins was thoroughly investigated by means of a multitechnique approach, including chemical methods such as Nuclear Magnetic Resonance (NMR), Electron Paramagnetic Resonance (EPR),
UV-vis, Circular Dichroism (CD), Electrospray Mass Spectrometry (ESI-MS) and biochemical assays. Results showed that the two protein isoforms bind Cu differently. Moreover, Cu binding to Ang affects the intracellular localization of protein decreasing its nuclear translocation and Ang-Cu(II) system negatively affects the protein-induced angiogenesis, as well as endothelial cells migration. Copper also reveals the ability to modulate the Ang transcription. These results highlight the close relationship between Cu and Ang actually circulating in the human plasma, pointing out the biological relevance of Ang-Cu system in the regulation of endothelial cell function. This reveals a possible new mechanism at the basis of vascular pathologies and development of new targets in cancer therapy.
Oral Session 2

The Legal Framework

Chairpersons
Antonella Di Gioia
Viktor G. Mihucz
The Italian Society for Applied Pharmacological Science (SSFA): its main goals, activities, working groups and cooperation

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The Italian Society for Applied Pharmacological Science (Italian acronym, SSFA) was founded in 1964 in Milan, where it is based, after the idea of some experts in pharmacological sciences (mainly pharmacologists) working in the pharmaceutical industry.

The changes in the Italian pharmaceutical industry over the last decades affected also SSFA membership. Now the majority of SSFA members are no more involved in basic research activities, but rather in drug development areas like clinical development, regulatory affairs, drug safety and quality assurance.

SSFA main task is to promote and coordinate, by means of courses, seminars and conferences, scientific activities in the area of biomedical science applied to the research and development of new drugs in order to:

- support scientific research;
- contribute to the scientific knowledge in the country;
- sustain the value of scientific research of experts in biomedical areas who are devoted to research and development of new drugs.

As a scientific association SSFA:

- is active by means of several working groups;
- is formed by about 850 members working mainly in pharmaceutical industries, CRO, public institutions, hospitals and universities;
- organizes a national conference every 3 years;
- has a website (www.ssfa.it);
- publishes a bimonthly bulletin (SSFAoggi, i.e., SSFAtoday).

SSFA current activities are as follows:

- working groups;
- master courses at the Catholic University (Rome) and at the Bicocca University (Milan). SSFA members are in the Executive Board of these masters, which have the Pharmatrain recognition as “Centre of Excellence”;
- several SSFA members are also lecturing at master courses at Catania, Camerino, Naples, Novara, Tor Vergata University of Rome;
- collaboration with AIFA (Agenzia Italiana del Farmaco, i.e., Italian Drug Agency);
- collaborations with national research institutes;
- collaborations with national learned societies, namely, Società Italiana di Farmacologia (SIF), Società Italiana Attività Regolatorie (SIAR), Società Italiana di Farmacia Ospedaliera (SIFO), Associazione Farmaceutici Industria (AFI).

The SSFA Working Groups are the following ones:
- Institutional Affairs;
- Legal Affairs;
- BIAS (Biostatisticians);
- Medical Devices;
- Pharmacoeconomics & Market Access;
- Drug Safety;
- GIQAR (Italian Group for Quality Assurance in Research);
- Pharmaceutical Medicine;
- Nutraceuticals;
- Scientific Journals;
- Observational studies.

SSFA has connections with the following European associations:
- EMA (European Medicines Agency, www.ema.europa.eu);
- EQAS (European Federation of Quality Assurance Societies, www.accreditation.info);
- EFSPI (European Federation of Statisticians in the Pharmaceutical Industry, www.efspi.org);
- IFAPP (International Federation of Associations of Pharmaceutical Physicians and Pharmaceutical Medicine, www.ifapp.org);

The Present Executive Committee (2014-2016) is as follows.
- President, Marco Romano;
- Vice-President, Anna Piccolboni;
- Treasurer, Luigi Godi;
- Secretary, Salvatore Bianco;
- Other members, Giuseppe Assogna, Rossana Benetti, Marie-Georges Besse, Sergio Caroli, Domenico Criscuolo, Gianni de Crescenzo, Paolo Primiero.

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Among the several new provisions foreseen by the new Regulation (R) 2014/536 on Clinical Trials (CTs) which will replace the European Directive (ED) 2001/20 and ED 2005/28, we want to highlight some aspects which in our opinion could lead the future European CTs out of the ethical procedures and principles agreed at the international level in the human experimentation 1-3.

1. EDs foresee the obligation to be in compliance with GCP detailed guidelines, while the new R foresees the obligation to be in compliance only with the GCP principles. Many of the GCP detailed aspects have been transposed in the R and the R provides to be in compliance with GCP quality standards (art. 47). Nevertheless, other GCP aspects, which have direct or indirect ethical implication in CTs, will not be mandatory in the European Union (EU).

2. While ED 2005/28 (art. 2) provides that “Clinical trials shall be conducted in accordance with the Declaration of Helsinki (DoH) on Ethical Principles for Medical Research Involving Human Subject”, the new R does not.

3. Consequently, the ethical principles of DoH, which so far are a mandatory requirement for CTs in EU, will become optional in the near future.

4. EU D 2001/20 (art. 15) provides that the inspections on CTs have the objective to verify compliance with the provisions of GCP, while the new R does not (art. 78). This could break up the alignment of EU inspections from GCP inspections of countries outside EU and currently joining the GCP area.

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1 Note for Guidance on Good Clinical Practice, CPMP/ICH/135/95, July 1996.
2 Council of Europe Convention on Biomedicine (CETS164) and its Additional Protocol on Biomedical Research (CETS 195).
3 Helsinki Declaration, Fortaleza, Brazil, October 2013.
Good Laboratory Practice (GLP) is unique within the regulatory world in so much as it offers an established international framework which ensures that non-clinical health and environmental safety studies will be accepted by regulatory receiving authorities if they have been conducted in a GLP facility which is part of a national monitoring programme recognised by the OECD GLP Working Group (WG). This mutual acceptance of data between countries ensures that duplication of studies can be avoided, saving time and resources. The central premise to ensuring this system works is confidence in the quality of data regardless of where it has been generated. That confidence is built on the fact that all signatories to the OECD’s Mutual Acceptance of Data (MAD) Agreement establish inspection programmes which monitor the compliance of non-clinical health and environmental safety studies with the OECD Principles of GLP.

One of the primary challenges that the OECD GLP WG faces is to ensure interpretation of the OECD GLP Principles is consistent in all member countries. Lack of consistency is likely to undermine confidence in data quality and potentially put facilities in one country at a commercial disadvantage to those in another. A number of approaches have been implemented by the WG to promote the harmonisation of GLP inspections and ensure that test facilities have detailed information and guidance on the maintenance of GLP compliance.

The OECD have published a series of consensus and advisory documents which cover a range of topics and have recently started to complement these documents with frequently asked questions which are periodically published on the OECD web site. Additionally the OECD GLP WG have established a GLP discussion group on harmonisation issues which is specifically designed to allow non-government stakeholder around the world to highlight areas where they believe regulatory expectations are not harmonised or where the current regulations may restrict the development of new techniques. This presentation provides details on how these initia-
tives have made a positive contribution to ensuring the harmonisation of standards across the OECD GLP community.
Meeting multiple needs with a quality management system: a challenge for an evolving scientific organisation

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The Institute for Health and Consumer Protection (IHCP) is one of the seven scientific Institutes of the Joint Research Centre (JRC), which is the European Commission’s in-house Science Service (https://ec.europa.eu/jrc/).

The mission of the JRC is to provide European Union (EU) policies with independent, evidence-based scientific and technical support throughout the whole policy cycle; the IHCP particularly focuses on the protection of the interests and health of EU citizens in the areas of food, consumer products, chemicals and public health.

In support of its activities, the IHCP has developed and implemented a Quality Management System (QMS) certified according to the ISO 9001:2008 standards. The Institute also maintains the ISO 17025 accreditation for two EU Reference Laboratories (EURLs), while a third one has been inspected and confirmed as compliant with the OECD Principles of Good Laboratory Practice (GLP).

The QMS assists the organisation in meeting the requirements of its customers, namely other Directorates General of the European Commission, other EU Institutions and public and private bodies operating in the fields relevant to IHCP expertise.

Different tools are contributing to constitute the QMS backbone, namely:
- the activity planning (Work Programme);
- processes and procedures;
- indicators and reviews;
- internal audits;
- Information Technology (IT) applications for managing documents, records and instrumentation.

Over the last years, the whole Directorate General JRC and, consequently, also the IHCP has been interested by significant changes concerning: i) mission; ii) objectives; iii) portfolio of activities; iv) organisation.
In this context, the instruments of the QMS have proven particularly effective in supporting the Organisation in its evolution, in terms of:
- traceability and quality assurance of its outputs;
- meeting customer, statutory and regulatory requirements;
- treatment of non-conformities and continual improvement of processes and performances.

Furthermore, an effective quality policy is beneficial also in view of a continual harmonisation in the *modus operandi* of the different scientific Units constituting the Institute.

A common QMS for all Institutes and Central Services of the JRC is currently being designed. By entailing the harmonisation of processes, procedures and management tools across the entire Directorate General, this process will lead to a unique ISO 9001 certification for the whole JRC.
Drug development is a most regulated process. In order for an active substance to get registration as a drug, preclinical development should be followed by three phases of clinical trials. The preclinical part involves animal pharmacology and toxicology studies raising questions of animal ethics. The clinical phase I recruits healthy volunteers or patients in certain conditions, whereas well defined patient populations are participants in later phases of clinical development. All clinical studies have to be authorized by the competent authority and evaluated by an independent ethics committee. No clinical trial can be initiated in the absence of a favorable opinion from the responsible ethics committee of the country where the study would be conducted.

The ethics committee is an independent body in a European Union (EU) Member State, consisting of healthcare professionals and non-medical members, whose responsibility is to protect the rights, safety and well-being of human subjects involved in a trial, expressing an opinion on the trial protocol, the suitability of the investigators and the adequacy of facilities, as well as on the methods and documents to be used to inform trial subjects and obtain their informed consent. Each and every protocol should be individually evaluated considering the rules of the profession and the disease and state of the patients concerned. All that evaluation is based on the World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. The Declaration is reduced to practical approach by EU regulation and national laws. This presentation gives examples of how ethical considerations influence the protocol, the way the trial is conducted and eventually the drug development process. In Hungary, the ethics committee for clinical pharmacology believes that helping the sponsors and CROs act ethically and put the patients in the focus promotes drug development and medical science.
Science-driven and well-conducted toxicity testing is the best tool to minimize the risk to volunteers participating in clinical trials. However, with humanized biotechnology products, advanced therapies and even personalized medicines, ensuring the adequate safety margin to the clinical subject is a challenging task which often requires a process of continuous updating, an open communication among the different stakeholders and the ability to overcome the traditional approach of preclinical toxicology.

Public pressure to limit animal use is acknowledged by regulatory bodies which encourage a wise conduct of in vivo testing and inclusion of relevant and translatable biomarkers analyzed with bioanalytical methods the quality level and robustness of which have to be proven to ensure reproducibility. Preclinical Contract Research Organisations (CROs) are engaged as valuable partners in this delicate process. General and specific cases are presented.
The monitoring of data collected from the Member States of the European Union (EU) to evaluate the quality of surface waters are not always fit for purpose in terms of quality and territorial coverage in the EU. Monitoring data are particularly lacking for many emerging pollutants. The Commission implementing Decision 2015/495 of 20 March 2015 has established a watch list of substances for EU-wide monitoring in the field of water policy. The substances selected include estrogens such as 17-Alpha-ethinylestradiol (EE2), 17-Beta-estradiol (E2) and Estrone (E1).

In the context of the Water Framework Directive (WFD) Implementation Strategy a technical report on the use of aquatic effect-based tools has been published that includes bioassays, biomarkers and other biological tools such as metagenomics. These monitoring tools could be used as screening tools or also to establish early warning systems or even to detect the effects of chemical mixtures and chemicals not directly determined.

With the aim to evaluate the field application of these tools in the context of the WFD and to collect monitoring data needed for the watch list, an international project for the monitoring of E2, EE2 and E1 is in progress. This project should promote reliable screening methods for the monitoring of endocrine disrupting chemicals in wastewater and surface water and link reliable effect-based tools with regulatory needs and chemical monitoring.
Residues and metabolites of pharmaceuticals can pollute environmental compartments, food commodities and workplaces (hospitals in the first place), thus posing a serious threat to human health and environmental integrity. Needless to say, this challenge is increasingly attracting the attention of the international scientific community, the decision makers and the layman and much concern has been expressed over the past few years over the deleterious consequences of the discharge of medicinal products, often as unused or expired products, into the various environmental compartments, not to mention their occurrence in foodstuff and feedstuff and their undue presence in the workplace. This widespread occurrence inescapably raises a number of questions among which of prime importance is the reliable quantification of such residues and metabolites in the various media in order to assess whether and to what extent they can endanger biota and humans.

This work summarizes the key findings of an overview of the scientific literature in this field over the past three years (2013–2016) with particular regard to the most fit-for-purpose analytical approaches currently resorted to for the detection, identification and quantification of residues of pharmaceuticals in food commodities and the assessment of their noxious potential. A total of 986 papers published in scientific journals were scanned.

Results show that liquid chromatography techniques have the lion’s share with a full 40%. In turn, methods based on High Performance Liquid Chromatography (HPLC) and Ultrahigh Performance Liquid Chromatography (UHPLC) techniques show basically the same percentage of use, each with 16% of the total. In all cases Diode Array (DA), Fluorescence (F), Ultraviolet (UV), Mass Spectrometry (MS) and tandem MS (MS/MS) detectors were employed.

A similar percentage (15%) characterizes biochemical tests, primarily in vitro and immunoassays including the Enzyme-Linked Immunosorbent Assay (ELISA), this last making about one fourth of the total in this group.
Other analytical approaches were by far less popular, i.e.: i) MS [High Resolution (HR), Time-of-Flight (TOF) alone or coupled with Matrix-Assisted Laser Desorption (MALDI)], 4 %; ii) Capillary Electrophoresis (CE) and Capillary Zone Electrophoresis (CZE) [Electrochemiluminescence (ECL), F, UV and MS detection], 2 %; iii) Gas Chromatography (GC) with various detection systems, 2 %; iv) electrochemical methods, 1 %; v) other techniques (High Performance Thin Layer Chromatography (HPTLC), Infrared (IR) spectrophotometry, microscopy, Raman spectroscopy, UV spectrophotometry), 4 %.

From a general viewpoint, an overall increase in the detection power of the most advanced analytical techniques can be observed. It is expected that this trend will keep the pace with future emerging needs to the point that the question will arise of whether extremely low levels of pharmaceuticals can still exert adverse effects on human health and the environment and are therefore worth measuring.
Oral Session 3

Advances in Analytical Techniques

Chairpersons
Roger Fuoco
Gyula Záray
Breath and saliva share a series of features that allow them to provide complementary and clinically valuable information. Their components originate from normal or abnormal physiology, ingestion and metabolization of food and beverage, exposure to pollutants and bacterial activity. The rapid equilibrium with blood provides these biological fluids with a high potential for the diagnosis of diseases as well as the monitoring of disease progression and therapy. The combination of non-invasive sampling and the fact that their composition mirrors almost in real time processes occurring inside the body makes breath and saliva analysis suitable for monitoring drug therapies, e.g., to detect potential adverse effects or to provide insights on the pharmacokinetics or on the mechanism of action.

From the analytical point of view, breath and saliva offer the advantage of reduced matrix effect and chemical interferences (compared to blood), but this is usually paid with lower concentration levels of the target compounds. Sampling procedures of both fluids are critical, as they may have remarkable effects on the composition of the collected specimens. Storage of samples is also critical in the case of breath, whereas saliva does not generally require ad hoc treatments.

In this work, the use of breath and saliva analysis in the pharmacological field is reviewed and practical applications are shown. After a brief introduction concerning the basics of breath and saliva analysis, much more attention is paid to the sampling procedures and their effects on sample composition. A series of illustrative applications in the pharmacological field conclude the work showing the present use of these techniques and trends for the future.
FT–IR/Raman spectroscopy coupled with quantum mechanics modelling for assessment of complexation ability of β-lactam analogs and cyclodextrins

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Assessment of ability of solid-state complex formation by Cyclodextrin (CD) and Active Pharmaceutical Ingredients (APIs) by using spectroscopic methods such as Fourier Transform-Infrared Spectroscopy (FT-IR) and Raman spectroscopy is a difficult task due to often insignificant changes in spectra of API-CD complexes in comparison to spectra of isolated pure substances and their physical mixtures. Hence, the goal of the present work was to develop a mathematical model describing the ability to form API-CD complexes based on data obtained from experimental as well as theoretical FT-IR/Raman spectra.

The model was based on Artificial Neural Networks (ANNs) coupled with evolutionary algorithm to ensure optimal ANN architecture and input feature selection. Analogs from a group of β-lactam antibiotics such as cefuroxime axetil, cefetamet pivoxil and pivampicillin were used. The CDs selected as the complexation agents were α-CD, 2-hydroxypropyl-α-CD, β-CD, 2-hydroxypropyl-β-CD, methyl-β-CD and γ-CD and 2-hydroxypropyl-γ-CD. The complexes of the above-mentioned ingredients were prepared through co-precipitation at a 1:1 stoichiometric ratio.

Differential Scanning Calorimetry (DSC) was applied as a reference method confirming the formation of API-CD complexes. Data from DSC analysis were used as output data for machine learning algorithm calibration. The changes of positions and intensities of bands on FT-IR and Raman spectra of API-CD complexes as well as computationally obtained data were used as input data for ANNs training. The main advantage of this approach is leveraging additional information derived from
chemical structure and theoretically computed spectra to increase model predictive ability without the need for conduction of any experimental measurements but FT-IR/Raman spectra acquisition. Results showed that the proposed model can correctly assess with a high probability the complex ability of binary systems of β-lactam antibiotics and CDs.

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Hollow-fiber field flow fractionation, an analytical tool for the characterization and quality control of new biotechnological protein-based drugs

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The rapid development of protein-based pharmaceuticals highlights the need for robust analytical methods to ensure their quality and stability. Among proteins, used in pharmaceutical applications, an important and ever increasing role is played by monoclonal antibodies and large proteins, often modified to enhance their activity or stability when used as drugs. The bioactivity and the stability of those proteins are closely related to the maintenance of their complex structure despite the influence of many external factors that can cause degradation and/or aggregation. The presence of aggregates in these drugs could reduce their bioactivity and bioavailability and induce immunogenicity. The choice of the proper analytical method for the analysis of aggregates is fundamental to understand their (size) dimensional range, their amount and whether they are present in the sample as generated by an aggregation or are an artifact due to the method itself. Size-Exclusion Chromatography (SEC) is one of the most important techniques for the quality control of pharmaceutical proteins, although its application is limited to relatively low molar mass aggregates.

Among the techniques for the size-characterization of proteins, Field-Flow Fractionation (FFF) represents a competitive choice: due to the absence of a stationary phase, in fact, the separation mechanism is soft with total maintenance of native properties of analytes and the dimensional range of applications is higher, from nanometer to micrometer sized analytes. The microcolumn variant of FFF, the Hollow-Fiber Flow FFF (HF5), is coupled on-line with Multi-Angle Light Scattering
(MALS) for the development of methods for the characterization of protein-based drugs\textsuperscript{1,2}. The HF5-MALS was shown to be able to size-separate therapeutic protein samples and their aggregates and to evaluate the nature of aggregates. The analytical performances, such as resolution, reproducibility and limits of detection, were also determined in the framework of quality control of protein-based drugs.


Integrated method development for chemical characterization and oxidative potential determination of indoor particulate matter

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There is an increasing concern on indoor air quality as in our modern world people are spending about 80% of their time in a built environment. Particulate Matter (PM) encompasses a lot of different chemical components and physical characteristics, many of which are potential contributors to adverse health effects. Chemical compounds in ambient PM are capable of generating Reactive Oxygen Species (ROS) causing cellular damage via oxidative stress. Trace elements can act like catalysts in ROS production and cause antioxidant depletion from the respiratory tract lining fluid. Correlation between Oxidative Potential (OP) and elements is influenced by water solubility, chemical form, oxidation and reduction behavior, capacity of ROS generation or induction in Fenton-type, Haber-Weiss reactions etc. For reduced Glutathione (GSH), affinity to its sulfhydryl groups is important. Metals that can reportedly bind to GSH are Ag, As, Cd, Cr, Cu, Hg, Pb, Se and Zn. So far, little is known about interaction of Ascorbic Acid (AA) and urate with trace elements. Hereby, an integrated approach is reported for the determination of elemental composition, major inorganic ions, Elemental/Organic Carbon (EC/OC) as well as OP in
the same Quartz Fiber Filter (QFF) loaded with indoor PM having an aerodynamic diameter less than 2.5 μm (PM$_{2.5}$). This was achieved by Inductively Coupled Plasma Sector Field Mass Spectrometry (ICP-SF-MS) after microwave-assisted aqua regia or sonication-assisted water extraction, Ion Chromatography (IC), Thermal - Optical Transmittance (TOT) as well as High Performance Liquid Chromatography (HPLC) and enzyme-linked 5,5'-dithio-bis(2-nitrobenzoic acid) assay by measuring the AA and GSH depletion, respectively. The low mass of PM$_{2.5}$ collected, the elemental blank values of the QFFs and the sample requirements of the ICP-SF-MS limited the elemental determination. This procedure was successfully applied to the determination of Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Rb, Sn, Sr, V and Zn at the ng m$^{-3}$ level in indoor PM$_{2.5}$ collected in offices equipped with mechanical ventilation across Europe according to sampling protocols relevant for epidemiological studies. Several ions (Cl$^-$, NO$_3^-$, SO$_4^{2-}$, Ca$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$, NH$_4^+$), OC and EC were determined at μg m$^{-3}$ level in all samples (n = 29). OPAA and OP$_{\text{GSH}}$ could be measured in all samples as the PM$_{2.5}$ critical sample mass to achieve quantitative determination of elements was about 500 μg.
Monitoring of Warfarin therapy by saliva analysis: open issue on the pathway to clinical practice

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In many clinical conditions, Warfarin (WAR) is an essential and not replaceable drug that requires a therapeutic monitoring due to its narrow therapeutic range. The evaluation of the International Normalized Ratio (INR) is the primary assay used for monitoring WAR therapy. New Anticoagulants Drugs (NOACs) have appeared on the market in the last decade, but they are not expected to replace WAR for a large number of patients due to the following drawbacks: i) costs; ii) collateral effects; iii) absence of specific antidotes; and iv) quick loss of the anticoagulant effect if a single dose is missed.

The aim of this study was to verify whether safety and effectiveness of the WAR therapy could be improved by monitoring the plasma and Oral Fluid (OF) concentrations of both the WAR and its active metabolites (i.e., Warfarin alcohols, WAROHs). For this purpose, 9 patients (5 males, 4 females) undergoing WAR therapy were prospectively enrolled for a period of three months. WAR and WAROHs were determined in stimulated OF and plasma samples (both unbound fraction and total content) by High Performance Liquid Chromatography (HPLC) with Fluorescence Detection (FD) after a suitable sample preparation. It was found that each patient had a different but constant dose-effect ratio of WAR and attained the desired anticoagulant effect with a different WAR plasma concentration largely independent from the dose. The INR variations over time mirror the variations of the plasma
concentrations of WAR and in particular of its diastereomer metabolite, namely RS/SR-WAROH. Even if statistics is insufficient to draw a firm conclusion, these results suggest that potentially useful clinical information can be obtained from this kind of measurements. For a clinical application, OF analysis would allow minimum invasiveness and easy sampling, which would represent an advantage for the quality of life of patients undergoing a long-term therapy.
Main challenges of analytical method development for multiresidue analysis of pharmaceuticals in environmental water samples

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Pharmaceuticals, Personal Care Products (PCPs), pesticides, herbicides and pharmaceutical drug residues can reach detectable concentrations in rivers and lakes, if their production and use are sufficiently large and the compounds show some mobility and persistence in the aquatic environment. Simultaneous analysis of multi-class PCPs with quite different physical-chemical characteristics often requires compromises to be adopted among the multiresidue method development steps.

Gas Chromatography (GC) and (Ultra) High Performance Liquid Chromatography (HPLC) coupled with (tandem) Mass Spectrometry (MS) detection are the appropriate choices to simultaneously analyze pharmaceutical residues with a high diversity of chemical structures, polarities and acidic properties.

An overview - with a number of examples of our own experience - are given about the main aspects and challenges of the most important method development parameters, such as:

- selection of target compounds and their metabolites along with acquiring method selection of MS techniques (full scan vs. selective- or multiple-ion monitoring and tandem methods);
- choice of sampling strategy in order to collect representative homogenous samples;
- stability of analytes in samples and in standard solutions and mostly used preservation strategies;
- different sample pretreatment methods based on extraction techniques;
- selection of derivatization reagents for GC-MS analysis;
- matrix effect and selection of calibration methods.
Ciprofloxacin residues detection in cow milk: development of a novel and rapid optical β-galactosidase-based screening assay

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Screening methods (as defined in Commission Decision 2002/657 by European Union) play an important role as part of surveillance and monitoring at the primary production levels of the food supply chain (e.g., dairy supply chain). The optimizations of routinely used screening methods as well as the development of innovative methods are highly recommended by the European Community. Fluoroquinolones, especially ciprofloxacin, are among the most widely used antibacterial substances of synthesis in the veterinary practices for the treatment of bacterial infections in livestock. Various commercial kits are available for the detection of fluoroquinolones residues in foods, but these are time-consuming and most kits fails to detect residues at Maximum Residue Levels (MRLs) concentrations that are allowed by food safety authorities.

We developed a novel and rapid optical microbiological screening assay for the detection of fluoroquinolone ciprofloxacin residues in cow milk samples. This screening assay is based on monitoring of *Escherichia coli* ATCC 11303 cell proliferation by optical measure of endogenous β-galactosidase enzymatic activity. A linear correlation between the optical density of *E. coli* cultures (cell proliferation) and the
induction of endogenous β-galactosidase activity was experimentally established. β-galactosidase enzymatic activity was determined using a chromogenic artificial substrate which changes the color of culture medium. The presence of ciprofloxacin residues in tested samples inhibits the E. coli cell proliferation which represents reduction in β-galactosidase activity due to a lesser amount of enzyme (compared to control). The β-galactosidase induction (normally obtained using isopropyl β-D-1-thiogalactopyranoside (IPTG)) was performed exploiting the lactose present in milk. The experimental results with lactose (from milk samples) were comparable to IPTG as synthetic inducer.

The ciprofloxacin β-galactosidase-based screening assay was performed by testing three different concentrations of ciprofloxacin in spiked cow milk samples and two different chromogenic β-galactosidase artificial substrates, O-nitrophenyld-β-D-galactopyranoside (ONPG) and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal). The screening assay is able to detect ciprofloxacin residues within one hour at one MRL concentration using ONPG (cheaper than X-gal). This method can be adapted for the detection of ciprofloxacin residues in cow milk samples at primary milk production site.
Oral Session 4

Medical Devices, Current Challenges and Future Prospects

Chairpersons
Salvatore Bianco
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Development of medical devices containing active pharmaceutical ingredients

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Recent years witnessed a remarkable increase in the development and clinical use of Medical Devices (MDs). To date about 500,00 different types of MDs are commercialised in the EFTA area, with a global turn-over of about 100 billion Euros. More and more frequently, manufacturers develop devices incorporating Active Pharmaceutical Ingredients (API). The correct regulatory classification of these products is often difficult to define. The difference among these MDs and the Medicinal Products (MPs) can be subtle and matter of discussion with the Competent Authority (CA). At the same time, a correct classification, is crucial for the development strategy and for the subsequent pre-clinical and clinical studies. In fact, the pre-clinical and clinical development plan, the size and number of studies, the reference guidelines and the CAs are different for MDs and MPs. This is important for manufacturers and especially for independent developers. The Authors present case studies of development of MDs incorporating an API, with an overview of the differences in terms of development time and methodology. Special attention is devoted to: i) the methodology for clinical evaluation; ii) the classification of the products according to the relevant European Guidelines; iii) the interaction with the relevant CA1-5.

Microbiological quality control of different types of medical devices

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The microbiological quality control represents a cornerstone in the production process of pharmaceutical products and Medical Devices (MDs). In both the pharmaceutical and MD industries, along with evolving regulatory requirements, products of greater complexity are elevating the challenges related to maintaining microbiological integrity. For a given bacterial inactivating treatment, the probability of bacterial survival is determined by the number and resistance of microorganisms and by the environment in which the organisms reside during treatment. Nevertheless, the sterility of an individual item in a population of sanitized products cannot be ensured in the absolute sense. Thus, sanitization, but especially sterilization, of a processed population is defined in terms of probability of there being a viable microorganism present on a product item. Following health authority warnings, several kinds of MDs were examined for investigating their real compliance with the sterility requirements expected for the specific pharmaceutical product category.

According to the European Pharmacopeia, mesophilic viable microorganisms, faecal bacteria indicators, moulds, P. aeruginosa, S. aureus and other parameters of interest depending on the circumstances were determined. Culture methods and biochemical confirmation tests were performed. The sterility expected for some MDs under test, such as saline solution for contact lens and dentistry graft devices, was not always confirmed and pathogenic parasites and opportunistic pathogenic bacteria were isolated and identified. The microbiological evaluation of some MDs for which the sterility was not required (auricular cones, vaginal douching and denture adhesive powder) was performed assimilating them to pharmaceutical products for topic use. In some cases, the limits for microbiological parameters were exceeded, while in others, even in the absence of indicators/index parameters, the presence of environmental microorganisms with potential pathogenic role was observed. The microbiological analysis of a dietary supplement for abdominal gas pains revealed the absence of any pathogenic microorganisms and a regular probiotic microor-
ganisms load as declared by the manufacturer. On the other hand, analytical identification procedures pointed to the presence of two distinct species of probiotic microorganisms differently from what was specified. These results emphasize the fundamental role of good manufacturing practices in the pharmaceutical production area and stress the need for closely monitoring any step of the production chain from treatment of raw matter to storage and distribution of final products.
Medical Devices (MDs) are nowadays more and more important in the healthcare industry and the related processes for worldwide regulation and certification are a topic of great interest. In particular, the need for regulation harmonization between European Member States (European Regulation), as well as worldwide, is very important both for Regulatory Authorities and for the industry world.

This presentation covers a typical process for an MD development and industrialization phase, providing some case studies and taking into consideration all steps from design up to start-up of the production phase and process validation. All these activities are necessary for the product certification process.

The discussion emphasizes the aspects related to the Quality System, Production, Validation and Quality Control, proposing an integrated approach, which combines the GMP and ISO requirements (e.g., ISO 13485 and ISO 14971), following a Quality Risk Management (ICH Q9) and, where applicable, an integrated Pharmaceutical Quality System (ICH Q10) structure.
Italian guidelines on microbiological quality of the water used in the medical devices industry

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The microbiological quality of the water used in the production of Medical Devices (MDs) plays a prime role for the prevention of risks to human health arising from the production process of several types of MDs. Water is used in MD production as a solvent and a diluting agent, to rebuild the products during the synthesis, as an ingredient of preparations and as a cleaning agent for equipment, distribution circuits, primary packaging, for generating steam etc.

Water must have specific microbiological features to guarantee that the MD or its use does not pose a risk to human health. However, there are no specific guidance provisions on microbiological parameters to be used for the evaluation of water quality during industrial production. The assessment of water quality used for MD production is currently based on the same microbiological parameters required for pharmaceutical industry.
In 2012 a joint project between the National Institute of Health (ISS) and the Ministry of Health was launched to promote better knowledge on quality of water used in the production of MDs. Thus, an investigation was undertaken to evaluate the microbiological quality of industrial water used in the production of some types of MDs in Italy. In the first phase of the project a number of non-injectable and non-sterile topical-use MDs were selected. Furthermore, also MDs involving the use of water as a component as well as at the production stage were selected. The microbiological characterization of microorganisms during this investigation involved not only a microbiological parameter required by Pharmacopoeia, i.e., Total Bacterial Count (TBC) at 37 and 22 °C, but also the faecal microbiological indicator \( E.\ coli \) and Enterococci and the pathogenic bacteria \( \text{Salmonella spp, Pseudomonas spp} \) and \( \text{Staphylococcus spp} \). The ISS organized a national conference where the results of study were discussed and shared with the stakeholders of the MDs industry. It was thus possible to develop the first Italian guidelines on microbiological aspects of water used in the MD industry. The full text of the guidelines can be downloaded from the web site of the Ministry of Health (http://www.salute.gov.it/imgs/C_17_pubblicazioni_2421_allegato.pdf).
Evaluation of subacute and subchronic systemic toxicity of metal dental alloys: guidelines and reference standards

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Biocompatibility is based on the interactions between a material and a biological environment. The analysis of the local and systemic tissue response to a biomaterial has long been recognized to play an important role in biocompatibility testing. The biocompatibility of dental materials is a topic of increasing importance for dentists, patients, public health experts and manufacturers. The oral cavity presents some features widely described in the literature for biocompatibility evaluation of metallic materials. The normal wound-healing response in the oral cavity is a dynamic phenomenon in which cells and their products interact to repair damaged tissues. Furthermore, fast cellular turnover, immunological reactivity, microbial contamination, thermal excursion and cyclic load induce different tissue response that may cause a series of iatrogenic effects including inflammation, fibrosis, coagulation and infection.

The number of patients with alloplastic materials in situ for long periods of time and the great variety of materials enhance the opportunities to get adverse effects on the short or long-term, sometimes related to professional treatment and daily dental hygiene procedures. Corrosion of a dental alloy is an extremely complex phenomenon and depends on a variety of physical and chemical factors, e.g., the combination of two different alloys/welding or the presence of pits and crevices in a single alloy. Corrosion plays a key role in biocompatibility as the release of elements from the alloy can cause adverse biological effects such as toxicity, allergy or mutagenicity or alterations of strength of the dental alloys till to brittle fracture. Among the local oral effects of corrosion one should include burning, metallic taste, halitosis, sialorrhea, aphthous ulceration, glossitis, stomatitis, lichen ruber planus, leucoplakia, xerostomia and sometimes irritation of the mouth region, like neuralgic pain or electric shock dental pulp. Corrosion may be assessed by observing the alloy surface, by electrochemical tests that measure the released elements indirectly through the flow of the released electrons or by tests that measure the release of
the elements directly by spectroscopic methods. However, the biological response mainly depends on what elements are released, their amount and the duration of exposure. Thus corrosion is a necessary, but not a sufficient, condition for adverse biological effects of dental alloys.

In dentistry, the evaluation of biocompatibility of Medical Devices (MDs) is regulated by international standards and international guidelines that direct manufacturers/sponsors and investigators in the correct choice of procedures to be applied to ensure the safety of the MD, the effectiveness of performance and protection of the environment. The pre-market biocompatibility evaluations are therefore to be considered as an essential requirement for the placing on the market of a new MD. To assess systemic toxicity (subacute or subchronic systemic toxicity of materials or devices in contact with the oral mucosa or with hard or soft tissue), it is necessary to follow specific harmonized ISO standards and OECD guidelines. The preliminary assessment of what is the best technical standard to be followed is defined in the ISO 7405 Standard Dentistry - Evaluation of biocompatibility of medical devices used in dentistry that specifies test methods for the evaluation of biological effects of medical devices used in dentistry. The ISO Standard 10993-11 Biological evaluation of medical devices - Part 11: Tests for systemic toxicity likewise specifies requirements and gives guidance on procedures to evaluate potential causes of adverse systemic reactions. In addition to the ISO standards also specific OECD guidelines can be applied that confirm and describe the methods for obtaining an adequate assessment of acute toxicity and chronic toxicity. In this work the Authors relate their experience in the evaluation of subacute and subchronic toxicity of non-noble metal dental alloys along with other biomedical observations.
Oral Session 5a

Personalized Medicine
from Genetics to Analytics

Chairpersons
Isabella Andreini
Katalin Monostory
The concept of personalized medicine optimizing medication types and dosages for individual patients based upon genetic, biomarker and other patient-related factors has received increasing attention. Application of these to the field of pain management would be necessary, but has been unrealized in practice. Several genotypic and phenotypic factors that could potentially serve as predictors of therapeutic success within a personalized pharmacotherapy of pain and inflammation are discussed.

Opioids and non-steroid anti-inflammatory/analgesic drugs are the “classic” analgesics still in usage for treatment of pain. No drug has been introduced with a selective site of action on nociceptive primary afferent neurons in striking contrast to the enormous therapeutic benefit of drugs with targets on the efferent side of the peripheral nervous system. The highly selective site of action of capsaicin, the pungent principle of hot peppers and the chemoanalgesia/desensitization which followed the state excitation were the clues and research tools to reveal potential targets on nociceptors for analgesics. Cloning the capsaicin receptor/Vanilloid Receptor 1 (VR1) cation channel almost twenty years ago was a real breakthrough. It is now renamed to Transient Receptor Potential Vanilloid 1 (TRPV1). The receptor of capsaicin turned out to be an integrative nocisensor protein in the plasma membrane of the major nociceptive subgroups of primary afferent neurons and provided means for High Throughput Screening (HTS) for the drug industry. Special emphasis is made to the unique features of TRPV1-gating and on the remarkable long-term analgesic effect of TRPV1 agonists. The latter scope is underlined by the fact that an 8 % capsaicin dermal patch has recently been introduced for lasting pain relief in some severe neuropathic pain conditions. Perspectives and new achievements in the field of TRPV1 antagonists are also outlined and some promising recent findings for novel initiatives are briefly summarized.

A number of neuropeptides have been implicated in the genesis of inflammation, such as tachykinins and calcitonin gene-related peptide. Development of their receptor antagonists could be a promising approach to the anti-inflammatory
pharmacotherapy. Anti-inflammatory neuropeptides such as vasoactive intestinal peptide, pituitary adenylate cyclase-activating polypeptide, alpha-melanocyte-stimulating hormone, urocortin, adrenomedullin, somatostatin, cortistatin, ghrelin, galanin and opioid peptides are also released and act on own receptors on the neurons as well as on different inflammatory and immune cells. Promising therapeutic impacts of these compounds as potential candidates for the development of novel types of anti-inflammatory drugs are also discussed.
Genetics of tailored medicine: the CYP system

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The genetics of cytochrome P450 (CYP) gene superfamily is a very active area of multidisciplinary research, overlapping the interest of medicine, biology, pharmacology and genetics. The hepatic CYP enzymatic system, encoded by these genes, is responsible for the metabolism of more than 80% of the commercially available drugs and variations in this system may account for the interindividual differences observed in drug efficacy, including severe clinical consequences such as Therapeutic Failures (TFs) and Adverse Drug Reactions (ADRs), worldwide primary causes of morbidity and mortality in western countries. We explored some basic concepts of human genetics that have important implications in the genetics of CYP. An attempt to transfer these basic concepts to the genetic data reported by the Home Page of The Human Cytochrome P450 (CYP) Allele Nomenclature Committee was also made, focusing on the current knowledge of CYP genetics.

These studies demonstrate that the targeted analysis of specific CYP genes may be common to several clinical disciplines for the identification of subjects responder/non-responder or showing TF/ADR, among patients with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)-related gastro duodenal bleeding in treatment with
NSAIDs, patients with Alzheimer’s diseases in treatment with acetyl cholinesterase inhibitors, patients in treatment with opioids for post-operative pain and patients with bipolar disorders in treatment with antidepressant, neuroleptics and antipsychotics. In particular, it is well known that the prevalence of both TF and ADR strongly increased in the elderly, clearly related to the presence of multiple pharmacological treatments, a common status in subjects aged 65 and over, thus also making geriatrics a special task for pharmacogenetics. Thus, the analysis of CYP may be useful to identify patients to be addressed towards alternative treatments, this being the base for the clinical applications of pharmacogenetics in which personalized drug treatments constituted the main aim. The status of what we know and what we need to know to forward the application of pharmacogenetics in clinical practice is finally overviewed in order to introduce a personalized treatment project that might be particularly interesting for its application in the elderly.
Molecular evidence-based targeting of rare driver gene alterations in cancer

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The census of cancer genes counts today around 500 cancer genes. Molecular alterations of 138 genes have been reported to provide direct growth advantage to cancer cells by bioinformatic analysis and therefore have been described as “driver” cancer genes by Bert Vogelstien. Each cancer cell can have up to 8 driver genes. The number of different somatic mutations reported in COSMIC database is over 3 million. Even if only half of these are functionally relevant, it is obvious that at least half of cancer patients have driver alterations, which are less frequent than 1 % in the total population. It is impossible to conduct clinical trials for all of these combinations. Therefore, personalized drug selection for these patients has to be based on molecular evidence and the unique clinical experience has to be stored in a database.

Molecular pharmacology is becoming part of the clinical work of oncologists and scientists of molecular pharmacology will participate in molecular tumor boards. We propose a novel algorithm and a free software tool to rank driver alterations, druggable targets which are associated with these driver alterations in order to always suggest the treatment options based on the highest evidence and to identify novel targets for drug discovery.
Pharmacogenetics as a tool to tailor the immunosuppressive therapy: focus on azathioprine and tacrolimus

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Pharmacogenetics research looks at variations in the human genome and ways in which genetic factors might influence individual responses to drugs. Two pharmacogenetic studies on the immunosuppressive drugs azathioprine and tacrolimus were performed to investigate the role of drug-metabolizing enzyme polymorphisms, alone or in combination with physiological factors, in avoiding severe drug reaction or in reducing pharmacokinetic (PK) variability.

Azathioprine is a thiopurine drug, used mainly in acute lymphoblastic leukemia, organ transplant and autoimmune disorders. The thiopurine methyltransferase (TPMT) enzyme is one of the major metabolizing enzymes of azathioprine and its activity level in human tissues is controlled by a common genetic polymorphism. Therefore, an azathioprine dose reduction is recommended to avoid severe adverse drug reactions in patients carrying mutations with a decreased TPMT activity. The TPMT genotype-phenotype relationship in a healthy unrelated population was investigated and a concordance rate of 71.6 % between genotype and phenotype was observed. Interestingly, genetic factors seemed to be the major determinant of TPMT phenotype variability in adults, whereas wild type children showed a significantly higher TPMT activity compared to wild type adults, showing also significant differences according to the gender. Therefore, not only genetic factors, but also physiological features, such as age and gender, should be taken into consideration when assessing the TPMT phenotype.

Tacrolimus is an immunosuppressive drug widely used in patients undergoing solid organ transplantation. This drug has a narrow therapeutic index, exposing patients to acute graft rejection and toxicity in cases of low or high blood concen-
trations, respectively. Therefore, it has been suggested to analyze some genetic factors, such as polymorphisms in genes coding for biotransformation enzymes like cytochrome P450 isoenzyme 3A5 (CYP3A5), in order to reduce the great interindividual PK variability after the initial tacrolimus dose.

The influence of the CYP3A5 donor genotype in pediatric patients after liver transplantation was also studied correlating this with tacrolimus disposition on the first day of treatment. It could be confirmed that tacrolimus starting dose in pediatric liver transplant patients may be influenced by the liver donor’s CYP3A5 genotype, but, again, also sex and age appeared to be associated with tacrolimus disposition.

These studies emphasize the fact that tailoring the azathioprine or tacrolimus dosage according to genetic and physiological factors in pediatric patients could improve the efficacy of these immunosuppressive therapies.
The fundamental innovation behind any drug is the molecular structure of the drug substance itself. Besides that, the need for determining molecular structures (such as related molecules, metabolites or degradants) crops up in numerous respects during the discovery, the development, the clinical studies, the patenting, the quality control etc., of a drug. But how exactly are these structures determined and how sure can we be about their correctness?

In this presentation a conceptual look is taken at the roles that the two most powerful and frequently used methods, Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) play in the structure determination of small molecules in an innovative pharmaceutical industrial setting. The discourse will not only focus on the capabilities of modern NMR and MS, but also on the inherent, but often overlooked role, of the “human element” in these investigations. On that pretext, and in a somewhat philosophical stance, a unique way of thinking is outlined about and practicing science in general, and NMR and MS in particular. This approach, called “anthropic awareness”, has been cultivated for a while in our research facility and has proved to be highly useful not only in our everyday professional lives, but also in our non-professional everyday lives. The aim is to develop a keen mindfulness of how our human nature secretly influences our thoughts in science in general and on how this influence can lead even the smartest and most knowledgeable scientist into what we call “mental traps”, resulting in cognitive mistakes ranging from widely held scientific misconceptions to faulty personal or team-level deductions. By understanding and analyzing the nature of these mental traps, one can develop the enlightening faculty of detecting and avoiding them both in one’s own and others’ thoughts. Based on a freshly published book written...
by our team\textsuperscript{1}, in this talk the reasons behind the mental traps are briefly discussed along with some of the most important traps that are relevant to the application of NMR and MS.

Oral Session 5b

Pharmaceuticals and the Environment

Chairpersons
Paola Bottoni
Anna Barra Caracciolo
Pharmaceuticals are used in high quantities in our society. After the use they can be excreted unchanged and/or as active metabolites in urine and faeces and are directly conveyed to Wastewater Treatment Plants (WWTPs). They can escape degradation during wastewater treatment and end up in surface and ground water. In the year 2000, our group detected for the first time pharmaceuticals in the north of Italy in drinking water, river water and sediments and soon after a survey was conducted in the river Po basin. Due to the huge amount of pharmaceuticals used, a procedure was adopted to establish priorities and restrict studies to a limited number of hazardous molecules. This procedure was recently updated to take into account changes in the market and a predictive approach was applied to identify the current priority substances in Italy. Sophisticated analytical techniques based on liquid chromatography tandem mass spectrometry were developed and used to monitor behaviour and fate of the priority pharmaceuticals in the environment. Several WWTPs in Italy were monitored to assess the removal rates of pharmaceuticals and the amounts discharged in the environment with treated wastewater. The presence of antibiotics was also investigated in a specific study and it was estimated that 7 to 14 tons of active principles are discharged annually in the aqueous environment in Italy.

More recently, pharmaceuticals were monitored, along with other classes of micropollutants, in the most urbanized area in Italy, the metropolitan area of Milan. A mass balance of the emissions was performed in untreated and treated wastewater, surface and groundwater to evaluate the impact of this metropolitan area in the River Lambro basin, that collects all treated wastewater, and in groundwater. Pharmaceuticals were the most abundant compounds and removal in WWTPs was affected by the type of advanced treatment adopted and by the nature of each substance. For instance, no removal was observed for bezafibrate, hydrochlorothiazide, furosemide and carbamazepine. Scattered sources of contamination other than the
city of Milan were found in the river Lambro basin and contributed highly to environmental loads of pharmaceuticals discharge into the river Po (6.6 kg/day). Finally, groundwater resulted also affected by the contamination. An Environmental Risk Assessment (ERA) was performed for each individual substance measured in surface water and for mixtures of substances. Several substances were observed to exceed the threshold levels indicating a potential risk to the environment.
Potentially toxic elements in the soil–water–sediment system by sequential extraction

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Environmental pollution by Potentially Toxic Elements (PTEs) is a very hot question in Hungary after the Tisza River contamination in 2000 and the Ajka red mud disaster in 2010. The magnitude of adverse effects caused by PTEs in the soil–water–sediment system depends on the various chemical species that can arise. Long-term biological impacts are strongly influenced by mobilization-immobilization processes in the aquatic environment. These can be studied through sequential extraction procedures to model their pathways1. In Europe the simplified extraction scheme proposed by BCR in 1993 and modified in 2001 is mostly used for this purpose2. Samples are gradually decomposed and four different fractions of PTEs can be identified, i.e.: i) water-soluble and carbonate-bound fraction; ii) fraction associated with reducible Fe- and Mn-oxides; iii) organic complexed fraction and sulphides; iv) fraction soluble in oxidative acids. The sediment-based Certified Reference Material (CRM) BCR 701 was produced to check the accuracy of the fractionation procedure. The stand-

ardization process was hindered by methodological problems, *i.e.*: i) the solvents used in the BCR system do not mirror completely the natural mobilization processes and partly modify the original chemical species; ii) the batch leaching extraction steps is exceedingly time-consuming (4-5 days); iii) the above CRM, certified for the fractionation of Cu, Cd, Cr, Ni, Pb and Zn, is available only for sediment and cannot be applied to other environmental matrices and other PTEs. Further studies aimed at improving the BCR methodology are planned, in particular to: i) develop a continuous flow system for sequential extraction of water-soluble and carbonate-bound PTEs fractions; ii) accelerate the BCR leaching steps by sonication; iii) extend the BCR procedure to other environmental matrices (soils, red mud, composts, biofilms) and further PTEs by multielemental (ICP-OES) detection. This approach was applied to the assessment of long-term changes in the environmental mobility of PTEs at two contaminated areas. It showed much potential for the quantification of PTEs released into environmental compartments by metal-containing pharmaceutical products.

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Effects on the natural microbial community of a fluoroquinolone antibiotic in an urbanized stretch of the river Tiber

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Fluoroquinolones are broad-spectrum antibacterial agents widely used for the treatment of bacterial infections inhibiting the activity of key enzymes involved in bacterial DNA replication. These pharmaceuticals are used in both human and veterinary treatments. Fluoroquinolones are partially metabolized in the treated-organisms and through human excretions reach Waste Water Treatment Plants (WWTPs). Most of conventional WWTPs have a limited removal efficiency and through their effluents these drugs can reach surface water. The widespread detection of pharmaceuticals in terrestrial and aquatic systems has engendered significant scientific and regulatory concern. Overall, knowledge concerning the ecotoxicology and sub-lethal effects in water is scarce, but some experimental studies show that antibiotics can induce pathogen resistance and they can also have detrimental effects on natural microbial communities and their key functions. Owing to their chemical structure (e.g., the fluorine atom) and consequent intrinsic biocide property fluoroquinolones can be considered not biodegradable and just a few studies report some microorganisms able to transform them.

The main aim of this study was to investigate the effects of the fluoroquinolone ciprofloxacin (CPF) on the natural microbial community of the river Tiber occurring in its most urbanized stretch. For this purpose, different river water microcosms were set up both in the presence and in the absence (river water sterilized) of the natural microbial community and treated with 500 μg/L of CPF. In order to compare the microbial community in the presence/absence of CPF, microbiological control microcosms were also set-up with no-antibiotic treated river water. At fixed times water samples were collected for measuring CPF residual concentrations over time by using High Performance Liquid Chromatography coupled to Fluorescence Detection (HPLC-FLD). The disappearance time of 50% of the initial CPF concentration
was more than 90 days, thus showing it is a quite persistent compound. Moreover, the effects of the antibiotic on the natural microbial community were assessed in terms of cell vitality, abundance and phylogenetic diversity.
Pharmaceuticals have been detected in all aquatic compartments and the presence of mixtures of several biologically active compounds raises concern about the potential long-term adverse effects to both humans and aquatic organisms from continuous environmental exposure. A majority of studies on these emerging contaminants focus on concentrations detected in wastewater treatment plants, while few studies take into account the role of the natural river microbial community in drug removal and the possible impact of these chemicals on it. An abundant and varied natural microbial community is a prerequisite for ecosystem self-purification processes. In fact, they play a key role in water quality control and, consequently, provide the ecosystem services of regulating (water purification).

Gemfibrozil, a blood lipid-regulating agent, is among the pharmaceuticals most frequently detected in European waters. Although the International Agency for Research on Cancer (IARC) has classified it as belonging to group 3 (i.e., not classifiable regarding its carcinogenicity for humans), it has been shown to have some detrimental effects on non-target aquatic organisms. In this context, the present work aims to evaluate whether the natural microbial community of the River Tiber is able to degrade Gemfibrozil, alone or in the co-presence of Naproxen (a polar acidic pharmaceutical used as an anti-inflammatory and antipyretic drug) and to assess whether the co-presence of the latter has an influence on Gemfibrozil degradation. For this purpose, water samples from a river stretch inside the city of Rome, located downstream from the Magliana wastewater treatment plant, were collected in two seasons (spring and autumn) and used for degradation experiments over a period of more than three months. Water microcosms were set up (in the presence/absence of the natural microbial community) and treated with 100 μg/L of Gemfibrozil alone or in the co-presence of 100 μg/L of Naproxen in order to evaluate the disappearance time of 50% of the initial concentration (DT_{50\%}) under different conditions. At fixed...
experimental times an SPE pre-concentration and purification procedure and then a RP-HPLC with fluorescence detection analysis were performed on microcosm water samples to detect the drug residual concentration. Moreover, the effects of these chemicals on the microbial community structure in terms of variations in bacterial abundance and composition were also assessed. Epifluorescence microscope methods were used for assessing the total microbial number (DAPI counts), the cell viability (Live/Dead method) and the bacterioplancton phylogenetic composition (Fluorescence In Situ Hybridization, FISH method).
Pharmaceuticals and other emerging pollutants: combining passive sampling and tandem mass spectrometry for their determination in waters

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The determination of new compounds used in everyday life, the so-called emerging pollutants, have recently become more and more relevant. Some of these pollutants, such as pharmaceuticals, can easily reach the aquatic compartment from wastewater treatment plants since they are not entirely absorbed by human body\(^1\)\(^-\)\(^3\).

The very low concentration levels and possible matrix interferences represent the main analytical problems. Thus, highly sensitive and selective detection techniques must be preceded by reliable sampling and preconcentration steps. Conventional methods for water analysis involve spot sampling, providing only a snapshot of the levels of pollutants at the time of sampling. Passive sampling represents an alternative approach providing the average concentration of the monitored compounds as a function of the exposure time, enabling also the in-situ preconcentration of target analytes.

A sensitive method using liquid chromatography-tandem mass spectrometry has been developed in our laboratory for the determination of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and other emerging pollutants in waters. Different aquatic environments were studied using the innovative passive sampling approach; in particular, Polar Organic Chemical Integrative Samplers (POCIS) were deployed for two or four weeks in different water matrices (river and surface water, seawater, effluents from water treatment plants) enabling the detection of analytes at ultra-trace levels. Analyte concentration measured in the passive sampler can provide Time Weighted Average (TWA) concentration of contaminants in water if

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the sampling rates (Rs) are previously calculated. The Rs values for each analyte were obtained by means of a simple calibration system developed in our laboratory. As an example, Time Weighted Average concentration of NSAIDs in the river Arno (Italy) was estimated to be in the range 0.33-0.46 ngL\(^{-1}\). This and other case studies are presented and discussed.
Environmental risk assessment of human and veterinary medicinal products – analytical challenges during the ecotoxicological studies

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Assessment of the environmental impact of medicinal products for human and veterinary use is a legal obligation and must be performed to evaluate and limit potential adverse effects of medicines in the environment.

The Environmental Risk Assessment (ERA) is performed in a stepwise approach in Europe. This starts with an initial screening phase (Phase I), aimed at identifying the environmental exposure of pharmaceuticals based on their potential for bioaccumulation and persistence in the environment. If, following this preliminary assessment, significant environmental exposure is anticipated or if specific risks are identified due to compound-specific characteristics, a number of studies should be performed (Phase II) based on the guidance documents issued by European Medicines Agency (EMA).

The Phase II tests identify the fate of medicinal products in the environment and their potential effects on representative organisms (e.g., fish or daphnids for the aquatic environment). For this purpose, the results of various internationally accepted test methodologies (laid down mainly by the Organisation for Economic Co-operation and Development, OECD) form the basis of the risk-assessment process, which may be further extended on a case-by-case basis, depending on the outcome of the assessment.

The authors illustrate what are the specific analytical requirements in terms of environmental fate and behaviour studies of different medicinal products as well as the different approaches of the analytical work in the ecotoxicological studies necessary for a correct ERA.
Photocatalytic decomposition of water contaminants over immobilized TiO₂

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More and more toxic materials come into the freshwaters that could not be mineralized by traditional biological and physicochemical wastewater treatment procedures. Therefore significant developments in chemical wastewater treatment technologies and their applications are required. For economical, technological and health reasons, it is important to use limited type and quantity of chemical additives and to have a good applicability against very different pollutants at low energy consumption. Advanced oxidation processes can meet these requirements. A common characteristic of these processes is the production of oxidative radicals, predominantly hydroxyl radicals, using solar energy or other kinds of energy, that oxidize a great variety of organic compounds. The heterogeneous photocatalysis became an intensively studied research field at the end of the XX century. One obstacle to the in-field use of the method is that the catalyst separation from the liquid phase is difficult and makes the technology more expensive. This problem can be eliminated if the catalyst is immobilized. The PVA-TiO₂ composite catalyst was prepared from poly(vinyl-alcohol) with 146 000-186 000 molecular weight, 99 + % hydrolysis degree and Degussa P25 TiO₂. During heat treatment the colour of the foils changed from white to brown. Upon stirring with distilled water and irradiation in a reactor, the colour of the composite catalyst gradually bleached and the organic carbon content significantly increased in the liquid phase.

Unsaturated alcohols, aldehydes, and carboxylic acids were identified in the GC-MS spectra of the liquid phase. After the pre-treatment of the foil the degradation of Triton X-100 was investigated. The model compound is one of the most widely applied man-made non-ionic surfactants that can hardly be degraded by biological treatment under anaerobic conditions and even in aerobic systems it can be just partly mineralized. Thus, it can reach natural waters damaging various living or-

ganisms therein. It has been established that Triton X-100 could be totally mineralized by TiO$_2$ mediated heterogeneous photocatalysis. The process was monitored by following the spectral changes, the organic carbon content and the actual concentration (UHPLC method). The photocatalytic efficiency of the foil can be sustained through several cycles.

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Oral Session 6

Metabolomics

Chairpersons
Stefano Angelo Santini
György Heltai
Pharmacokinetics of biological medicinal agents

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Due to the complex interactions of the biological agents with the biological system from which these agents are derived, the evaluation of their pharmacokinetic behavior is highly complex and drug assays and bioassays have frequently to be used jointly. Biologicals are usually administered by parenteral routes. Both the absorption and distribution from the Subcutaneous (SC) and intramuscular injection sites are dependent on the molecular characteristics of the drug, its size and charge. The bioavailability of SC administered biologicals varies from 50 to 70 %, while for Monoclonal Antibodies (MABs) the range is even wider, 20 to over 90 %.

The large size of the MABs mostly limits their distribution to the vascular system and their volume of distribution is similar to that of albumin. The pharmacokinetics of MABs which contain IgG proteins is closely related to the presence of the so-called neonatal Fc receptors (FcRn) abundantly expressed in endothelial cells and many other cell types. Following the binding of the IgG on the cell surface or via fluid phase uptake, the MABs enter the cells and are engulfed into newly formed endosomes. Within the low pH of the endosomes the free proteins are degraded while much of the MABs are protected by their strong binding to the FcRn. The endosomes recirculate to the surface and at the neutral pH of the interstitial fluid the MABs are released back into the circulation. This mechanism is responsible for their unusually long (>10 days) half-lives.

Target Mediated Drug Disposition (TMDD) including binding of the molecule to the cell surface targets, subsequent internalization and degradation play a major role in the metabolism and elimination of biological agents. Another important site of metabolism is the Reticulo-Endothelial System (RES). The biological proteins are catabolized to smaller peptides which are then excreted by the kidney (<30 kDa), less frequently through the bile, or are further metabolized to aminoacids. The aminoacids in turn can be reused for protein synthesis.

The Immune Complexes (IC), especially if containing an Antidrug Antibody (ADA), are rapidly taken up by the Kupffer cells of the liver due to the binding of the
IgG part. However, under some circumstances, primarily determined by the nature of the antigen-ADA binding, the produced ICs cannot be eliminated and are bound to various tissues which might lead to pathologic organ function.
Possible application of out-of-plane metalloporphyrins in medical sciences

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Porphydrins and their derivatives represent one of the most significant families of compounds in biochemistry. Distorting effects on any steps in their complicated biosyntheses or their biodegradations result in various porphyrias. This discovery inspired the development of medical photochemistry.

Free-base porphyrins have planar structures with extended conjugated π-electron system and aromatic character, as well as a size-limited coordination cavity for binding of various metal ions. However, the radius of several metal ions is too large (over 80 pm) for the coplanarly fit. These are useful in medical sciences, e.g., Eu, Gd(III) or Mn(II) porphyrins as MRI contrast agents, lanthanide(III) ions for biomedical optical imaging owing to their nIR f-f luminescence (details can be found in Melitta Patrícia Kiss’ poster), Bi(III) for radioimmunotherapy; Ag(I) for photodynamic therapy (see also Zsolt Valicsek’s poster). These metal ions form Out-of-Plane (OOP or Sitting-atop=SAT) complexes with labile, dome-distorted structure and higher reactivity than the in-plane ones. In such case, the fixation of metal ions used to be solved by picket substitutions on the macrocycles.

In our research group, we investigated the formation, the UV-Vis absorption and emission, as well as the photochemical properties of typical OOP complexes of post-transition and lanthanide ions. We determined common characteris-
tics for these complexes, mainly enhanced photoactivities, which may be also useful for their application in photodynamic therapy and photocatalytic sterilizations.

This work was supported by the Hungarian Scientific Research Fund (NN107310) and the Austrian-Hungarian Action Foundation (90öu2).
Comparative study of anticancer copper chelators

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Although the success of cis-diaminedichloroplatinum(II) complex is highly effective for testicular cancer treatment, due to its dose-limiting side effects and limited use because of inherited or acquired resistance the investigation of alternative metal-based drugs came into the spotlight. Thus, there is a high demand on other molecularly targeted metal based-drugs, preferably complexes of endogenous metals such as Cu. Moreover, molecules with chelate structures have been identified as efficient molecules to combat multidrug resistance.

In vitro efficacy of several Cu chelating agents belonging to different classes such as thiocarbazone, thiosemicarbazone, quinoline, phenantroline and thiocarbamate were investigated alone and in the presence of Cu on various cancer cells. Among these compounds, Dp44mT is has a paramount importance.

In the present study, characterization of complex forming properties was performed to establish possible linkage between complex stability/composition and Cu-dependent toxicity. Generally, Cu complexes with 1 : 1 stoichiometry are more toxic than those with 1 : 2 Cu : ligand stoichiometric composition. On the other hand, it is important to investigate the relationship between metal transport capacity of chelators and metal-induced cellular toxicity. Thus, cellular toxicity seems to correlate with Cu accumulation and Zn/Fe depletion. Copper accumulation depends largely on the different metal : ligand molar ratios and this phenomenon was investigated in detail. Another key aspect is the Cu localization within the cells. According to μ-XRF imaging experiments, Cu could be detected solely in the nuclei. However, our experiments pointed out that there is a selective sensitivity of certain cells to Cu poisoning.
Chiral capillary electrophoresis in metabolic research

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Due to its extremely high separation efficiency capillary electrophoresis well fits the requirements of chiral analysis where enantiomers are to be separated. Enantiomeric resolution is achieved by interactions with a chiral pseudostationary phase, thus the separation is based on both electrophoretic and chromatographic principles further increasing the peak capacity of the method. In addition to its high separation power flexibility is another advantage of chiral capillary electrophoresis that results in rapid method development. On the other hand, analysis of biological samples with complex matrix can raise a significant challenge even to this powerful separation technique, thus careful method development is essential to achieve chiral resolution and ensure appropriate chemical selectivity.

More and more data suggest the biological role of some D-amino acids in mammalian brain. D-Aspartate and D-serine can modulate the function of N-Methyl-D-Aspartate (NMDA) receptors and may participate in neuronal development and plasticity. We aimed at developing a chiral capillary electrophoresis method for separation and determination of D-aspartate and D-serine in various biological samples to study their role in neuronal function.

Enantiomeric resolution of aspartate and serine was achieved after their fluorescent derivatization by using an amine modified cyclodextrin as chiral selectors. Quantitative performance of the method was validated according the FDA guidance.

D-Aspartate and D-serine levels were assayed in the brain areas of animals. Concentration of D-serine was much higher than that of D-aspartate in all areas but cerebellum. Higher levels of both amino acids were detected in the brain regions involved in neuroplasticity.

Neuronal differentiation is also studied in vitro using SH-SY5Y neuroblastoma cell line. Differentiation of neuroblasts in cell culture was accompanied by accumulation of D-amino acids in the cells suggesting their role in neuronal development and maturation.
The use of omics-based approaches in regulatory toxicology

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Since their first applications, more than a decade ago, genomic technologies have been rapidly evolved as powerful tools for discovery- and hypothesis-drive perspectives in research. More recently, the concern about animal welfare and the need to provide mechanistic insights into the process of toxicity prediction, led to new viewpoints in the use of these high-throughput approaches in combination with in vitro tests.

In vitro tests, toxicogenomics and computational methods have been included in the current REACH regulation as a possible approach to refine, reduce or replace animal studies. The possibility to predict the adverse effects in humans by applying a bottom-up approach, based on the use of the combination of in vitro approaches and toxicogenomics, has been incorporated in the vision of Tox21.

Several regulatory agencies encourage the use and submission of complementary toxicogenomic data in an effort to establish guidelines. The use of omics methods has been included in the OECD approach to the identification of adverse outcome pathways and within integrating testing strategy. It has been proposed as an additional tool to the grouping and prioritization of toxic substances and it is regarded as a reliable approach to improve the drug approval process at lower cost and higher level of safety.

Toxicogenomics has already been included in studies aiming at addressing complex topics, such as the relevance to humans of results from animal studies, the effects related to exposures at low and very low doses, the toxicological behavior of complex mixtures. Even if the holistic approach of toxicogenomics, which aims at integrating all the information from all the omics components, including transcriptomics, epigenomics, proteomics and metabonomics, is still difficult, the use of single components is regarded as a precious tool in personalized medicine and chemoprevention intervention.
Novel tools of radiochromatography in pharmacokinetic and drug metabolism research

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This lecture sums up the possible novel tools of the radiochromatography in the pre-clinical and clinical pharmacokinetic and drug metabolism research. The essential pharmacokinetic and drug metabolism information of different species contribute to the final drug registration process.

The high detection power (sensitivity) pg/mL, fg/mL, at/mL) and highly selective hyphenated techniques such as Liquid Chromatography (LC)/Triple Quadrupole (Quad)-Jet Stream-Electrospray (ESI)-Mass Spectrometry (MS) and Gas Chromatography (GC)/MS-MS) required for quantitative pharmacokinetic studies had replaced the conventional methods of detection such as GC and High Performance Liquid Chromatography (HPLC).

Nowadays in the course of drug development the radioactive isotopes (beta and gamma single and/or double source) labeled (\(^{3}\)H, \(^{14}\)C, \(^{99}\)Tc, \(^{131}\)I) pharmacokinetic studies performed by means of the new generation of triple-Quad MS techniques combined, e.g., with GC, LC and Over Pressure Layer Chromatography (OPLC) are essential. A number of related case studies are presented.

The former high quality Imaging Techniques, e.g., Digital Autoradiography (DAR) and Phosphorus Imaging Technique (PIT) and the new generation of \textit{in vitro} – \textit{in vivo} Imaging Techniques, namely Matrix-Assisted Laser Desorption/Ionization (MALDI) Imaging Technique, nanoScan, Positron Emission Tomography (PET)/Magnetic Resonance Imaging (MRI) in animal and human studies are also presented.

A complex multi-step process is illustrated from separation, purification, isolation to structure elucidation, e.g., GC-MS, LC-MS/MS, LC-Nuclear Magnetic Resonance (NMR) of minor and major metabolites derived from animal and human biological matrices. The addition of the above systems to the off-line and on-line separation and radioactivity detection possibilities of OPLC-DAR/PIT, OPLC-Radioactive Detector (RD), HPTLC-DAR-MS and GC-RD, HPLC-RD and the combined multi-hyphenated techniques OPLC-DAD-RD-MS/MS, OPLC-DAD-RD-NMR as
well as LC-DAD-RD-MS/MS and LC-DAD-RD-NMR resulted in a new, flexible and rapid high-performance complex approach to metabolism research.
Profiling the toxicity of new drugs in an animal-free testing strategy: the relevance of integrating dynamics and biokinetics

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When developing testing strategies, kinetics is considered the crucial body of information for the design and performance of toxicological tests and for toxicity data interpretation. Indeed, toxicokinetics is defined as the quantitation of the time course of a toxicant in the body during the processes of its absorption, distribution, biotransformation and excretion or clearance. The end result of the toxicokinetic processes is a biologically effective dose of a chemical; therefore, the knowledge of the bioavailability and kinetics should represent the starting point for any toxicological testing, both in vivo and in vitro.

Nevertheless, only very few in vitro studies have addressed this issue and the nominal applied concentrations rather than the actual level of cell exposure are usually associated to an observed effects. In this respect, the role of in vitro biokinetics has been explored within the FP7 Project PREDICT-IV, aimed at developing a strategy to allow Adverse Drug Reactions (ADRs) to be identified in the early stage of development, by using in vitro methods integrating kinetics and dynamics data.

One of the Work Packages (WPs) was coordinated by ISS and devoted to measuring or estimating the real exposure of cells to drugs and/or their metabolites in some in vitro test systems, as a key element for the extrapolation of in vitro results to the in vivo situation. An experimental approach has been developed in order to measure the actual intracellular concentration, possibly affected by both abiotic processes (e.g., chemical stability, interactions with medium/plate) and by physiological cellular processes (transport across the membranes, biotransformation, bioaccumulation) after acute and repeated treatments of cellular models from the liver (rat and human primary hepatocytes and HepaRG cells), the Central Nervous System (CNS) (3D and 2D cell models) and the kidney, the three organs mainly involved in drug attrition and removal from the market for ADRs with model compounds (e.g.,
ibuprofen, amiodarone, ciclosporin A and chlorpromazine). The obtained biokinetic information were used as input for in vitro kinetic modeling, allowing the estimate of the actual intracellular concentration over 14 days of repeated treatment. Results are presented showing how the use of kinetic data could allow a better interpretation of, e.g., differences in effects observed among cell models.

This approach made possible to derive a No Observed Effect Concentration (NOEC) in in vitro experimental models, preferentially based on human cells representative of in vivo target organs, from which the corresponding in vivo dose can be extrapolated by means of Physiologically-based Pharmacokinetics (PBPK) modelling.
Oral Session 7

Nutraceuticals

Chairpersons
Ornella Abollino
Viktor G. Mihucz
Evaluation of different approaches for the elemental characterization of dietary supplements

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The content of toxic and potentially toxic trace elements namely, As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, V and Zn was determined in nine different brands of dietary supplements purchased in Argentina, Brazil, Canada and USA. The aim of the study was also to establish a reliable procedure for sample digestion and trace element determination in this kind of samples. To this end, various Microwave (MW)-assisted digestion approaches using different acid mixtures were applied and compared.

Several analytical techniques including plasma-based techniques Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Hydride Generation/Cold Vapour Atomic Absorption Spectrometry (HG/CV-AAS) were selected for the determination of total element concentration. The overall approach was tested in tablets of three brands of multivitamins, cholesterol control tablets, Se supplement, multivitamin + multimineral supplement, herbal pills and Ca-based tablets.

For checking the accuracy of measurements, aliquots of the Certified Reference Material MURST-ISS-A2 (Antarctic Krill) were subjected to the same MW treatment and included in the overall analytical process. For most elements, a good agreement was found between certified and found values.

Mercury concentrations in all of the analyzed samples resulted below the Limits of Detection (LODs) for this element (35 ng g⁻¹). Elemental concentrations of the other elements investigated showed a great variability depending on the dietary supplement and the trademark ranging from 9.0±0.8 ng g⁻¹ (Cd) to 0.75±0.06 % (Zn). It is important to note that Cr (0.27-52.8), Cu (0.71-1307), Mo (0.09-16.9), Ni (2.5-7.7), Se (0.06-328) and V (0.10-10.4) were found at μg g⁻¹ levels in the range of concentrations shown in brackets with an important variability between supplements.

For several dietary supplements no information on the content of trace elements
is mentioned by the manufactures. When reported, for most elements concentra-
tions agree with found values. The major problem found was that significant dif-
ferences in metal concentrations were detected among tablets in one and the same
container, this being a serious problem for consumers.

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(Brazil) and Mariana Achad (Argentina)
In vitro estimation of element bioaccessibility in ayurvedic products

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One of the most popular traditional medicine systems in the world is Ayurveda, which was developed in India more than two thousand years ago and is still used nowadays. In the last decades, Ayurveda has become increasingly popular worldwide, and ayurvedic formulations are available from chemists, ethnic markets, practitioners, health food stores and the Internet. Such formulations can be divided into two main classes: herbal-based (kasthausadhi) and metal-based (rasausadhi). The latter contains herbs deliberately combined with metals (e.g., Fe, Hg, Pb, Zn), minerals (e.g., mica) and gems (e.g., pearl). Over recent years, some cases have been published reporting patients poisoned by heavy metals after ingesting metal-based ayurvedic preparations. On the other hand, such products are being used by millions of people in India without apparent side effects.

For these reasons, we focused our attention on the inorganic components of 17 ayurvedic products sold through different distribution channels: Indian ayurvedic medical shops, an Italian drugstore and the Internet. A chemical element in an ingested product may not be totally available for absorption by the organism and therefore its total concentration is not sufficient to assess its potentially harmful effects; the amount actually assimilated can be estimated in vitro by measuring the

bioaccessibility, i.e., the fraction of a compound that is released from its matrix in the gastrointestinal tract\(^2\). We firstly determined the total concentrations of 25 elements in the investigated products.

Five medicines, among those purchased in India, contained high total amounts of As, Cu, Hg, and Pb; the concentrations of potentially toxic elements in products purchased on the Internet and in the Italian drugstore were below the safety limits fixed by international authorities: ayurvedic medicines marketed in Western countries have to be declared “metal free”. We then assessed element bioaccessibility in the above mentioned five products by extraction into solutions simulating gastric and intestinal fluids. Two products had bioaccessible As concentrations greater than the corresponding maximum admissible intake level. Element extractability was higher in gastric juices than in the intestinal medium and was influenced by the form of administration. Concentrations were determined by Optical Emission Spectrometry (OES) or Atomic Absorption Spectrometry (AAS). Experimental results were interpreted by means of chemometric techniques.

\(^2\) Shi-Wei Li, Jie Li, Hong-Bo Li, Ravi Naidu, L. Q. Ma, Arsenic bioaccessibility in contaminated soils: coupling in vitro assays with sequential and HNO\(_3\) extraction, *J. Haz. Mat.*, 295 (2015), 145-152.
Diet-related bioactive compounds are intensively examined in vitro, although their in vivo and in vitro effects are different and the mode of actions in in vivo are not yet known in detail. Metals are also important both in free radical formation and in antioxidant defence in signal transduction. It is a proven fact that methylating agents play an important role in preventing cancer and improve redox homeostasis. The root of the table beet plant has been used for centuries as a traditional and popular food in many national cuisines.

Beneficial medical effects of table beet are due to betaine, betanins, betacyanins, betaxanthins, vulgaxanthine, flavonoids, polyphenols, vitamins (thiamine, riboflavin, piridoxine, ascorbic acid, biotin and folic acid) as well as soluble fibre, pectin and different metal elements.

In this clinical study 10 g natural table beet lyophilized product (361/004/2003 BFAEE GPS POWDER) was administered twice daily for 1 month to 24 patients (mean age 68±8 years) with hormone-resistant and metastatic prostate cancer treated with taxan chemotherapy, who reported their complaints themselves first, mean 3.6±2.8 years before. The information of 18 men was amenable after treatment for evaluation. In addition to routine laboratory examination, values of HbA1c, 9 cytokines and levels of 3 growth factors, the global parameters of redox homeostasis, few metal elements and level of free and Zn-protoporphyrins, transmethylation ability were determined before and one month after treatment (Permission number: Semmelweis University 127/2006). Low transmethylation ability, high free and Zn-protoporphyrin concentrations and high induced free radical level of erythrocytes are very important indexes of cancer.

The favourable impact of table beet is enforced and significantly high levels of free and Zn-protoporphyrins decrease. Furthermore, transmethylation processes fasten in cancerous patients. These results clearly show that Fe, folic acid and betaine components as well as colourful compounds with antioxidant activity of table beet lyophilized products demand more attention as a preventive therapy in chemo-
therapy induced anaemia. Table beet will have a great impact and application in hu-
mman cancer, but because of the increasing values of Epidermal Growth Factor (EGF) 
and parallel Prostate Specific Antigen (PSA) level in some taxane treated patients, 
close medical control is necessary for patients especially during chemotherapy.

Table beet bioactive compounds affect numerous biochemical reactions, en-
zyme activities and metabolic pathways, therefore this vegetable can be considered 
as functional food.

_The support of the Semmelweis University Doctoral School to this investigation is gratefully acknowledged._
In vitro and ex-vivo models to study sucrosomial® iron pharmacokinetic

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The Caco-2 cell monolayer is currently being considered a useful model for studying Fe uptake by human intestinal cells. In fact, an experimental protocol that conjugates the simulation techniques of the gastrointestinal digestion with the Fe internalizing phase by the Caco-2 cells has been set up. Such a protocol appears very useful and predictive of the in vivo behaviour so far as it takes into account the phase of Fe³⁺ dissolution in the gastro intestine and the subsequent permeation across the epithelium. Indeed it cannot be taken for granted that the latter phase is slower than the former and hence that it is the rate-determining one. It is known that at pH values higher than 3 the Fe³⁺ ion tends to form Fe(OH)₃, the water solubility of which is practically null. For this reason the organism has developed an efficient transport system. Quite hypothetically another type of Fe³⁺ transport across the intestinal epithelium could involve the cellular endocytosis of a Fe³⁺ carrier (sucrosome, e.g., nano- or micro-particulate). For these reasons the Caco-2 cells model might not predict the in vivo bioavailability of Fe³⁺ which could reach the general circulation by internalization and subsequent release from the intestinal epithelial cells of the nano or micro-particulate Fe³⁺ carrier. Therefore, to evaluate the ability of the carrier to transport Fe³⁺ across the epithelium an ex vivo model based on excised rat intestine could be more predictive.

The present work compares the two models, namely that based on the Caco-2 cells monolayer and that based on the excised rat intestine, for their ability to predict the absorption of Sucrosomial® Iron from different formulations supplied by Pharmanutra SpA. For Caco-2 cells absorption studies, Caco-2 cells were seeded in well plates, maintained in cell culture medium and used in the Fe uptake experiments at 14 days post-seeding. An aliquot of the intestinal digest was pipetted into the upper chamber and after 24 h-incubation, cells were harvested for analysis. For permeation studies, the intestinal mucosa was excised from non-fasting male Wis-
tar rats. The excised intestine was cut into strips and mounted in Ussing-type chambers without stripping off the underlying muscle layer. Data described in this work has shown that the Sucrosomial® Iron formulations have the ability to promote the Fe$^{3+}$ absorption across the intestinal epithelia, probably thanks to the endocytosis of the microparticulate Fe$^{3+}$ carrier.
Trace determination of skin-irritating metals in tea tree oil by GFAAS

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Tea tree oil, originating from Australia, is nowadays used worldwide as all-round home remedy. It is applied diluted or undiluted for the treatment of skin and nail infections, against lice, scabies, athlete’s foot and ringworms. Furthermore, it is used topically as a local antiseptic for cuts and abrasions, for burns and insect bites. Tea tree oil is considered safe when put on the skin, but it may cause also skin irritation and swelling. In rare cases skin dryness, itching, stinging, burning and redness have been observed. Skin irritation is mainly associated with the organic compounds, but may also be caused by allergenic metals, such as Co, Cr and Ni.

This study focuses on the determination of selected skin-irritating metals in tea tree oil samples available on the European market. Measurements were performed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Although only a single-element method, this technique offers substantial advantages for the given analytical task. The oil sample can be applied directly without any sample pre-treatment, reducing time and labour as well as the risk of contamination by the reagents used for digestion. The method is optimised for each analyte regarding drying, pyrolysis and atomisation step and the application of modifiers. The obtained characteristic masses for the elements analysed are close to the theoretical ones. Thus, the proposed method can profitably be used for the analyses of tea tree oil. It is supposed that this method is also appropriate for investigating other oils used for instance in aromatherapy or for massages.
The genus *Hypericum* (Guttiferae) is one of the most representative species in temperate zones\(^1\)-\(^2\). Due to the increasing commercial value of *Hyperici herba* (*Hypericum perforatum*), many other *Hypericum* species have received considerable renewed interest in the last years. In fact, several species have been recently investigated especially for their content in hypericins and flavonoids as potential substitutes of the well-established *H. perforatum*. However, particular attention should be given also to their volatile constituents, which could play a crucial role not only in the chemotaxonomic classification, but also as new target compounds for quality control procedures. The present preliminary study points out some *Hypericum* volatiles as potential inter-species discriminating phytochemicals to perform two quality control tasks, namely:

- to define volatile fingerprint of new valuable species with similar composition in the typical standardisable active principles (hypericins, hyperphorins and flavonoids);
- to avoid contamination in *H. perforatum* raw plant material with other wild collected *Hypericum* spp. the well-established medicinal use of which has not yet been evaluated for the EU market.


Exhausted roots of licorice: an unexplored potential source of bioactive compounds

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Licorice, the dried roots of Glycyrrhiza species (Fabaceae family) is one of the oldest and most widely used herbal drugs in both eastern and western countries. Due to its sweet taste, it is also used in the preparation of candies and as a flavoring additive in food and tobacco industries. Numerous authors have reported that the chemical constituents of licorice have biological effects such as anti-inflammatory, anti-ulcer, anti-hepatotoxic, anti-microbial, anti-viral and anti-oxidant benefits.

The traditional and industrial productions of candies mainly use hot water or steam distillation in order to produce licorice extract. The result of this process is an aqueous extract and a solid residue consisting of exhausted licorice roots. This residue is normally used as soil conditioner in local agricultural activities; some alternative uses have been proposed, like the production of activated carbon, but nothing concerning the extraction of bioactive compounds.

In order to characterize the chemical composition of the exhausted licorice roots, a metabolomic approach has been used that has proven to be a fast, reliable and highly efficient method for the simultaneous determination of a high number of compounds. High Performance Liquid Chromatography - High Resolution Mass Spectrometry (HPLC-HRMS) measurements, combined with statistical methods, revealed the metabolites composition of the licorice residues. Many of the identified compounds in this matrix are known for their effects on human health, like glycyrrhizin, the main – and the most studied - constituent of licorice roots.

Two different extraction solvents were tested and the relevant extracts were analyzed to elucidate the composition differences and the extraction efficiency. The
dried extracts were also tested for biological activity. Preliminary results show a moderate bioactivity for the methanol/water extracts on some *Staphylococcus aureus* strains.

This is the first report concerning possible alternative uses of this kind of material and we believe that the residues analyzed in this work can be characterized as raw material for the extraction of some chemical compounds with pharmacological activity.
Poster Session 1

Pharmacological Research and Innovation

Coordinator
Giacomo Pozzoli
Mixed metal oxide nanoparticles as pharmacologically active biocides

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Metal oxides, well-known for decades as catalysts, possess an astonishing pharmacological potential due to their peculiar properties, especially when used in their nanosized form. Envisaged applications include treatment of chronic inflammation and pathologies associated with oxidative stress¹. Recently, investigations were performed on the use of metal oxide nanoparticles, such as CuO and ZnO, as biocides with respect to Gram-positive and Gram-negative bacterial strains²,³. Here we report the enhanced biocidal effects of purposely synthesized nanosized mixed Cu/Zn metal oxides. Escherichia coli was chosen to test the biocidal efficacy for its rapid growth rate and simple nutritional requirements. The E. coli number decline was estimated by measuring at OD600 at 0, 4, 8 and 24 hr and by a culture method after cells exposure to the nanosized mixed metal oxides. The 5-7 nm diameter mixed oxide nanoparticles exhibit a higher biocidal power against E. coli compared to the corresponding single oxides and are promising for efficient pharmacological applications. Furthermore, with the increase in concentration of nanoparticles, the bacterial inhibition rate also increased. The highest viability reduction values (> 99 %) were obtained at the highest doses with the longest exposures.

Investigation of in vitro copper toxicity induced by chelators in human cancer cells

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Copper is found in all living organisms and it plays a pivotal role in mitochondrial respiration, Fe absorption and free radical scavenging. At high concentrations, Cu is also toxic since it is able of producing Reactive Oxygen Species (ROS) causing lipid peroxidation and direct cleavage of DNA/RNA. Thus, the Cu levels in human organisms are strictly regulated. Several molecules, typically chelators, are known to overcome this strict regulation inducing Cu-dependent toxicity exploited for targeted cancer treatment.

For the present study, HCT-15 and HT-29 colon adenocarcinomas, HT-1080 fibrosarcoma as well as MCF-7, MDA-MB-231 and ZR-75-1 human breast adenocarcinomas were used. As chelators, 2,2’-biquinoline, 8-hydroxyquinoline, APDTC, Dp44mT, dithizone, neocuproine and D(-)- penicillamine were used, the latter as control. Generally, cells were incubated without fetal calf serum by adding 2 μM Cu(II) and chelators at a concentration of either 5 or 50 μM.

Evaluation of in vitro cytotoxicity and cytostatic effects of the chelators as well as long time antiproliferative effects were assessed. Independently of the performed experiment, Dp44mT was the most efficient chelating agent. For the rest of the investigated compounds, the following toxicity order could be established: neocuproine > APDTC > 8-hydroxyquinoline > dithizone,> 2,2’ biquinoline, whereas D(-)- penicillamine showed no toxicity. The IC₅₀ values decreased considerably in the presence of Cu. As in the case of cytotoxicity, a similar tendency could be observed for cytostatic. The colony forming ceased with the 6-hr-long treatment for Dp44mT, whereas the decrease in colony forming, and consequently cytostatic activity, was
observed for the rest of the Cu chelating agents only with the 24-hr-long treatment. Elevated Cu concentration (from 0.5 μM to 50 μM) caused higher apoptotic cell ratios in combination with Dp44mT and 8-hydroxiquinoline already after 20 min. Thus, the key to explain the toxicity mechanism of Dp44mT should be connected to the intracellular binding of Cu ions. Each investigated chelator induced ROS generation in the presence of Cu(II). Circular dichroism spectra demonstrated that the investigated chelators - except for neocuproine - restored DNA damage induced by free Cu(II).
The effect of portal vein occlusion on drug-metabolizing function of rat liver

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Cytochrome P450s (CYPs) plays an important role in the oxidation of structurally diverse xenobiotics (e.g., drugs, pesticides, environmental pollutants). Pathophysiological conditions, such as disease or surgical interventions, can influence drug-metabolizing function of the liver. The Portal Vein Occlusion (PVO) has been proven to be useful as a preoperative treatment for major hepatic surgeries with impaired liver function. PVO induces atrophy of portal deprived lobes, while the non-ligated part of the liver shows hyperplasia. These processes might accompany with alteration of hepatic drug metabolism.

The aim of the present work was to investigate drug-metabolizing function and CYP activities in male Wistar rats 24, 48, 72, 168 and 336 hr after PVO. In vivo functional sleeping test (30 mg pentobarbital /kg bw) was used to characterize overall hepatic CYP3A activities. CYP selective activities (midazolam 1'- and 4-hydroxylase for CYP3A1/2, pentoxyresorufin O-dealkylase (PROD) for CYP2B1/2 and ethoxyresorufin O-deethylase (EROD) for CYP1A1/2 were determined in microsomes isolated from ligated and non-ligated lobes of the liver.

PVO resulted in weight decrease in the atrophic part (ligated) of the liver with concomitant increase in the hypertrophic (non-ligated) part; however, the total liver weight did not change throughout the study. The sleep time significantly increased 48 and 72 hr after PVO and was normalized by 336 hr. Midazolam hydroxylation of CYP3A significantly decreased in ligated lobes, whereas substantial increase was
observed in non-ligated lobes 72 hr after PVO. Furthermore, midazolam 1'- and 4-hydroxylase activities were much lower in portal deprived lobes than in non-ligated lobes at each time point. In ligated lobes, significant decrease in PROD activities was observed 168 hr and 336 hr after PVO. In non-ligated lobes, significant increase in EROD and midazolam 1'- and 4-hydroxylase activities was measured 168 hr after PVO, which decreased to the starting activity by 336 hr.

In conclusion, PVO resulted in some fluctuation in CYP activities in ligated (decreased activities) and non-ligated (increased activities) lobes of the liver; however, two weeks after PVO, CYP3A, CYP1A and CYP2B activities were normalized. The overall CYP3A activity characterized by in vivo functional sleeping test indicated transient decrease at the early post-PVO period.

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The role of purinergic mechanisms in maternal Poly(I:C) evoked rodent model of autism spectrum disorder (ASD)

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition caused by the interaction of large set of genes and environmental factors. Symptoms are highly variable, but most frequently include individualized behaviours from deficits of social-emotional reciprocity, verbal and non-verbal communication, deficits of development and maintenance of relationships. Typical symptoms are stereotype and repetitive motor movements, ritual behaviour patterns, fixated interests with abnormal intensity and hypo- or hyperreactivity to sensory input of the environment. Comorbidities are various from Gastrointestinal (GI) disturbances, increased seizure susceptibility, sleep disorders, anxiety, motor and sensory impairment, reduced nociception, altered mitochondrial dynamics, hypopimmunoglobulinaemia, neuroinflammation, Purkinje cell loss etc.

Recent studies have revealed that Suramin, a broad spectrum P2 receptor antagonist relieves the majority of symptoms in different animal models of autism. Our aim was to establish a reliable model of ASD utilizing behavioural, neurochemical and anatomical parameters in order to investigate the role of different P2 receptors in autism. We measured the effect of prenatal Poly(I:C) (PIC) treatment in 8-weeks-old wild-type and P2X7 receptor knockout (P2X7R KO) mice, respectively. We performed social preference test, rotarod test, self-grooming and marble-burying tests. After behavioural experiments animals were sacrificed, para-sagittal sections of the cerebellar vermis were cut and Purkinje cells were counted. Half-brain synaptosome fractions were examined by Electron Microscopy (EM). Monoamine contents of the striatum and hippocampus were measured by High Performance Liquid Chroma-
tography (HPLC). We compared PIC treated offsprings with saline treated animals (n=10-16 animals/group) and measured the effect of Poly (I:C) in P2X7R KO animals.

PIC treated animals showed decreased sociability and impaired motor coordination. Maternal Poly(I:C) treated animals showed increased repetitive behaviour in the marble-burying and in the self-grooming tests. Quantitative Purkinje cell dropout was found in PIC treated mice and EM of half brain synaptosome fractions revealed ultrastructural abnormalities in them. Disturbances in monoamine levels were found in PIC treated offsprings. Mice lacking the P2X7 receptor did not show the above mentioned changes.

Maternal Poly(I:C) treatment reproduced autistic features described in the literature in the majority of the offsprings. Poly(I:C) treatment did not elicit autistic-like behaviour on mice lacking P2X7 receptor. This suggests that the P2X7 receptor plays a role in neuronal development and the formation of ASD.
Is CYP2D6 phenotype predictable from CYP2D6 genotype?

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The genes of cytochrome P450 (CYP) superfamily code approximately sixty isoenzymes in human beings. Most of them are highly polymorphic resulting in clinically significant modifications in drug metabolizing capacities. CYP2D family members are CYP2D6 and two pseudogenes (CYP2D7P and CYP2D8P). Although CYP2D6 presents only a small proportion of hepatic CYP enzymes, CYP2D6 is one of the most important drug metabolizing enzymes, catalyzing the metabolism of approximately 25-50 % of the drugs on the market, such as beta-blockers and antipsychotics. Hitherto more than one hundred different allele variants have been identified. Allele variants show different phenotypic appearance; thus, the population is divided into four metabolizer groups: poor (PM), intermediate (IM), extensive (EM) and ultra-rapid metabolizers (UM). Comparing to other CYP genes, CYP2D6 has some special properties. Both Single Nucleotide Polymorphisms (SNP) and gene Copy Number Variations (CNV) (such as gene deletion and multiplication,) frequently occur. On the other hand, the transcription of the CYP2D6 gene is not inducible by xenobiotics; thus, CYP2D6 expression particularly depends on the genetic factors. Consequently, the question is raised whether CYP2D6 phenotype is predictable from CYP2D6 genotype.

For identification of loss-of-function mutations in CYP2D6 gene occurring most frequently in Caucasian populations (CYP2D6*3,*4,*6,*10,*41), Real-Time PCR methods have been developed. The presence of gene deletion (CYP2D6*5) or duplication/multiplication was also evaluated by CNV analysis. CYP2D6 phenotype was de-
CYP2D6 genotype and CYP2D6 activities of 138 Hungarian liver donors were evaluated. CYP2D6 phenotypes were partially confirmed by CYP2D6 genotypes. Donors with low CYP2D6 activity (PM) often carried only one non-functional allele, albeit this genotype is generally considered to be IMs. PM phenotype was evoked by some additional non-functional allele variants. UM phenotypes were not confirmed in all cases by gene duplication/multiplication. Some authors have found relationship between UM phenotype and the -1584T>C SNP in the CYP2D6*1 allele’s promoter region. This mutation may lead to increased gene expression and higher activity.

Identification of the most frequent allele variants of CYP2D6 is not appropriate for reliable prediction of CYP2D6 phenotype. Thus, the monitoring of genotype should be more comprehensive and determination of other allelic variants is necessary.

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Utility of *in vitro* pharmacokinetic data in prediction of *in vivo* hepatic clearance of antipsychotics

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*In vivo* drug clearance is a significant pharmacokinetic parameter which largely determines the drug exposure. In the early phase of drug development, hepatic stability screening is a widely used method to assess the metabolic stability and to predict *in vivo* hepatic clearance of a drug candidate. Primary hepatocytes offer a useful *in vitro* model because the cells possess the full complement of drug-metabolizing enzymes and contain cofactors at the physiological concentrations. Additional advantages of hepatocytes applied in suspension are the rapid distribution of the test compound and rapid sampling for kinetic studies. *In vitro* pharmacokinetic data obtained from human hepatocytes can be used for prediction of *in vivo* hepatic clearance in human, whereas *in vitro* clearance data from hepatocytes isolated from rat, dog or rabbit can be useful to identify the laboratory animal(s) with similar pharmacokinetics to human beings and to select the animal model most relevant to human.

The utility of primary hepatocytes for the prediction of *in vivo* clearance was investigated by using 14 antipsychotic drugs of disparate structures (aripiprazole, biperiden, carbamazepine, clonazepam, clozapine, duloxetine, fluoxetine, haloperidol, mianserin, mirtazapine, olanzapine, paroxetine, quetiapine, risperidone). *In vitro* pharmacokinetic parameters (elimination half-life, intrinsic clearance and hepatic clearance) were determined in human, rat, dog and rabbit hepatocytes by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) and hepatic extraction ratios as well as bioavailability values were predicted.
The fastest elimination and the lowest elimination half-lives were observed in rabbit and rat hepatocytes, whereas the elimination of these drugs was one or two magnitude orders slower in human liver cells. In vitro clearance values obtained from rat hepatocytes displayed strong correlation with in vitro human clearance values. The human hepatic extraction ratios of antipsychotics ranged widely from the lowest values for carbamazepine and clonazepam (<0.1) to the highest for quetiapine (0.7). The human bioavailability values predicted from in vitro pharmacokinetic data were in good agreement with clinical bioavailability data. Altogether, the predicted bioavailability obtained from human hepatocytes showed an excellent rank order with in vivo findings. Furthermore, rat was considered to be the most relevant animal model to human subjects.

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How to choice a clinical study management system

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The scope of a Clinical Trial (CT) is to answer most of the questions created by scientific research in drug development. This may be done by generating data with the objective to demonstrate or deny a hypothesis.

Data managing is a critical element in CT management. The quality principles must be applied to all of the data managing phases, such as acquisition, digitization, archiving, analysis, presentation and use.

It is easy to understand that the quality of the generated data plays a very important role in the trial results and that data managing covers a substantial part of the whole study.

To improve data quality the greatest importance should be attached to prevention, control and correction. From this viewpoint, it is quite easy nowadays to find a fit-for-purpose Clinical Data Management System (CDMS).

The following approach is recommended:
- Phase 1, User Requirements definition;
- Phase 2, CDMS Information Technology (IT) structure definition and its connection to the Company IT structure;
- Phase 3, CDMS simplicity.

A good CDMS may reduce errors in data entry, thus increasing precision and requiring less quality control activities. This may well result in saving time and cost in data managing.
Contribution of CYP3A enzymes and NAT2 alleles to clonazepam metabolism

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Clonazepam is a benzodiazepine-type drug, acting as a post-synaptic GABA₆ receptor modulator. It displays potent anticonvulsant, muscle relaxant, anxiolytic and hypnotic properties and as such it is often prescribed for the treatment of panic attacks, generalized anxiety, social phobias and epilepsy. Clonazepam is metabolized to 7aminoclonazepam by CYP3A enzymes catalyzing nitro-reduction, and then Nacetylated to 7acetamidoclonazepam by the polymorphic NAT2 enzyme. The differences in clonazepam metabolism can be explained by the genetic polymorphism of CYP3A5 and the changes in CYP3A4 expression due to various environmental and endogenous factors. Consequently, some patients’ drug-metabolizing capacity can be poor (individuals with low or no activity for a given isoenzyme) or extensive (individuals with faster metabolism rates) comparing to the majority of the population. CYP3A5*3/*3 homozygous mutant genotype, resulting in the lack of CYP3A5 expression, occurs in 90% of the white population. The presence of CYP3A5*1 allele results in CYP3A5 enzyme expression, which may lead to an increase in the metabolism of CYP3A substrates since the enzyme activity of CYP3A5 is added to CYP3A4 activity. N-acetyltransferase 2 enzyme encoding by NAT2 gene contributes to both the activation and deactivation of arylamine and hydrazine compounds. Polymorphisms in NAT2 gene segregate the population into slow and rapid acetylator phenotypes. The NAT2*4 allele results in the expression of wild-type enzyme, whereas the two most common alleles, NAT2*5B and NAT2*6A lead to non-functional enzymes.
The CYP3A-status (CYP3A5 genotypes and CYP3A4 enzyme expressions) (CYPtest™) and NAT2 genotypes (for NAT2*4, NAT2*5B and NAT2*6A alleles) were determined by quantitative real-time PCR in 94 psychiatric patients treated with clonazepam. Relationship between the serum levels of clonazepam or 7aminoclonazepam and patients' phenotypes for these enzymes was investigated.

Our results show that CYP3A5 genotype does not influence clonazepam serum levels, whereas a strong association (p< 0.0001) was found between CYP3A4 expression and clonazepam serum levels normalized by body weight. Patients with low CYP3A4 expression required lower clonazepam doses for therapeutic blood concentrations than patients with normal CYP3A4 expression (0.03 mg/kg vs. 0.05 mg/kg, respectively). Individuals qualified as slow acetylators for NAT2 and having normal CYP3A4 expression displayed higher 7-aminoclonazepam/clonazepam ratios (1.212) than the rest of the patients (0.5249). Hence, CYP3A4 expression is the key determinant of clonazepam blood concentration, whereas NAT2 contributes to blood levels of 7aminoclonazepam.

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Formation and photoinduced properties of silver porphyrins as promising PDT reagents

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Photodynamic Therapy (PDT) is a medical treatment which employs the combination of light and a drug (i.e., a photosensitizer) to bring about a cytotoxic effect to cancerous or otherwise unwanted tissues. A vast number of photosensitizers used in PDT are porphyrins or related compounds. Despite the general prejudice against heavy-metal ions used in drugs, Out-of-Plane (OOP or Sitting-atop, SAT) metalloporphyrins might even prove to be better photosensitizers, or rather photocatalysts, compared to the in-plane complexes because of their higher photoactivities and red-shifted UV-Vis absorption bands\(^1,2\). One group of the most promising possible PDT reagents, among the OOP metalloporphyrins, contains Ag complexes owing to their suppressing effects on undesired infections\(^3\).

We investigated mainly the complexation reaction between Ag(I) and an anionic porphyrin, this process being very complex as the consequence of the possible coordination of two Ag(I) ions to a porphyrin as well as due to the rare disproportion of Ag(I) in this dinuclear intermediate. The end product is a relatively stable, although slightly distorted, OOP Ag(II) complex. We determined the rate constants for each step at various metal ion and porphyrin concentrations and increasing ionic strength. We also studied the coordination of a few potential axial ligands as well as their effects on the absorption properties of Ag porphyrin. Photoinduced properties of the water-soluble, sulfonato-phenylated derivative were also investigated; however, the lipophilic tetracyanophenyl porphyrin, tetrakis(3-hydroxyphenyl)porphyrin and 5,10,15,20-tetrakis(3-hydroxyphenyl)chlorin were examined concurrently in preliminary experiments. The latter ligand is especially promising as it has been

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used under the name Foscan® (international non-proprietary name, temoporfin) for over 15 years for the treatment of squamous cell carcinoma of the head and neck.

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Poster Session 2

The Role of Analytical Chemistry in Pharmacology

Coordinator
Viktor G. Mihucz
Simple hydrazone based tweezer type receptors for the recognition of cyanide ion

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Hydrazones are an important class of compounds in medical biotechnology to couple drugs to target antibodies (e.g., in cancer therapy). Substituted hydrazones are known to be associated with a broad spectrum of activities in biological systems, which include antioxidant, antiviral, antibacterial, activities etc. Due to the presence of acidic N-H moiety in hydrazones functional groups, it can interact with negatively charged anions through hydrogen bonds.

Among a variety of anions, which are present in biological systems and environment, CN⁻ is known for its acute toxicity. The toxicity of CN⁻ arises because of its affinity for Fe present in cytochrome C, thus causing hypoxia. The extreme toxicity associated with CN⁻ demands easier methods for its detection in order to assist the environmental remedial efforts to improve human health. Since hydrazone derivatives are known for their beneficial effects on biological systems, an effort was made to investigate their applicability to detect CN⁻. Therefore, hydrazone derivatives were designed with a suitable cavity to selectively detect anions through hydrogen bond with the N-H group.

The structure of the synthesized receptors was established using ¹H, ¹³C-Nuclear Magnetic Resonance (NMR), High Resolution Mass Spectrometry (HR-MS), CHN elemental analysis and single crystal X-ray crystallography. The single crystal X-Ray crystallographic studies revealed the formation of polymorphs at different temperatures. The receptor molecule at 100 K crystallized in the P(-1) space group, while at 298 K, the receptor molecule crystallized in the P₂₁ space group. The crystal structure obtained at room temperature displayed both the exclusion and the inclusion complex between the receptor and the DMSO, while the structure obtained at 100 K displayed only exclusion complexes. The formation of the hydrogen bond between the receptor and the DMSO molecule was observed in both the type of pol-
ymorphic crystals. The crystal packing is characterized by the presence of several short intramolecular and intermolecular contacts.

The anionic affinity was evaluated initially through a naked eye detection technique followed by UV-Vis spectroscopy. Through the naked eye detection, the formation of dark red color in the presence of CN was observed, while color change was not observed in the presence of other anions. The complex formation between the receptor molecule and the CN- initiated with the displacement of the DMSO present in the receptor cavity, which led to the development of dark red color. The UV-Vis spectroscopy investigations, the formation of a new absorption band at 550 nm in the presence of CN was observed, while no absorption shift was observed in the presence of other anions. The stoichiometry of the complex was determined through Job’s plot method as 1:1. NMR spectroscopy was used to investigate the mechanism of complex formation, while Discrete Fourier Transform (DFT) studies confirmed the complex formation between CN- and the receptor molecule. Thus, the proposed substituted hydrazine derivative has the potential for developing drug structures suitable in pharmacology for removal of CN-.
In this study, samples collected from different blocks of hashish seized by the Guardia di Finanza (Finance Police) in different requisitions were analyzed by means of Head Space – Solid Phase Micro Extraction (HS-SPME) followed by Gas Chromatography coupled with Mass Spectrometry (GC/MS). The samples were taken from different blocks, which had already been registered with an internal batch number; for each block, the titration of the Trans-Δ^9-tetrahydrocannabinol (THC) content had been performed by the laboratory of Forensic Toxicology. Overall, we identified more than 170 Volatile Organic Compounds (VOCs) belonging to different chemical classes: terpene (monoterpenes, sesquiterpenes and diterpenes, both oxygenated and hydrocarbons) and non-terpene derivatives. We carried out a multivariate statistical analysis on the results, using both the Hierarchical Cluster Analysis (HCA) and the Principal Component Analysis (PCA) methods.

The objective of this study was to evaluate the possibility to determine the differences or the similarities between each sample based on its spontaneous volatile emission profile: this could help the Investigative Judge to evaluate the existence of one or more dealers. This method is fast and does not involve chemical or physical treatment of the sample, thus its simplicity would be a great advantage. The results of the statistical evaluation are very promising as they show a sharp tendency of the volatile profiles of the samples to gather in clusters based on their batch of origin.
Investigation of macro, micro and toxic element concentrations of fermented traditional beverage kefir by using an inductively coupled plasma optical emission spectrometer to improve food safety in Turkey

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Fermented foods are important components of daily diets in many parts of the world. Kefir is a viscous and self-carbonated beverage with a smooth, slightly foamy body and whitish color. Kefir is prepared by inoculation of cow milk with kefir grains. Kefir grains consist of different species of yeasts and lactic acid and acetic acid bacteria.

The aim of this study was to compare various sample pre-treatment methods on home-made and commercial kefir samples. Macro, micro and toxic element contents of kefir were determined by Inductively-Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Several procedures were tested, namely, wet digestion, dry digestion and microwave-assisted digestion. The best results were obtained from microwave digestion. Results obtained for samples of home-made and commercial kefir were (in mg kg⁻¹): Al, 0.262±0.036 - 0.326±0.027; Ba, 0.090±0.009 - 0.110±0.007; Ca, 1160±37 - 1129±47; Cu, 0.120±0.010 - 0.184±0.012; Fe, 3.173±0.004 - 3.165±0.003; K, 1040±46 - 1108±58; Mg, 116±1 - 147±10; Na, 248±6 - 274±22; and Zn, 2.406±0.134 - 2.471±0.272. The microwave digestion method was validated by means of both the Certified Reference Material NCS ZC73015 Milk Powder and recovery experiments with satisfactory results in all cases.

Oil-in-water emulsion broken by heating into three-separated-phase procedure: a new approach for simultaneous separation/preconcentration of iron in solid oil samples

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Traces of metals like Cu, Co, Fe, Mg, Mn and Ni in butter oils directly affect their quality by stimulating lipid oxidation and thus decreasing the shelf-life of the commercial products. These oxidation processes may produce peroxides, aldehydes, ketones, acids, epoxides and other products that can adversely impact the digestive system and also react with protein and pigments with the ensuing stimulation of the action of some carcinogens. Sensitive spectrometric techniques are required for determination of metal concentrations in solid oil samples because of the low concentration changes in the key elements and the complexity of the oil matrix. On the other hand, most of the spectrometric techniques available require sample preparation prior to analysis.

The procedure based on the oil-in-water emulsion broken by heating into three separate phases (OWEBH-TSP) is considered to be a new approach for the simultaneous separation and preconcentration of Fe in solid oil samples (animal fat, butter, margarine etc.) by using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). This procedure exploits the formation of an oil-in-water emulsion prepared by mixing of the oil samples with acidic surfactant solutions. The oil-in-water emulsion is then broken by heating. Three well separate phases are thus obtained, namely: i) an organic solvent-rich top phase; ii) an aqueous-extracted metal middle

phase; and iii) a surfactant-rich bottom phase. Iron was separated/preconcentrated in the aqueous phase and quantified by GFAAS. Several parameters that could affect the extraction efficiency and the time required to break the emulsion were investigated such as the concentration and nature of the surfactant, the type and concentration of the acid and the heating temperature and time. In addition, recovery tests were performed by spiking the oil samples with known amounts of the metal in the form of organo-metallic standards.
A completely automatic optical biosensor system for stressed bacteria in marine water

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Laboratory techniques used for the quantification of pathogenic and environmental bacteria commonly give results within 18-72 hr after sampling. In some cases, as for bathing waters quality control, the time needed for results can represent a not negligible problem, since microbial contamination can be confirmed when public health has been already jeopardized.

To overcome this problem, in the absence of analytical methods faster than those approved by law, cutting-edge technologies can offer today a wealth of innovative solutions. Recent developments in electronic engineering, for instance, have led to the miniaturisation of detectors, giving the possibility of designing analytical instruments with features of higher detection power, smaller size and better portability. Biosensors, in this context, are among the instruments that mostly exploit miniaturized technology, thus enabling easy and rapid analysis directly on site, as required for biomedical or environmental analysis. The advantage of using biosensors is that the analytical procedure leading to target’s identification is usually highly specific and reduced to few, extremely simplified steps, not requiring specialized personnel. On this basis, we developed a novel, completely automatic optical biosensor system able to rapidly detect faecal bacteria in marine water.
The developed system (patent pending) is able to autonomously perform the entire analytical procedure usually followed in a microbiological laboratory, from sample incubation in culture medium to the analysis itself. The autonomy is guaranteed by the provision of a standalone supply box, including battery, solar panels, substrate stock and a communication system that constantly interacts, by the Universal Mobile Telecommunications System (UMTS) network, with a web server application storing and showing data. The principle of the analysis is based on the hydrolysis of the substrate β-D-glucuronide contained in the culture medium added to the sample. The hydrolysis, operated by the enzyme β-D-glucuronidase (a specific marker for *E. coli*), leads to 4-methylumbelliferone, the fluorescence of which is measured, thus giving evidence of the presence of *E. coli*, assumed as an indicator of faecal contamination.

In order to validate the system, results were obtained in parallel with those gained with a standard fluorogenic substrate method; the microbial growth was quantified on this latter medium. The developed biosensor system reliably detected *E. coli* as low as 1 cfu/mL of marine water sample. This study paves the way to the development of an automatic monitoring platform, remotely controllable, intended for the management and control of water resources (seas, rivers, lakes) and able to act as an early warning system launching alarm signals when preset threshold values of pathogens are exceeded.
Effective mineralization of benzenesulfonic acid by heterogenous photocatalysis

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In the pharmaceutical industry benzenesulfonic acid is mostly used for producing other specialty chemicals. A variety of pharmaceutical drugs are prepared as salts of benzenesulfonic acid and are known as benzilates. Heterogeneous photocatalysis is a worldwide researched Advanced Oxidation Process (AOP) and it can be successfully applied for the degradation of benzenesulfonic acid. Although the efficiency of this method is not sufficient, it can be increased and thus gain the flexibility for being used in practical application (e.g., for pharmaceutical wastewater treatment). Efficiency can be increased by modifying the catalyst with precious metals or by combining heterogeneous catalyst with other oxidative procedures such as ozonation.

Our photochemical research team has already determined the order of the degradation steps of the benzenesulfonic acid¹. The first one is hydroxylation, then followed by the opening of the benzene ring. The last one is probably the outcome of the reaction between the hydroxylated intermediates and oxidative radicals \([O_2^-/\mathrm{HO}_2^-/O_2(1Dg)]\).

The mineralization process was studied in detail with the following methods: heterogeneous photocatalysis, ozonation, combination of the previous two methods and in the suspension of TiO₂ modified with Ag. The hydroxylation rate of the model compound was the following: \(O_3^-+UV < TiO_2^-+UV < O_3^-+TiO_2^-+UV < Ag-TiO_2^-+UV\). On the other hand, the order of the mineralization rate (decrease of Total Organic Carbon, TOC) was different: \(O_3^-+UV < Ag-TiO_2^-+UV < TiO_2^-+UV < O_3^-+TiO_2^-+UV\). The formation rate of the highly oxidative \(\cdot OH\) radicals under the said circumstances was also high. As a scavenger reactant coumarin \((C_9H_6O_2)\) was used². The amount

of the formed ‘OH radicals increased in the following order: \( \text{O}_3 + \text{UV} < \text{TiO}_2 + \text{UV} < \text{O}_3 + \text{TiO}_2 + \text{UV} < \text{Ag-TiO}_2 + \text{UV} \).

Based on these results, the most efficient procedure turned out to be the application of Ag-TiO\(_2\) + UV at the first stage to promote the hydroxylation of the model compound, then combination of this photocatalysis with ozonation to increase the rate of the ring-opening by generation of additional oxidative radicals.

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Determination of Warfarin and its active metabolites in dried blood spots

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Warfarin (WAR) is the most common oral anticoagulant drug prescribed for the treatment of many diseases (e.g., atrial fibrillation and pulmonary embolism). The narrow therapeutic index coupled with the large individual variability of response to treatment and the delayed anticoagulant effect of WAR (<72 hours) pose serious risks of hemorrhagic events or thrombi formation if WAR dose is not correctly balanced. Thus, the effectiveness and safety of the therapy must be frequently controlled by monitoring the International Normalized Ratio (INR) and adjusting the WAR dose accordingly. An interesting alternative to the INR assay could be the determination of WAR and its active metabolites (Warfarin alcohols) in blood as it was previously demonstrated that a good correlation exists between these two parameters.

The aim of this study was to develop an analytical procedure for the determination of WAR and its metabolites in Dried Blood Spots (DBSs) by Ultra-High-Pressure Liquid Chromatography (UHPLC) with Electrospray Ionization (ESI) combined with tandem Mass Spectrometry (MS/MS). The collection of a DBS is less invasive than the collection of a blood sample and its transport and storage is much easier. For these reasons, the collection of DBSs may represent a convenient strategy for drug therapeutic monitoring, especially when blood samples must be collected frequently. DBS samples were produced by pipetting 10 μL of blood sample (collected from ten patients undergoing WAR therapy) on a paper filter (42 ashless Whatman, 70 mm in diameter) and the resulting spots were dried at room temperature. The analytes were then extracted using 500 μL of a methanol-acetonitrile mixture (3:1 v/v) and diluted 5-folds with 0.1 % formic acid in water prior to analysis.

The analytical figures of merit highlighted the reliability of the proposed method for the determination of WAR and Warfarin alcohols in DBS samples. The results
pointed out the influence of the hematocrit levels on the spot size and recovery of the compounds from the spot. Good correlations between the concentration of both analytes measured in patient DBS and plasma samples were found when a correction for the hematocrit value was applied, confirming the possibility of using DBSs for monitoring the WAR concentrations in blood.
Sulfur determination in some nuts and dried fruits sold in Turkey by high resolution graphite furnace molecular absorption spectrometry

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This work describes the determination of S in some nuts and dried fruits using High Resolution Continuum Source Electrothermal Atomic Absorption Spectrometry (HR-CS-ET-AAS). The two rotational molecular bands of CS at 257.959 nm and 258.056 nm were simultaneously evaluated. Sulfur was determined in a W coated graphite tube/platform at the two wavelengths mentioned above using thiourea as a calibrant, Pd/citric acid as a matrix modifier and applying a pyrolysis temperature of 800 °C and a molecule forming (vaporization) temperature of 2200 °C.

The calibration curve prepared from thiourea was linear up to 2500 ng of S. The Limit of Detection (LOD) and characteristic mass of the method were 21.6 ng and 7.4 ng of S, respectively. The accuracy of the method was tested by analyzing certified reference materials in a matrix of spinach, milk powder and tea applying a linear calibration technique based on a thiourea aqueous standard. Results were in good agreement with the certified values.
Spectrophotometric investigation of early lanthanide(III) porphyrins for potential biomedical applications

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Larger-sized metal ions are not able to coplanarly fit into the cavity of the porphyrin ring; they are located out of the ligand plane, resulting in Out-of-Plane (OOP or Sitting-atop, SAT) complexes with dome-distorted structure, thermodynamic instability, kinetic lability, typical photophysical features and photochemical reactivity. OOP position promotes the formation of bis- or oligoporphyrins, so-called sandwich complexes. Lanthanide(III) ions offer good opportunities to finely tune the OOP distances utilizing the well-known lanthanide contraction. From optical and photophysical aspects, porphyrins are able to efficiently sensitize the near-IR luminescence of lanthanide ions, which can be widely applied, e.g., in biomedical optical imaging. From a photochemical viewpoint, the enhanced photoactivity of OOP metalloporphyrins, compared to that of the in-plane complexes, makes possible their more effective usage in photodynamic therapy.

In this work, we studied the effect of the presence and absence of potential axial ligand on the formation as well as the photophysical and primary photochemical properties of water-soluble, anionic porphyrin complexes of early lanthanide(III) ions (La-Gd). The bidentate O-donor acetate and glycol, as well as the monodentate ethanol and chloride, can enhance the coordination of the first porphyrin ligand due to their axial effect, but they can hinder the connection of an additional porphyrin. In the presence of non-coordinating perchlorate ions, bisporphyrins can form, which have slightly redshifted and broadened UV-Vis absorption bands compared to those of the monoporphyrins. Also the bisporphyrins display a blueshifted and less intense $S_1$-fluorescence, related to the free-base porphyrin, similarly to the monoporphyrins as a consequence of the special type of aggregation. The formation and

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the transformation between the mono- and bisporphyrins are very slow reactions in dark at room temperature\(^3\). These reactions are accelerated by the photolysis of the equilibrium system; they are considerable by-processes accompanying the photoredox degradation. The mechanism of the photochemical reactions must be thoroughly described in \textit{vitro} before the promising application of these complexes \textit{in vivo} can be exploited in the mentioned photodynamic therapy and biomedical optical imaging\(^3\).

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Radezolid (RX-1741) is a novel biaryloxazolidinone antibiotic which is over clinical development (two Phase-2 clinical trials completed). In the presented study N-[[(5S)-3-(3-fluoro-4′-[(1H-1,2,3-triazol-5-ylmethyl)amino]methyl)biphenyl-4-yl)-2-oxo-1,3-oxazolidin-5-yl]methyl]acetamide was synthesized and characterized using Fourier Transform (FT)-IR, Raman and Nuclear Magnetic Resonance (NMR) spectra in comparison with the spectra of the working standard of RX-1741. The FT-IR and Raman spectra in solid phase of the RX-1741 were recorded in the region 4000–400 cm⁻¹ and 4000–200 cm⁻¹, respectively. The ¹H and ¹³C NMR were recorded using a Varian VN-MRS 500 spectrometer. The analysis of position/intensity of characteristic bands and shifts were supported using density functional theory method and the B3LYP method employing the 6-31G(d,p) basis set. The most characteristic bands associated with vibrations of the bonds present in RX-1741 are located between 2000-500 cm⁻¹. The full assignment of proton and carbon signals were accomplished using the COSY, ¹H [¹³C] HSQC and ¹H [¹³C] HMBC experiments. The establishment of frontier molecular orbitals (FMOs) showed lacks in the location of electrons in the structural area of 1H-1,2,3-triazol-5-ylmethyl and methylacetamide groups. The difference in
energy gaps calculated for HOMO-LUMO was 4.91 eV. Moreover, the analysis of MEP maps of RX-1741 showed that the area of the 1H-1,2,3-triazol and 1,3-oxazolidine structures are electron poor regions while acetamide substituent is an electron-rich one. Other regions of RX-1741 molecule have almost neutral potentials.

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A sensitive and robust method for the simultaneous determination of acetylsalicylic acid (ASP) and its major metabolite salicylic acid (SAL) in human plasma using Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) was developed and validated. ASP and SAL were extracted by simple liquid-liquid extraction using tert-butyl methyl ether. The ions were detected in the multiple reaction monitoring mode at the m/z 179.0→137.0 transition for ASP and m/z 136.9→93.0 for SAL. The lower Limits of Quantification (LOQs) for ASP and SAL were 1 and 80 ng/ml, respectively.

The method was successfully applied for the characterization of the plasma concentration levels of ASP and SAL after oral administration of Aspirin Protect 100 to healthy volunteers. The present method can contribute to the improvement of ASP/SAL determination in patients under antithrombotic therapy and to the reduction of the risk for ASP resistance associated with bioavailability/exposure issues (non-compliance, underdosing, poor absorption).
Analysis of NMDA modulator D-serine and D-aspartate in biological samples using capillary electrophoresis–laser–induced fluorescent detection (CE–LIF) method

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Analysis of D-enantiomers of amino acids in biological samples is gaining increasing importance to elucidate their physiological functions in mammals. The majority of these works focus on determination of D-serine and D-aspartate as they possess neuromodulator functions in the Central Nervous System (CNS) where their main target is the N-methyl-D-aspartate type glutamate receptor. This plays an important role in neuroplasticity, memory formation, learning processes and some pathological conditions, e.g., Alzheimer’s disease and schizophrenia.

Earlier we developed a chiral capillary electrophoresis method to quantitate D-aspartate and D-serine in biological samples, among others in rat brain. For the sensitive Laser-Induced Fluorescence (LIF) detection of amino acids, derivatization with 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) was used. An amino-modified β-cyclodextrin, 6-monodeoxy-6-mono(3-hydroxy)propylamino-β-CD (HPA-β-CD) was found suitable for chiral analysis of various amino acids. Using 50 mM pH 7 HEPES buffer containing 6 mM concentration of this chiral selector provided baseline separation of aspartate and serine enantiomers. All determinations were accomplished in a polyacrylamide coated capillary using reverse polarity for the analysis of the negatively charged analytes. The method thus developed was validated. The Limits of Quantification (LOQs) were determined for D-enantiomers on the basis of acceptable accuracy and found to be 0.05 μM for both D-aspartate and D-serine. The Limits of Determination (LODs) given as S/N 3:1 were estimated to be 8 and 12 nM for D-aspartate and D-serine, respectively.

The method was used to quantify D-aspartate and D-serine in various brain areas of animals where Alzheimer’s disease was modelled and compared to quantities of these D-amino acids in control animals. Significant changes in D-aspartate content of several brain areas were found.
Poster Session 3

Environmental Contamination by Pharmaceuticals

Coordinator
Norbert Szoboszlay
Pharmaceuticals are indispensable for the maintenance of public health and the quality of life. Rapid advances in drug therapies to meet health challenges and their timely availability are essential for a healthy society. Veterinary pharmaceuticals prevent and treat disease and increase the efficiency of food production. Thousands of different active compounds are currently in use in large quantities to treat or to prevent diseases; however, they can also act as water micro-contaminants. In fact, following administration, a great amount of human pharmaceuticals are excreted unaltered or as active metabolites and end up in Wastewater Treatment Plants (WWTPs). Moreover, hospital and industrial wastewaters, uncontrolled and illegal drug disposal and aquaculture can also be a significant source of aquatic contamination.

Several works have been conducted recently to improve the chemical methodologies for detecting drugs in natural water and the widespread detection of pharmaceuticals in terrestrial and aquatic systems has engendered significant scientific and regulatory concern. Using an ecological approach for assessing the environmental effects of the occurrence of pharmaceuticals in the environment can be very useful. In light of this, the combined use of microbial ecology and chemical methods to investigate contaminated water can improve the evaluation of drug fate (transformation and/or degradation); this approach is also useful for evaluating the effects of different pharmaceutical loads on ecosystem functioning and for developing water remediation strategies. This work reports examples of application of this approach and an overview of methods for assessing antibiotic occurrence and fate in water ecosystems.
Bioaccumulation of endocrine disrupting compounds in the Greenland shark

*Somniosus macrocephalus*

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The rapid climate change causes stresses to polar ecosystems. Responses are expected at the molecule, organism and community levels. Differences between polar regions and the rest of the planet suggest that outcomes of stress may differ from those at lower latitudes. Polar ecosystems are key for stability and potential changes across the biosphere. The international TUNU-MAFIG Programme (Tromsø University, currently TEAM-Fish), involving dozens of scientists from ten nations, aims at studying the biodiversity of species, populations and communities, initially in Northeast Greenland and to date in the whole, poorly studied, Euro-Arctic region. Genetics, demography, ecology, trophic interactions and physiological adaptations are viewed on a broad evolutionary time scale in the context of climate and human stressors. In this work we report the bioaccumulation of Endocrine-Disrupting Compounds (EDCs) in muscle and liver of the Greenland shark. There are currently very few scientific papers on the distribution and transport of these compounds in the arctic marine food web and no such studies have been performed on the Greenland shark. This species is of interest for biogeography, migration, long- and short-term contaminant storage, physiology etc. This is an ideal model for multidisciplinary collaborations due also to its high position of top predator, feeding on a variety of items, in the trophic web: a long-living organism that may accumulate persistent organic contaminants. EDCs are defined as chemicals that affect endocrine system
structure or functions that are responsible for the maintenance of homeostatics and reproduction (including embryonic development, gonadal formation and sex differentiation). This class of contaminants includes also the organic derivatives of phenol that are classified as dangerous compounds, namely Nonylphenols (NPs) and Bisphenol A (BPA). Seventeen muscles and livers of the Greenland shark sampled in the western Greenland and four specimens (muscle and liver) from eastern Greenland were analysed to evaluate the presence of BPA, 4-NP, NPE1 and NPE2. Extraction with ASE and analytical determination using High-Performance Liquid Chromatography (HPLC) with Fluorescence detection and Liquid Chromatography-Mass Spectrometry (LC-MS) showed higher contamination levels in muscle than in liver and the specimens of western Greenland were more contaminated than those of eastern Greenland. In fact, the 4-NP content in liver of specimens sampled in the western Greenland ranged from a maximum of 158.2 ng/g fw to a minimum of 4.1 ng/g fw and from 59.2 to 3.6 ng/g fw for BPA, while in muscle samples the 4-NP concentration varied from 211.7 to 9.9 ng/g fw and from 101 to 4.4 ng/g fw for BPA. These results show the presence of EDCs in this species and in the Euro-Arctic marine trophic web.
Study of chlorobenzene removal by ferrate treatment from groundwater applying GC–MS

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Since chlorobenzenes were widely used as solvents in the chemical industry, they are the most frequently detected organic pollutants in groundwater. Their biodegradation is limited and therefore their removal needs different technologies based on adsorption or oxidation processes. Our research group developed an electrochemical approach for continuous production of ferrate [Fe(VI)] solution which can be mixed directly with polluted groundwater in order to oxidize the Cl-containing compounds.

Our investigation focused on the degradation of mono-chlorobenzene (MCB), 1,2-dichloro-benzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB) and 1,4-dichlorobenzene (1,4-DCB) applying model solutions and real groundwater samples. To determine the concentrations of chlorobenzene compounds before and after treatment a Solid-Phase – Micro-Extraction – Gas Chromatograph – Mass Spectrometry (SPME-GC/MS) system was used. During the optimization of ferrate treatment the effect of pH, ferrate concentration and ferrate/CB molar ratios were studied at treatment time of 30 minutes. The highest removal efficiency was achieved at pH 7 in the ferrate concentration range of 10-50 mg/L.

If the model solutions containing the four chlorobenzenes in concentration of 100 μg/L were treated with ferrate in concentration of 50 mg/L, 24, 35, 23 and 19 % removal efficiency was achieved for MCB, 1,2-DCB, 1,3-DCB and 1,4-DCB, respectively. In the presence of real groundwater matrix these removal efficiency values decreased by 4-9 %, because a part of ferrate was consumed by other organic compounds having similar or higher electron donor capacity than the chlorobenzenes.
Characterization of Cyclodextrin containing nanofilters for removal of pharmaceutical residues

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Nowadays an increasing number of chemicals, among which also pharmaceuticals, are released to the environment from different sources. For their removal from water phases the traditional treatment processes often proved to be insufficiently effective. Therefore, to increase the removal efficiency numerous methods, such as membrane processes and also a large variety of adsorbents (ion-exchange resins, zeolites, activated carbons) have been investigated.

In this work Β-Cyclodextrin Polymer Beads (BCPBs) and BCPBs-containing nanofilters were tested as adsorbents for the removal of pharmaceutical residues from different water types. Cyclodextrins (CDs) can be used in several industrial fields, e.g., in pharmaceutical industry (pharmaceutical manufacturing), or in medicine (removing pharmaceuticals from blood) and also in water treatment processes (removal of contaminants). CDs are cyclic oligosaccharides containing a cylindrical, hydrophobic inner cavity and having hydrophilic outer surface. Due to these properties they are able to form host-guest complexes with numerous types of substances without creating chemical bonds; hence, they are called nowadays “molecular capsules”.

The experiments were carried out with ibuprofen (IBU) and carbamazepine (CBZ) containing model solutions in order to optimize the nanofilter production by measuring the filters’ adsorption capacities. During these experiments the Total Organic Carbon (TOC) concentration was determined to follow the adsorption process of IBU and CBZ.

It was concluded that the adsorption capacity of BCPBs for ibuprofen and carbamazepine was 16.0 and 29.5 μmol/g, respectively. It could be also established that both the application of inorganic additives (NaCl, NaHCO₃, NH₄HCO₃) during
production and regeneration of nanofilters with ethanol increased the adsorption capacity of nanofilters. Depending on the chemical composition and thickness of nanofilters the adsorbed amount of IBU and CBZ after regeneration procedure changed to 1.7–9.1 μmol/g. The best results were achieved with nanofilters having chemical composition of 30 m/m % BCPBs embedded in ultra-high molecular weight polyethylene applying NH₄HCO₃ and NaCl as additives.
Photoinduced degradation of nitrofurantoin

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Many pharmaceuticals and Personal Care Products (PCPs) have been detected as aquatic pollutants all over the world. There are many pathways of introducing pharmaceuticals into the environment, mostly through excreta, disposal of unused drugs or from industrial processes. The class of compounds, known as the nitrofurans [furaltadone, furazolidone, nitrofurantoin (NFTs)], are expected to be found in the aquatic environment as a consequence of their production and use. Being biologically active, these pollutants can be harmful to the ecosystem. In the last decade, TiO\textsubscript{2}-based heterogeneous photocatalysis proved to be one of the most powerful techniques for remediating environmental pollution through both liquid and gas phase reactions.

In this work, the aquatic photochemical conversion and degradation processes of nitrofurantoin were investigated by application of direct photolysis as well as by photocatalytic oxidation in the UV-Vis range. The key intermediates and products of the photolytic and photocatalytic decomposition of NFTs were identified and monitored by various chromatographic and photometric methods. The effects of the irradiation wavelength and the presence of O\textsubscript{2} were studied in the case of direct photolysis. The degradation of the potential intermediates and the roles of the oxidative agents generated in the photocatalytic system were also investigated.

The spectral and chromatographic results clearly indicate that in direct photolysis the starting antibiotic compound undergoes structural transformations strongly deviating from the reactions in the case of TiO\textsubscript{2}-mediated photocatalysis. The first
quick step of the direct photolysis is photoisomerization on the C-N double bond, followed by a slow second step of photodegradation. The changes observed in the UV range of the absorption spectra upon heterogeneous photocatalytic decomposition of NFTs were much faster, indicating an efficient mineralization accompanied by a considerable decrease in pH. The key intermediates of the photolytic and photocatalytic decomposition of NFTs were identified by High-Performance Liquid Chromatography – Mass Spectrometry (HPLC–MS).

This work was supported by the Hungarian Scientific Research Fund (OTKA K101141).
A pilot plant scale experiment for the removal of pharmaceutical residues from biologically treated wastewater

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Since the conventional wastewater treatment technologies are not suitable for the total removal of pharmaceutical residues and other micro-pollutants, these compounds show up in the effluent wastewater. In our pilot plant scale experiment the removal of nine pharmaceutically active compounds was studied applying Beta-Cyclodextrin Polymer Beads (BCDBs) as sorbents.

Stock solutions of four Non-steroidal Anti-Inflammatory Drugs (NAIDs), namely, ibuprofen, ketoprofen, naproxen, diclofenac, and five other pharmaceutically active compounds, namely, bisphenol-A, beta-estradiol, ethinyl-estradiol, estriol and cholesterol, were added to biologically treated wastewater. The concentration of the above compounds was in the 5-10 μgL⁻¹ range after their addition. Sorption experiments were carried out in a sorption column filled with 336 g BCDBs (Cyclolab Ltd., Hungary) with a volume of 4.4 l, and a 0.035 ms⁻¹ flow rate. Samples were collected from the treated wastewater after 5 minutes and 1, 3, 6, 12, 24 and 48 hours contact time. The concentration of the pharmaceutical compounds in the untreated, spiked and treated wastewater was determined using a Varian 240 Gas Chromatography tandem Mass Spectrometry (GC–MS/MS) system after trimethylsilyl-(oxide)-ether/ester derivatization.

The 5 minutes contact time was enough the reach an 85% removal in the case of seven compounds (ibuprofen, diclofenac, bisphenol-A, beta- and ethinyl-estradiol, estriol and cholesterol). BCDBs were especially effective in the sorption of the four compounds having a hormonal effect (bisphenol-A, beta- and ethinyl-estradiol, estriol) for which the removal efficiency was above 95%. The concentration of ethinyl estradiol was below the detection limit after 5 minutes. By increasing the contact time a slight increase was observed in the removal efficiency of four pharmaceut-
ticals (naproxen, bisphenol-A, beta-estradiol and cholesterol). The concentration of beta-estradiol was below the detection limit after 3 hours.

In summary, out of the four NAIDs the ketoprofen and the naproxen were removed with lower efficiency than that allowed by the conventional biological wastewater treatment. The removal efficiencies were 59% and 33% during the biological treatment and 15% and 28% applying beta-cyclodextrin, respectively. In the case of ibuprofen there were no significant differences between the removal efficiency of the two treatments as 83% removal was achieved with beta-cyclodextrin and 82% with the biological treatment. Nevertheless, more than 85% of diclofenac was adsorbed by the BCDBs, while conventional wastewater treatment showed a highly fluctuating removal efficiency for this compound.
Kinetics study of ultrasound-assisted extraction of BCR sequential extraction procedures for potentially toxic element content of soil and sediment

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For characterizing the mobility of Potentially Toxic Elements (PTEs) in solid environmental samples the BCR-sequential extraction procedure is frequently used. Novel chemical information can be gained by sequential application of extraction reagents and by gradually increasing chemical aggressivity at each step. The generally applied batch leaching techniques requires time consuming shaking to achieve the extraction equilibrium and when a large number of samples are to be processed the time demand of the procedure can be extremely high. Therefore, the possible reduction in time demand was attempted by combination of preliminary ultrasound treatment with batch leaching. The experiments were performed using the Sediment Certified Reference Material CRM BCR-701. The prescribed BCR protocol was applied to control the kinetics study.

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagents</th>
<th>Shaking time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.11 M ( \text{CH}_3 \text{COOH} )</td>
<td>16 hr</td>
</tr>
<tr>
<td>2.</td>
<td>0.5 M ( \text{NH}_2 \text{OH}^* \text{HCl} )</td>
<td>16 hr</td>
</tr>
<tr>
<td>3.</td>
<td>after ( \text{H}_2 \text{O}_2 )-digestion, 1 M ( \text{NH}_4 \text{OAc} )</td>
<td>16 hr</td>
</tr>
<tr>
<td>+1</td>
<td>( \text{HNO}_3 / \text{H}_2 \text{O}_2 )</td>
<td>Microwave-assisted digestion</td>
</tr>
</tbody>
</table>

After 1 hr ultrasonic treatment the shaking time was gradually increased to 1, 2, 4 and 8 hr to study the release kinetics. In these preliminary experiments after 1 hr ultrasonic treatment without shaking 72 % of the element content extractable by regular BCR-leaching can be dissolved, but subsequent shaking of 1 hr increased this extraction efficiency to 83 %. Further studies with additional times are still ongoing, in particular with regard to the quantification of PTEs released to the environment by metal-containing pharmaceuticals.

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Poster Session 4

Medical Devices

Coordinator
Fabio Geremia
Microbiological quality of water used to manufacture medical devices

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The incoming water in industrial processes can originate from different sources and therefore, before being introduced in the network, undergoes a pretreatment process in order to reach the necessary quality requirements in terms of purity, salinity, chemical and physical parameters and microbial load. In addition, since the water can come into direct contact with products, the microbiological quality control of water is fundamental in the production process of Medical Devices (MDs).

The objective of this work was to evaluate the microbiological quality of water in the manufacturing of MDs. The test phase was preceded by an inspection of 16 manufacturers of MDs. Each company was asked to fill in a questionnaire about the management and control of the main activities related to the operation and maintenance of water treatment systems involved in the industrial process. The samples were taken once a month for three consecutive months. For each site five phases common to every company were selected, namely: i) water inlet; ii) water after treatment with reverse osmosis; iii) water after the second treatment (distillation, filtration); iv) sampling in the washing area; v) water outlet. The method used was based on Filter Membranes (FMs).

The microbiological parameters used were those provided by the Pharmacopoeia, i.e., the Total Bacterial Load (TBL) at 22 and 37 °C. In addition to this, protocols were developed for the detection of faecal contamination indicators, namely, faecal
Enterococci and E. coli and some pathogens such as P. aeruginosa, S. aureus and Salmonella spp. The results obtained showed the constant presence of Pseudomonas spp and Staphylococcus spp in the various sampling points, even when the TBL at 22 and 37 °C did not exceed the upper limits. While the occasional presence of Enterococci and faecal E. coli was observed, Salmonella spp was constantly absent in culture tests. Taking in account these results and given its ability to develop biofilms in hydraulic systems, it can be concluded that it would be appropriate to include Pseudomonas spp in routine monitoring programs of water sanitation.
Clinical investigations of medical devices, regulatory framework and methodological specificities

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The aim of this work is to describe the methodology and role of clinical investigations on Medical Devices (MDs) in the EU. Clinical Investigations (CIs) are one of the three pillars of clinical evaluation. Results of CIs, evaluation of scientific literature and combined analysis of data from CIs and from literature are overviewed through the systematic review of scientific literature and the analysis of websites of Competent Authorities. Methods to conduct CIs are described by UNI EN ISO 14155:2012, by European Guidelines MEDDEV and for Italy by specific national regulations, with important differences from medicinal products. In conclusion, CIs are governed by a complete system of regulations and personnel in charge of CIs must have a specific training.

A pilot study for the wastewater reuse by medical device manufacturers

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Treatment and reuse of industrial wastewater is an important practice in a sustainable water management, thus saving water resources and reducing the number of discharges. In Italy the treatment of wastewater and its potential reuse are regulated by the Legislative Decree 152/2006 and the Ministerial Decree 185/2003. Wastewater is classified according to its civil or industrial use.

This study aims at highlighting the potential use of treated or reclaimed wastewater discharged from plants manufacturing Medical Devices (MDs) taking into account microbiological water quality, ecotoxicological parameters and economic aspects. Reclaimed wastewater can be exploited for different uses such as irrigation or industrial processes.

To establish the financial sustainability of reuse, an economic assessment was performed on the basis of the Cost Benefit Analysis (CBA). CBA is an evalua-
tion method based on financial indices - usually referred to investment projects at the micro-economic level – able to show the convenience of water treatment activities. This approach compares the costs of the measures adopted - calculated on the basis of actualized investments costs and running costs to operate the treatment - to the benefits obtained, represented by saved costs for good quality water supply.

Samplings were performed at six manufacturers of MDs. Water samples were collected, where possible, at the inlet and outlet of water treatment plants. Microbiological analyses were performed on water samples collected before and after the treatment using the membrane filter technique. On the other hand, ecotoxicological effects were tested only on treated wastewater samples.

Preliminary results show a significant decrease in reclaimed water samples of all microbiological parameters and the absence of E. coli, although noxious effects can be still observed. CBA results, even though not yet conclusive, can be a useful and valuable tool in the reuse management of wastewater from manufacturers of MDs.
Medical devices borderline: regulatory aspects

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Medical Devices (MDs), cosmetics and medicinal products are three different product categories, which for some applications may overlap. Borderline products may belong to one of the three categories depending on the assigned intended use. Typical examples are some products for topical, gynecologic and ophthalmologic use that follow the Council Directive 93/42/CEE.

First of all, in order to define whether a product can be considered an MD, it is mandatory that the intended use fits with its definition, i.e., the product must be used in humans for diagnosis, prevention, control, treatment or alleviation of disease, injury or disability, for study, replacement or modification of the anatomy or a physiological process or control of conception.

Secondly, the MD must not achieve its principal intended action in/on the human body by pharmacological, immunological or metabolic means.

Thirdly, the composition of the product is an important aspect to consider. If a MD contains components and substances which are considered medicinal product when used separately, it is required that all these substances act upon the body in an ancillary way to that of the MD. An example is the presence of an antibacterial substance used not to act on the patient, but only to control microbiological contamination of the product during its lifetime.

The Directive defines four classes of risk (I, IIa, IIb, III) to distinguish and classify MDs and the higher class (III) defines more critical devices. The borderline devices containing ancillary substances are classified in Class III.

Except for class I, to be marketed, all MDs must be identified with the EC mark followed by the number of the Notified Body that must verify that the manufacturer implements a robust and effective management system, which ensures the manufacturing of a device in compliance with the requirements of the Directive.

There are many Notified Bodies in Europe, but only a few of them are authorized to issue EC certification for all classes and for all types of devices.

In Italy only the Istituto Superiore di Sanità may issue an EC certificate for all
classes and all types of MDs, including devices classified in Class III for the presence of a medicinal substance with an ancillary action.

The EC mark and the number of the Notified Body are the sign of the conformity to the essential requirements laid down by the EU Directive and of a continuous monitoring aimed at ensuring the safety and health of patients and users.
Microbiological risk linked to use of medical devices in beauty salons

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In the last 10 years the field of wellness (beauty salons, beauty farms etc.) has undergone a considerable development. This growth caused, however, also a proportional increase in the number of people who could potentially become infected in case of accidental contact with contaminated materials. Such activities may in fact pose a risk to public health for workers and for people undergoing treatments not performed under hygienically appropriate conditions. Consequently, the importance arose of summarizing the various national and regional regulations available in a unique standard, i.e., an ad hoc guideline for the microbiological risk assessment, primarily in small business.

This study investigated the activities of beauty salons in relation to the microbiological risk related to the use of Medical Devices (MDs), generally in Class I. This was achieved through the preparation and distribution of a questionnaire that allowed to gather important information for the purposes of health and hygiene safety and efficacy of experimental protocols for the evaluation of microbiological risk and microbiological tests. A significant number of beauty salons have been contacted and visited in the Province of Rome. The questionnaire focused on the identification of potential microbiological hazards such as ability to identify risk, identification procedures and equipment at risk, sterilization and disinfection procedures, environment and transmission of infection and handling of medical instruments.
Results identified the following risk factors: 30.8% of all centres surveyed were contaminated by fungal infections such as users or dermatitis. All centres that have encountered dermatitis have also found mycosis (89.2% of the centres), while 10.8% reported only mycosis. There were no people who had only dermatitis.

Insofar as risk management is concerned, 31.7% of centres surveyed responded. Out of these, 32.5% of those that reported infections have also managed microbiological risk. Among the centers that have reported infections of the skin of the users, only 2.6% of users undergo treatments with ongoing infections using Personal Protective Equipment (PPE), while the remaining 97.4% decide not to carry out the treatment and to invite the user to contact a dermatologist. Microbiological assays performed on wax have shown that a heating treatment up to at least 60 °C is enough to prevent potential cross contaminations between users. In conclusion, this study pointed to the need for training and informing not only operators, but also users.
Round Table 1

Pharmacology and Analytical Chemistry: the Quest for Quality

Participants
Sergio Caroli, Roger Fuoco,
József Posta, Valentine Sforza, Beáta Sperlágh
Round Table 2

Pharmaceuticals and the Environment: Challenges and Remedial Actions

Participants
Anna Barra Caracciolo,
Paola Bottoni, Sergio Caroli,
Viktor G. Mihucz, István Sebestyén, Gyula Záray
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