SESSION I

WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Danica Agbaba and Monika Waksmundzka-Hajnos

1.

Characterization and Classification of stationary phases in SFC (and HPLC)

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Packed column supercritical fluid chromatography (pSFC) is an attractive technique in drug discovery related analysis because it offers several advantages over the more commonly used high-performance liquid chromatography (HPLC) technique. Column characterization aims to obtain a quantitative understanding of the properties of a column that influence the selectivity of a separation. Determining column properties allows the rapid selection of dissimilar columns for method development for a particular application. Columns have been extensively characterized in HPLC using several approaches. However, limited column characterization has been done in SFC.

Common methods used to characterize stationary phases in HPLC include thermodynamic methods, spectroscopic techniques, and chromatographic test methods. The linear solvation energy relationship (LSER) model (Abraham's model) and the carotenoid method are two methods which are used to characterize columns in both HPLC and SFC. LSER is a quantitative structure retention relationship (QSRR) model in which solute parameters, such as polarizability, dipolarity, steric and hydrogen-bonding properties are linked to the solute's retention through linear regression in order to get a better understanding of the applied stationary-phase properties. A closer look at the LSER coefficients will help understanding the retention differences observed between SFC and HPLC conditions, for columns with similar chemistries. The carotenoid test consists of the analysis of carotenoid pigments and evaluates polar surface activity, absolute hydrophobicity and the steric separation factor of octadecylsilica (ODS) stationary phases.

The aim of this presentation is to give an overview of the approaches used to characterize stationary phases in SFC (and HPLC), and to highlight topics that may still need to be researched further.

2.

Method development for impurity profiling in supercritical fluid chromatography: The selection of a dissimilar set of stationary phases

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Supercritical fluid chromatography (SFC) is gaining considerable interest as a separation technique in the pharmaceutical industry. The use of SFC as a technique for drug impurity profiling is examined here. To define potential starting conditions in method development for drug impurity profiling, a set of dissimilar stationary phases should be screened in parallel.

This study evaluates the possibility to select a set of dissimilar columns in SFC using the retention factors (k-values) for a set of 64 compounds measured on 27 columns. Experiments were carried out at a back pressure of 150 bar and 25◦C with a mobile phase consisting of CO_2 and methanol containing 0.1% isopropylamine (5–40% over 10 min) at a flow rate of 3 mL/min.

The k-values of the drugs were then used to calculate correlation coefficients between two columns on the one hand and to perform principal component analysis on all column data on the other. The Kennard and Stone algorithm, dendrograms and correlation-coefficient colour maps were used to select a set of dissimilar stationary phases. Derringer's desirability functions, a multi-criteria decision making technique was used to rank stationary phases used according to overall column performance. The stationary phase characterization results from this study were compared to those from previous studies found in the literature. The dissimilarity of the selected subset of stationary phases was finally validated using mixtures of compounds with similar properties and structures, as one can expect in a drug impurity profile.

3.

Retention behavior of model compounds in thin layer chromatography with novel green mobile phases

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Nowadays, the green mobile phases have an increasing interest in separation science and this strategy fits the current ecological trends. The aim of the green chromatography is the use of non-toxic and environmental friendly substances in the mobile phase preparation.

As the literature on such trials in thin layer chromatography is very limited, the aim of our study was to check the ability to use simple and non-toxic organic substances in aqueous solutions as mobile phases on different adsorbents.

In this study, we used 35 simple model organic compounds (including drugs), which were also used in our previous research (see for example [1]) on separation behavior in TLC. They belong to different chemical groups and possess various chemical properties.

These compounds were separated on RP18, RP8, DIOL, CN, NH2 and cellulose plates with the use of: urea, N-methylurea, N,N-dimethylurea, O-methylisourea, 2-hydroxyethylurea, N,N'-bis(hydroxymethyl)urea and guanidine aqueous solutions as mobile phases, in concentration varying from 0.5 mol/l to 4 mol/l. Plates were developed in 9 cm distance in horizontal non-equilibrated DS chambers.

All the results were subjected to multivariate chemometric analysis methods which allowed explanatory data analysis and decomposed the retention data to several independent trends.

The best results were achieved on DIOL, RP8 and RP18 plates. CN plates gave well-established spots, but they suffer from long development time and curvy migration front. There is no significant difference in spots shape between used modifiers.

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4.

CHROMATOGRAPHIC COLUMNS FOR HPLC: An Overview Of Today's Columns Technology And Their Applications For Analysis Of Some Pharmaceutical Products In Drug Quality Control Laboratories

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It is true that the possibilities of HPLC continually being extended through the development in HPLC- column technology, advances in instrumentation design and performance. The HPLC column is the heart of the HPLC – instrument and essential to its success. Today's HPLC column technology offering high efficiency, high resolution, short analysis time, use of minute volumes and a wide pH range of mobile phase. Drug analysis in drug quality control laboratories of Kuwait have been benefiting from these advanced features of today's HPLC column technology considering the main advantages over conventional HPLC columns. This will be done by reporting some of our recent results obtained by using these columns (Symmetry , Symmetry Shield and XTerra columns) for analysis of some pharmaceutical preparation according to the requirements of drug manufacturers specification or drugs pharmacopeias. Determination of molecular size distribution as a quality control test for Human Albumin in pharmaceutical preparations was done using Size Exclusion (SE) columns. HPLC – column for Mass Spectrometry was employed as analytical column (C18, 150 mm X 2.1mm and 5um particle size, Symmetry 300 Waters, Milford, MA, USA) for screening studies which was conducted to investigate the presence of three synthetic PDE-5-inhibitors, Sildenafil (S), Tadalafil (T) and Vardenafil (V) illegally adulterated in natural herbal products. These herbal products have been a subject for registration by Kuwait Drug and Food Quality Control Administration (KUFDA) as natural herbal products for improving sexual performance for man in the period from 2003 to 2012. Analytes detection was done simultaneously by PDA and MS. Nowadays in our laboratories, instead of Atomic Absorption Spectroscopy (AAS), HPLC with conductivity detector and cation or /anion columns were employed for analysis of cations such as , Na^{+}, K^{+} , Mg^{++} , Ca^{++} in Balance Salt Solution (BSS) and anions such as Cl^{-} in Movicol sachets (for the relief of constipation). Based on Ion Exclusion Chromatographic mechanism (polymethacrylate – based weak acidic cation exchange resin HPLC column) with detection UV, a simple,selective and sensitive method for the determination of carboxylic acids in Renal Dialysis solutions was used in our laboratories. Finally and on the base of our results, it can be said that using today's column technology by the analysts in Drug Quality Control Laboratories, the lab productivity and accuracy will be increased.

5.

TNF-α analysis and characteristic by the use of combined chromatography and mass spectrometry generated under influence of various genospecies of *Borrelia burgdorferi* sensu lato

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A rapid separation and characterization method for online detection of TNF-α (fig.1) generated under exposition of various genospecies of *B. burgdorferi* s.l. was developed based on UHPLC, GPC and MALDI-ToF analyses (fig. 2).

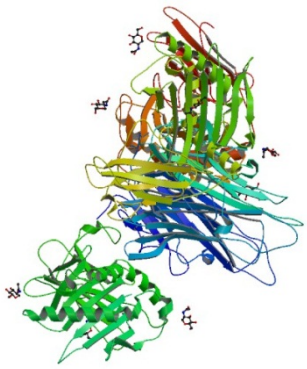

Fig. 1. Crystal structure of TNF-α

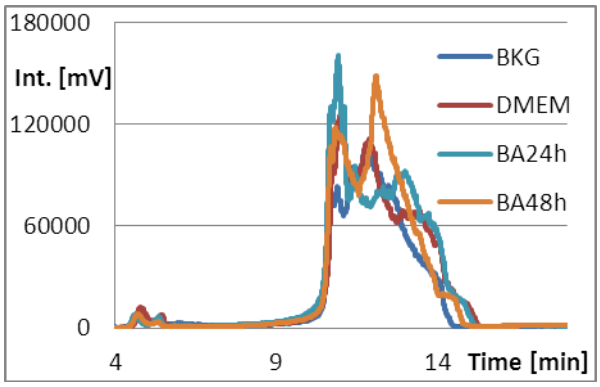

Fig. 2. GPC separation of TNF-α obtained after human fibroblasts (NHDF) were infected with genospecies of *B. burgdorferi* s.l.

TNF-α (tumor necrosis factor alpha) is a cytokine with multiple biological activities. It stimulates the immune cells to secrete cytokines, the epithelial cells to expansion adhesion molecules important for binding leukocytes, has an pyrogenic effect.

The HPLC equipped with micro mixer, DAD and FLD detectors was used as the tool for QA/QC analysis of TNF-α.HPLC Pinnacle DB PAH column dimensions: 50 mm x 2.1 mm, particle size:1.9 µm pore size:140 Å, temp.:30 °C sample eluent: physiologic salineconcentration from:800 ppm each component inj. vol.:1 µL, mobile phase A: water B: acetonitrile. flow rate: 0,2-0,9 mL/min was used. For identification of TNF-α excitation and emission wavelength, separated standard samples were investigated by the use of spectrofluorometer. Additional, for better separation and structure investigation UHPLC-GPC equipped with fraction collector and MALDI ToF^2 MS^2 techniques were used.

6.

Multi-dimentional chromatographic systems coupled with mass spectrometry

R. Zera

Shim-Pol A. Borzymowski

Początki obecnej firmy „SHIM-POL A.M. Borzymowski" E.Borzymowska-Reszka A.Reszka Spółka Jawna sięgają roku 1986. W tym roku mgr inż. Marek Borzymowski stworzył wyłączne przedstawicielstwo koncernu SHIMADZU w Polsce pod nazwą SHIMADZU Polska Analityka. W kolejnych latach, w celu zapewnienia swoim klientom kompleksowej oferty, nasza firma rozpoczęła współpracę ze światowymi liderami w zakresie produkcji sprzętu laboratoryjnego i innymi. Należy podkreślić, że SHIM-POL A.M. Borzymowski reprezentuje na zasadzie wyłączności koncerny międzynarodowe Shimadzu, Phenomenex®, Chromacol, ANTEC, PEAK SCIENTIFIC oraz jest dystrybutorem niewyłącznym kilku innych firm międzynarodowych oferujących akcesoria do aparatury analitycznej.

W 2001 roku firma zmieniła nazwę na SHIM-POL A.M. Borzymowski, w 2005 roku została utworzona spółka cywilna SHIM-POL A.M. Borzymowski s.c. Od roku 2007 nasza firma funkcjonuje pod obecną nazwą „SHIM-POL A.M. Borzymowski" E. Borzymowska-Reszka, A. Reszka Spółka Jawna.

Od początku istnienia, nasza firma zainstalowała w całym kraju już ponad 1700 aparatów w laboratoriach prywatnych, uniwersyteckich i instytucjach państwowych prowadzących badania z zakresu ochrony środowiska, farmacji, badania żywności, petrochemii oraz innych. Atrakcyjność aparatury SHIMADZU polega na jej różnorodności, wysokiej jakości, konkurencyjnych cenach oraz na profesjonalnej obsłudze serwisowej. Oferujemy naszym klientom nie tylko wsparcie w zakresie obsługi oferowanych przez nas urządzeń, lecz także zapewniamy wsparcie techniczne i aplikacyjne.

Oferujmy następujące rozwiązania:

chromatografy UHPC, HPLC i GC; systemy MS: GC-MS(MS), LC-MS(MS), LCMS-IT-TOF, MALDI-TOF-TOF, spektrofotometry UV-VIS, FTIR, RF i AAS, analizatory TOC; spektroskopy do analizy powierzchni ESCA-XPS, SIMS, ISS i Auger oraz analizatory fluorescencji rentgenowskiej (EDX). Oferujemy również akcesoria: PHENOMENEX, ANTEC LEYDEN, RHEODYNE, CHROMACOL, PEAK SCIENTIFIC – generatory, PIKE - akcesoria IR, HORIZON TECHNOLOGY - automaty do ekstrakcji, zatężania i osuszania próbek, w tym automatyczny koncentrator XcelVap, SUPERCRITICAL FLUID TECHNOLOGIES - aparaty do prowadzenia ekstrakcji cieczą w stanie nadkrytycznym oraz reaktory wysokociśnieniowe.

SESSION II

WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Huba Kalasz and

Ivan Vander Heyden

7.

Chemical changes in resins and relations with the mechanical properties of abrasive articles

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Aging of adhesives is important practical problem that influences on the functional properties of many products such as glues, furniture, bonded abrasive articles. In this paper aging of phenol-formaldehyde resins were studied. Tested resins are used as a binder in abrasive articles. Chemical changes occurring during storage of phenolic resins were assessed by different instrumental methods: Fourier Transform Infrared Spectroscopy (FT-NIR), Inverse Gas Chromatography (IGC), X-ray Photoelectron Spectroscopy (XPS). Thermo-mechanical changes of the model final products were determined by Dynamic Mechanical Thermal Analysis (DMTA). Presented results showed subtle chemical changes during storage. However, thermo-mechanical properties of model final products changed more significantly.

8.

Impact of gas sampling in polymeric bags on the quality of quantitative VOC measurements: a critical evaluation

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ABSTRACT

In the field of environmental analysis, researchers rely on the accurate qualitative and quantitative assessment of odorous compounds in the gas phase. In recent years, attention has been mainly given to advanced hyphenated analytical techniques and the development of new detectors (e.g. e-noses, SIFT-MS, etc.). However, the sample collection step is equally important and strongly influences reproducibility and accuracy. Whole air sampling using polymeric bags is still one of the most frequently used sample collection methods in the field. The degree of scalping, which is defined as sorption of the volatiles on the inner surface of polymeric sampling bag, is often underestimated, in particular in the field of environmental sampling. Own experiments revealed that after introducing a wide range of volatiles in a two-phase system containing Nalophan, recoveries decreased down to 57% in a period of 22 hours.

In this lecture chromatographic expertise is been used to develop a Phase Ratio Variation (PRV) method as a fast and efficient manner for predicting the degree of scalping for individual compounds, and thus enabling to compensate for sorption phenomena. This method requires limited measurements, without the need for time-consuming calibrations. Moreover, a correlation was found between partitioning coefficients and the liquid molar volume for a number of aliphatic, aromatic and oxygenated compounds.

9.

Non-chromatographic applications of porous monolithic materials

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Monolithic columns are commonly used in high performance liquid chromatography (HPLC). The development of monolithic stationary phases based on either silica or polymer skeleton is a relatively recent achievement in the preparation of chromatographic columns [1]. Monolithic stationary phases prepared from hydrophobic, hydrophilic or charged monomers can be employed for a wide range of applications.

Monolithic materials found also increasing role as sorbents for a sample preparation [2]. In most cases they are located in capillary column [3] or poly(ether ether ketone) (PEEK) tube [4]. The first report of a polymer monolith used for SPE was presented by Xie et al. in 1998 [4]. Less known use of monoliths was described by Svec in the review [1]. Miyazaki et al. prepared monolithic silica extraction tip. In this way the solid-phase extraction (SPE) tool was combined with pipette-tip shape [5]. Monolithic porous polymer for on-chip solid-phase extraction was invented by Yu et al. [6] Microextraction in a packed syringe (MEPS) was introduced by Abdel-Rehim [7]. In this device, a solid support is inserted directly into a syringe as a plug (with a filter at either end of the plug holding the solid phase), and fitted manually into the syringe. Stir cake sorptive extraction using monoliths as extractive medium was developed by Xiaojia Huang [8]. Shintani et al. dealt with monolithic silica column for in-tube solid-phase microextraction coupled to high-performance liquid chromatography [9].

Incorporation of a monolithic material in the needle for the extraction purposes is a quite new proposal [10]. In-needle extraction technique was up-today limited to bulk sorbents, which can be displaced in the needle or even removed from the needle. The application of monolithic filling of the in-needle device should prevent changes occurring in the sorbent layer and increase the efficiency of this sample preparation tool.

Monolithic scaffolds modified with nanostructures are not only used for chromatographic separations but are finding use in a broad range of applications [11]. Recently, monolithic scaffolds incorporating these nanoparticles were applied in cell cultivation and tissue engineering [12]. Porous monolithic inorganic/polymeric hybrid materials in a disk format were prepared using ring-opening metathesis polymerization (ROMP).

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SESSION III

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Kornelia Tekes and Hubert Paelinck

10.

Thin-Layer Chromatography with biological detection as a method for screening drugs candidates in natural mixtures

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Thin-layer chromatography coupled with biodetection is very useful for screening of wide range of plant extracts for fishing out bioactive compounds. It should be emphasized that TLC is most often the only method for detection of pharmacologically active compounds. TLC gives various possibilities to separate several samples in parallel in the same conditions and time to obtain a lot of results simultaneously. The experiments can be delivered with the crude plant extracts. It gives time and labor saving. Investigator has an open access to the adsorbent bed with separated mixtures and can perform observations in various conditions and obtain scans at various wavelength. Then one can perform biological detection. In such situation one wants to see only those compounds which possess desired activity, and does not care about the rest. The comparison of physicochemical vs. biological detection gives possibility to recognize which compounds or compound groups exhibit specific activity. It is the first step to obtain information about drug candidates before search of compound identity, isolation of it and investigation of activity which should follow-up preliminary experiments.

In such a way various active substances can be detected such as: new antibiotics and chemotherapeutics, new drugs for the therapy of: Alzheimer's disease, hypertension, diabetes, depression, obesity and free radical scavengers, which prevent deleterious effects of oxidative stress.

Detection of antibiotics can be performed by bioauthography (contact, agar-overlay or direct one) with bacteria growing on the chromatographic plate (or its imprint). After visualization using bacteria-coloring agent antibiotics appear as white zones against a color background.

Detection of enzyme inhibitors such as acetylcholinoesterase inhibitors, α- and β-glucosidase inhibitors, lipase inhibitors, xanthine oxidase inhibitors relays on the making contact of the plate with the selected enzyme and after that incubation under proper conditions of temperature and humidity followed by derivatization. Inhibitors should appear as white inhibition zones on the color background.

For detection of free radical scavengers various tests can be performed by use of stable radicals such as DPPH', ABTS or by β-carotene - linoleic acid assay.

The results of TLC-biodetection for various groups of natural compounds are also discussed.

11.

Analysis of food supplements by planar chromatography

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The endless possible combinations of the bioactive ingredients and excipients in different formulations such as hard/soft capsules, tablets, and liquids make the analysis of food supplements very difficult. Bioactive ingredients can be bioactive natural compounds, vitamins, minerals, plant extracts or even several plant extracts in one dietary supplement product, chemically modified compounds (e.g., encapsulated), etc.. Additional problems are the lack of available reference standards, standard reference materials (SRM), and marker compounds of particular plant materials, plant extracts or plant extract fractions, as well as a variety of chemical structures including isomeric compounds.

We will show the state-of-the-art and the potential of the planar chromatography in the analysis of food supplements with carotenoids, flavanols, stilbenes and methylxanthines as bioactive ingredients. The examples will include fast chemical fingerprinting, identification and characterization of biomarkers and analysis of adulterants with a focus on the instrumental detection possibilities (densitometry, image analysis, mass spectrometry) in solving the analytical challenges related to different combinations of bioactive ingredients in multi-ingredient food supplements.

12.

Detection and characterization of antibacterial components of *Onopordum acanthium*

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To fight against various diseases there is an increasing demand for effective compounds applicable in human and animal medicine as well as in plant protection. It is especially true for the antimicrobials, because the origin of a broad range of diseases is infection, and the incidence of (multi)drug resistance in pathogens is increasing against the widely used antimicrobials.

The aim of this study was to isolate and characterize the antibacterial components sourced from scotch thistle (*Onopordum acanthium*) plant extracts. High-throughput thin-layer chromatography - direct bioautography was utilized as bio-monitoring system for the detection, purification and isolation of the active compounds. The test organisms were Gram negative pepper pathogen *Xanthomonas vesicatoria*, the luminescence gene-tagged *Arabidopsis* pathogen *Pseudomonas syringae* pv. *maculicola*, the naturally luminescent marine *Vibrio fischeri* bacteria and the Gram positive soil bacterium *Bacillus subtilis*.

One major antibacterial component was found in each samples (root and leaf), which showed activity against all tested bacteria. The active compounds were enriched and isolated by means of gravimetric column and flash chromatography as well as preparative TLC, OPLC and HPLC. OPLC and HPLC are preferable, providing better separation and the possibility of the on-line fraction collection.

The isolated components with confirmed antibacterial effect were characterized and/or identified by LC-ESI-MS/MS (with positive and negative ionization modes) and NMR.

A lignin derivative and long chain aldehydes were found as active major components. Sesquiterpene alcohol and sesquiterpene lactones were also established as active components, compounds that are characteristic secondary metabolites of the *Asteraceae* family.

This work was supported by OTKA grant PD83487, and Á.M. Móricz was supported by Bolyai grant.

13.

Natural Products from Seagrasses

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Seagrasses are the only higher plants which are occurring in marine environments. Seagrasses are flowering plants (Division Angiospermae) belonging to four closely related plant families (Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, and Zosteraceae), which grow in marine, fully saline environments. There are 12 genera of seagrasses with about 60 species known. The secondary metabolite profile of seagrasses is of particular interest, a) because of their unique ecology as the only marine higher plants and b) from an evolutionary perspective, because seagrasses are descendants from higher land plants which have reverted to marine life. Thus, seagrasses are the only marine organisms featuring the spectrum of secondary metabolites also commonly found in higher land plants but on the other hand seagrasses contain very different secondary metabolites from sympatric other groups of plants such as brown, red, and green algae.

The secondary metabolite profile of seagrasses and the methods used to analyse it will be discussed. These chemosystematic data will be compared with data from the closest relatives of seagrasses, i.e. other families of the order Alismatales s.l., which predominantly encompass freshwater species such as the taxa of the Potamogetonaceae.

Seagrasses contain many groups of secondary metabolites commonly found in higher plants such as caffeic acid derivatives and flavonoids. Additionally, some rare groups of natural products including diarylheptanoids, unusual diterpenes, sulfated flavonoid glycosides, and sulfated phenolic acids have been found. Though seagrasses are of particular ecological relevance for the marine environments they are growing in, little is known about the ecologial role of secondary metabolites from seagrasses, both in response to abiotic factors as well as in the response to other marine organisms feeding on seagrasses.

Besides the description of seagrass secondary metabolites and the discussion of analytical studies for their detection and quantification, the presentation will strive to identify gaps in the current knowledge of the phytochemistry of seagrasses and their ecological role, and to identify some priorities for future research.

14.

Hyphenated techniques in phenolic profiling of wild fruits grown in Serbia

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Wild fruits are unfairly forgotten and pushed aside in comparison to other cultivated species. Our study on *Morus alba* species showed that mulberries are rich in health beneficial secondary metabolites [1]. The characteristic phenolic acids and flavanoids were identified using Ultra High-Performance Liquid Chromatography coupled with Linear Trap Quadrupole and OrbiTrap mass analyzer (UHPLC-LTQ OrbiTrap MS). Quantification of polyphenolics was realized using UHPLC coupled with a diode array detector and triple-quadrupole mass spectrometer (UHPLC DAD-MS/MS). Differences in the contents of anthocyanins, phenolic acids and non-anthocyanins among all the samples were evident. Except for derivatives of chlorogenic acids, this was the first report on the identification of individual hydroxycinnamic acid esters in *Morus alba* L. fruits. Our latest research in the field of phenolic profiling of indigenous fruits from Serbia was conducted on Elderberry (*Sambucus nigra*), Rose hip (*Rosa* sp.), and Cornelian cherry (*Cornus mas*). UHPLC DAD-MS/MS was used in order to quantify characteristic phenolics. In order to trace other polyphenols accurate mass search was performed using LTQ OrbiTrap MS. As a third hyphenated technique thin-layer chromatography coupled with mass spectrometry gave rise to distinctive profiles of the extracts. Monomeric and polymeric flavan-3-ols were found to be the major polyphenols extracted from the Rose hip samples, while anthocyanins were characteristic for Elderberry and Cornelian cherry.

[1] Maja M. Natić, Dragana Č. Dabić, Adele Papetti, Milica M. Fotirić Akšić, Vladislav Ognjanov, Mirjana Ljubojević, Živoslav Lj. Tešić, Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruit grown in Vojvodina, North Serbia, Food Chemistry 171 (2015) 128-136.

15.

Innovative sample introduction, extraction and separation technology for analytical systems such as HPLC, MS, GC and NMR

E. Cybulska

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SESSION IV

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Irena Vovk and Łukasz Komsta

16.

Update of a Chiral Separation Strategy in Capillary Electrochromatography using Chlorinated & Non-Chlorinated Polysaccharide-based Selectors

Debby Mangelings[1], Dima Albals[1], Ans Hendrickx[1], Bezhan Chankvetadze[2], Yvan Vander Heyden[1]

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Capillary electrochromatography (CEC) is a hybrid separation technique that combines the separation principles of two techniques capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Advantages of CEC are highly efficient separations due to the plug-like electro osmotic flow (EOF) as driving force in the capillary column, a higher sample loading capacity due to the presence of a stationary phase, and overall low mobile-phase and sample consumption.

Chiral drug molecules often display different pharmacological properties. Because most pharmaceutical compounds possess chiral properties, the development of chiral separation methods is an important research topic the pharmaceutical industry to isolate the therapeutically active enantiomer, the eutomer, from the other, the distomer. Consequently, the possibility to commercialize safe drugs with less or no side effects will be achieved.

The development of chiral separation methods is often a trial-and-error procedure. For that reason, a generic chiral separation strategy was proposed earlier using CEC as separation technique. For the separation of acidic compounds, a low pH mobile phase was needed while for the separation of basic compounds, a high pH mobile phase was used. The final CEC strategy was therefore composed of two sub-strategies, i.e. one for acidic and one for non-acidic compounds.

In this study, an update of the existing CEC strategy was conducted by evaluating the potential of newer types of chiral stationary phases (CSP) that use chlorinated polysaccharide derivatives as chiral selector. In a first part, the earlier defined screening conditions were tested on the newer types of CSP. Then the most enantioselective and most complementary CSP were chosen to update the screening steps for acidic and non-acidic compounds. In a second phase, the applicability of the existing optimisation steps was verified and adapted where necessary. The final result was an updated CEC strategy with a higher success rate, which uses both chlorinated and non-chlorinated polysaccharide-based CSP.

17.

Molecular Lipophilicity Profile in Drug Discovery: A Comparative Study for a Set of the Terephthalamides Derivatives

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Lipophilicity is generally regarded as a first-rate physicochemical parameter increasingly relevant in specification both the pharmacokinetic (ADMET) and pharmacodynamic aspects of drug-receptor/enzyme interactions which often correlates well with the bioactivity of chemicals. Its quantitative descriptor (logP), considerably used in the early stages of drug development, indicates the ratio of neutral solute concentrations in the organic (apolar) and aqueous (polar) phase of a two-component (n-octanol/water) mixture under equilibrium conditions. Unfortunately, the experimental procedures for the partition coefficient estimation are basically time- and/or material-consuming and require a high purity of the solute; therefore the alternative lipophilicity descriptors have been provided using mainly *in-silico* predictive models e.g., Hansch's π constant derived for chemical constituents as an additive property. On the other hand, it is possible that some methods for theoretical calculation of lipophilicity might be more or less suitable for specific series of compound analyzed, thus a variety of approaches should be employed and subsequently compared with the empirical data.

However, the routine application of various logP predictors requires a continuous evaluation of their credibility by comparison with empirical data taken as a reference. Partition coefficient can be measured experimentally using at least several procedures ranging from 'shake-flask' technique to popular thin-layer (TLC) or high-performance liquid (HPLC) chromatographic methods. The determination of the partition coefficient by direct measurement using the 'shake-flask' faces issues such as poor reproducibility; therefore the advantageous application of non-polar stationary phase and polar mobile phase in so called reverse phase TLC (RP-TLC) or *vice versa* in normal phase TLC is an attractive and reliable alternative to troublesome procedures.

Apart from the purely structural design and synthesis, the additional objectives of the presented investigation was the experimental determination of the lipophilic profiles of the amides offspring and subsequent critical assessment of the relationship between the retention parameters and the corresponding numerical values. The analyzed compounds were coded using SMILES line-notation while the spatial energy-minimized geometry was stored in Sybyl MOL2 file format. The molecular lipophilicity profiles for the entire ensemble of molecules were calculated using different software packages (clogPS, Molinspirations, OSIRIS, HyperChem 7.0, Sybyl X) for predicting logP value whereas the physicochemical properties were specified with DRAGON generator. The experimental logP values were related with the corresponding calculated values and physicochemical properties using MATLAB programming environment.

The chromatographic data were determined for the investigated set of the amides derivatives by RP-TLC method and related with theoretical partition coefficient calculated by means of *in-silico* procedures. Statistically, significant correlation was found between experimental R_{MO} values and the quantitative descriptor of lipophilicity (logP) specified by OSIRIS and Sybyl predictors. The impact of the calculated physicochemical and structural descriptors on the retention parameters was elucidated by variable elimination procedure IVE-PLS, indicating the involvement of various factors on hydrophobic forces.

18.

Testing of complementarity of PDA and MS detectors using chromatographic fingerprinting of genuine and counterfeit Viagra®

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Counterfeit medicines pose a huge threat to public health worldwide. Their safety, efficacy and quality cannot be guaranteed. High amounts enter the European market, which is why characterization of these pharmaceuticals is a very important issue.

In this study a High Performance Liquid Chromatography – Photodiode Array (HPLC-PDA) and a High Performance Liquid Chromatography – mass spectrometry (HPLC-MS) were developed for the analysis of genuine and generic products of Viagra® and counterfeit samples. The acquired fingerprints were included in the chemometric data-analysis which aimed to test whether PDA and MS are two complimentary detection techniques. The MS data comprise both MS1 and MS2 fingerprints; the PDA data consist of fingerprints measured at 254nm, 270nm and 290nm. The applied chemometric techniques are Partial Least Squares – Discriminant Analysis (PLS-DA) and k Nearest Neighbours (kNN).

Chemometric analysis of all three single wavelengths showed that the best model was obtained by kNN for the 254nm data with a correct classification rate of 97,37% for cross validation and 96,55% for external validation. Combining all three single wavelengths did not result in an improvement of the model. A perfect model was obtained by PLS-DA for the MS data when both MS1 and MS2 were included in the analysis. This model resulted in a perfect prediction of all three sample classes (genuine – generic – counterfeit). Both PLS-DA (98,25% cross validation and 93,10% external validation) and kNN (97,37% and 96,55%) resulted in good prediction model for the combination of 254nm and MS1 fingerprints.

This study shows that a good discrimination between three groups of samples can be obtained by kNN for PDA data and PLS-DA for MS data. However, when combining PDA and MS data, both PLS-DA and kNN are capable to discriminate between genuine, generic and counterfeit Viagra® samples.

19.

Characterizations of *Ipomoea reptans* extracts through HPLC and HPTLC fingerprint development, phytochemical profiling and in vitro cytotoxicity and antioxidant activity determination

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For several centuries, natural products have played a very important role in our daily life. The secondary metabolites of medicinal plants form a main source of natural antioxidants compound. The present study aims developing and finding the best conditions to separate bioactive compounds from various *I. reptans* fractions by both high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) approaches. The results revealed that the HPLC fingerprint analysis produces more peaks and a better separation than HPTLC, while HPTLC analysis helped identifying the classes of the active compounds in some fractions and confirming the similarity between fractions. The evaluation of phytochemical bioactive constituents of the *I. reptans* fractions was performed using standards methods. The results showed the presence of carbohydrates, alkaloids, phenolics and flavonoids in the ethyl acetate-methanol and methanol fractions. Terpenoids and cardiac glycoside constituents were found in hexane-dichloromethane, dichloromethane-ethyl acetate, ethyl acetate, ethyl acetate-methanol, methanol and aqueous fractions. The cytotoxicity of different *I. reptans* fractions was tested using the brine shrimp assay and the results revealed the ethyl acetate-methanol and methanol fractions as the more active fractions. Furthermore, the antioxidant scavenging activity of several fractions was determined by DPPH and ABTS assays. The DPPH results revealed the ethyl acetate-methanol and methanol fractions as the most potent, and this was confirmed by the ABTS results. In addition, the aqueous fraction possessed a higher ABTS radical scavenging activity. Current work focuses on the separation and purification of the bioactive compounds from the active fractions using reversed phase (RP-18) open column chromatography (OCC), thin layer chromatography (TLC) and HPLC according to the same method that was used to develop the chromatographic fingerprints. In the final stage, the chemical structures of isolated pure compounds will be elucidated by several techniques, like Nuclear Magnetic Resonance (1D-NMR & 2D-NMR), Infrared Spectroscopy (IR) and Mass Spectrometry (MS).

POSTGRADUATE STUDENT SECTION

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Maja Natić and Adam Voelkel

20.

How to read and to write scientific publications: a personal view on an important piece of our individual scientific lives

Huba Kalász

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One of the most important activities in our every-day scientific carrier is reading and writing. Well-planned and properly done activities greatly facilitate our lives. Reading, writing and understanding scientific and other sources are of importance. The "freshness" of any material may range from up-to-date to as old as several decades.

The validation of a **protocol** is generally an up-to-date instruction and, what is more, an order, unless otherwise instructed. It is an obligatory statement, irrespective of its author. A well-constructed protocol should contain the possibility of suggestions concerning its validity period and other conditions.

Scientific papers give an account of either original research or a review-type activity of the author(s). Accelerated and letter-type publications usually have a one-to-several months delay from their submission and acceptance. Regular papers give an account of the stage of a discipline, three-to-twelve months before publication. Special permissions (such as keeping ethical requirements, source of financial support, etc.) must be stated. It is the author's responsibility (in the first place) to keep the copyright law, that is not to borrow a text, a figure or a table without a written permission of the publisher.

Printed comprehensive **materials** are books or proceedings depending on the origin of materials. Delay in their topicality can be from zero to several years.

The evaluation of a manuscript has several basic points of view. Formal requirement (typing instructions) is a must. Understandable, clear, fluent and correct English (or the required language of publication) is an important aspect of decision for acceptance. The manuscript should correspond with the scope of the given journal or book. These requirements are normally controlled by the Managing Editor. Reviewers are authorized to express their opinions on the value of the manuscript, as well as to give a definite evaluation, such as accepted, accepted with minor/major revision, to be rejected. Reviewers' statements mean guiding, and the decision is solely made by the Editor. Schedule and actual of publication is the publisher's responsibility.

The form of scientific publications has shifted from printed copies to electronically accessible materials. In general, both forms exist in parallel for the time being. It is essential to know, that writing is the duty and privilege of the author(s). Success achieved and fame earned by scientific work through publications are primarily up to the author.

21.

Inverse liquid chromatography as a tool for biomaterials surface characterization

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The linear free energy relationship (*LFER*) proposed by Abraham is a well-known mathematical correlation used for surface characterization via chromatographic analysis. It combines the value of retention parameter with the force of interaction taking place in all systems occurring in liquid chromatography, i.e. solute – solvent, solute – stationary phase and solvent – stationary phase interactions. The materials being investigated in that way are most often commercially available stationary phases. The aim of our investigation was to prove that liquid chromatography might be applied as a tool for biomaterials surface characterization.

Two ceramic biomaterials applied as a bone tissue substitute were examined by inverse liquid chromatography. Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA) and β-tricalcium phosphate $Ca_3(PO_4)_2$ (β-TCP) were pelletized and in the form of crushed pellet were introduced to the stainless steel tube. Such prepared material served as a stationary phase which the surface properties were examined by inverse liquid chromatography method (ILC). Few types of mobile phase systems were used in these investigations. First, acetonitrile and the mixture of acetonitrile and water had been chosen to determine the physicochemical properties of biomaterials surface. The addition of water enabled to observe the influence of mobile phase polarity on test solutes retention and thereby a change of biomaterials surface properties. Additionally the attempts were made to investigate the physicochemical properties of HA surface in simulated body fluid as a mobile phase. It should help to estimate HA surface behavior in real system when being implanted into human body. Results of these experiments forced us to characterize the HA surface by using two mobile phases revealing much lower ionic strength than SBF – water and 0,1M Na_2HPO_4

22.

Determination of pesticides in herbs by gas chromatography

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The pesticides constitute a plentiful group of chemical compounds intended to destroy or incapacitate the organisms which are dangerous for humans or their surroundings. Application of pesticides has resulted in a rise of food production and simultaneously posed a serious threat to natural environment and people's health. A widespread using of pesticides in order to preserve herbal cultures makes the control of their residues an obvious duty [1].

As far as herbal matrixes are concerned and due to an occurrence of lipids, fatty acids, ether oils, etc. therein, they characterize by an unusual complexity. The presence of these compounds can lead to difficulties in pursuing analysis, so that the selection of a proper research procedure, a right sample preparation and selection of the extraction method and working conditions are important. The analysis of pesticides is challenging due to the low concentrations distributed in complex botanical matrices, so that the process of pesticide isolation from the matrix and enrichment for the chromatographic analysis is necessary. The main aim of the research is optimization of sample preparation, the extraction and the purification of extract for the qualitative and quantitative determination of pesticides' residues in medicinal plants, including the so-called research of homogeneity [2,3].

Gas chromatography is the most often applied technique in pesticide analysis, which allows to achieve verifiable qualitative and quantitative results with an adequately selected column (i.e. DB 5, HP-5 MS, DB-XLB) and detector (i.e. Electron Capture Detector – ECD, Flame Ionization Detector – FID, and Mass Spectrometry Detector). Monitoring pesticide residua is a tool which facilitates an estimation of the consumer exposure to pesticide residues in medicinal plants. Moreover, the results of such monitoring can spur modification of a scope of using the chemical plant protection products and change the value of acceptable levels of the pesticide residues [4].

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23.

Investigation of the peptide nanofibers and nanospheres formation by chromatographic and microscopic techniques

Agnieszka Godziek[1], Anna Maciejowska[1], Ewa Talik[2], Teresa Kowalska[1], and Mieczysław Sajewicz[1]

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In our earlier studies, we have proved that the low molecular weight chiral compounds (e.g., amino acids), can undergo spontaneous oscillatory condensation. This kind of reactions is characteristic of single compounds [1], or the mixtures of compounds [2] in aqueous or non-aqueous solvents. In our previous research on the pair of amino acids (*L*-Pro– *L*-Phe), it was shown that the investigated amino acids characterize with an oscillatory instability, which consists in spontaneous oscillatory oligopeptidization, i.e., in sequential formation and decay of homo- and heterooligopeptides as the products of spontaneous peptidization process. In our other research, it was found out that the amino acids not only undergo spontaneous peptidization, but also self-assemble to form nanostructures [3]. For example, the pair of amino acids (*L*-Pro-*L*-Phe) forms peptide nanofibers, while *L*-Cys forms peptide nanospheres.

In this study on three pairs of amino acids (*L*-Pro-*L*-Cys, *L*-Phe-*L*-Cys, and *L*-Phg-*L*-Cys), it was found out that *L*-Cys determines the formation speed and the shape of nanostructures. To prove that the obtained structures are peptides and have nanostructure characteristics, we used LC-MS and the scanning electron microscopy (SEM). To prove that this process has an oscillatory nature, we used HPLC-ELSD and turbidimetry.

The obtained results demonstrate that the investigated amino acids can undergo an oscillatory chiral conversion and condensation, with a consequence that these compounds may form peptide nano- and microstructures in an abiotic system.

Acknowledgement

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24.

Investigation of peptide structures resulting from spontaneous oscillatory reactions of biogenic amino acids

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The phenomenon of spontaneous oscillatory chiral conversion was for the first time reported with profen drugs [1]. Further research has shown that the oscillatory reactions are specific of hydroxy acids [2] and amino acid [3].

Our research also showed that the amino acids dissolved in aqueous organic solvents undergo two parallel processes of chiral conversion and peptidization. As a result of peptidization, amino acids are formed giving the nano- and microstructures.

In the reported experiment, we focused our attention on two pairs of amino acids (*L*-His-*L*-Thr and *L*-Met-*L*-Ser). The choice of these amino acids was dictated by their important functions in living organisms. *L*-Thr is essential in the synthesis of proteins, contributing to the growth and development of the muscles. *L*-His acts as a precursor of histamine and it is often present as a key amino acid in active centers of many enzymes. *L*-Met and *L*-Ser are necessary for the translation of proteins and they are important reagents in the synthesis of taurine and glutathione.

Observations of the oscillatory peptidization reactions of amino acids were possible with use of HPLC. We also checked the amino acid structures by means of HPLC-MS bcause with time, there appeared additional structures in both binary amino acid samples, which did not belong to the amino acid monomers. With HPLC-MS, we confirmed the presence of peptides in the solutions as a result of spontaneous peptidization. Moreover, we studied peptide nanostructures by means of SEM.

The obtained results expose the oscillatory changes of the monomeric amino acid concentrations in solutions, are indicative of spontaneous peptidization reactions, and they were confronted with predictions of the theoretical model proposed by Epstein et al. [4].

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25.

The GC-MS investigation of hydrogen–deuterium exchange among selected 1,10-phenanthroline-4,7-diones

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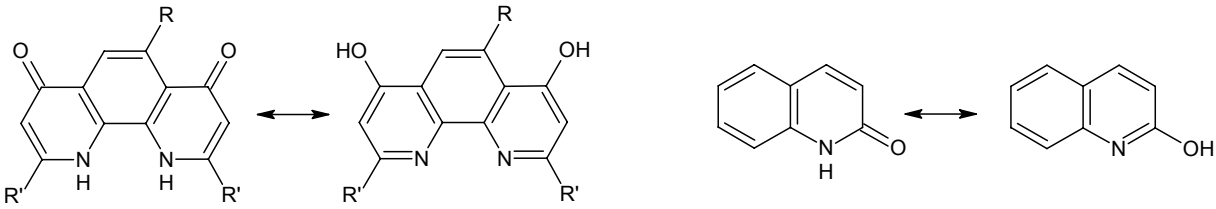
1,10-Phenanthrolines are one of the most significant compound among *N*-heterocyclics [1]. 1,10-Phenanthroline was first synthesized by F. Blau in 1898 [2]. Nowadays, 1,10-phenanthroline and their derivatives have wide range of applications that encourage many synthetic chemists to explore and plan more productive experiments to minimize the side-reactions and increase the yield of target products. New compounds can be precursors for innovative technologies in medicine or dying [3, 4]. Many of them are ligands in coordination chemistry as N, O or N, N atom donors for chelating with metal ions.

The presence of the hydroxyl group in the –ortho or –para position in the pyridine ring in phenantroline constitution generates tautomerism (Scheme 1).



Scheme 1. Tautomerism among 1,10-phenanthroline-4,7-diones and quinolin-2-one.

Our studies are focused on hydrogen/ deuterium (H/D) exchange reactions. We compare the GC-MS and NMR (^{1}H and ^{13}C) techniques. The predominant hydrogen–deuterium exchange reaction is observed for ortho position in phenol ring similarly to reference compounds (quinolin-2-one and quinolin-4-one). On presentation we will present the GC chromatograms and MS spectra comparing with NMR (^{1}H and ^{13}C).

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SESSION V

FRIDAY, MAY 29th, 2015

CHAIRPERSONS: Debby Mangelings and Agnes Moricz

26.

Distribution of selegiline in the rats and rabbits

Kornélia Tekes and Huba Kalász

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Selegiline is the generic name of L-Deprenyl, that is (R)-N-methyl-N-(1-phenylpropan-2-yl)prop-1-yn-3-amine, a selective and irreversible inhibitor of the MAO-B enzyme. It has been registered worldwide under the names of Anipryl, Apo-selegiline, Atapryl, Carbex, Eldepryl, Emsam, Jumex, Selgene, Zelapar, Zydis as a medicine to treat Parkinsonian patients. Its indication has been recently expanded for treatment of human depression, however Anipryl is a formulation for animal use (e.g. treats Cushing's Disease & Cognitive Dysfunction Syndrome in dogs).

Tissue distribution of selegiline was studied by a variety of methods, however no systemic examination of tissues with high MAO-activity was carried out. In our present study rats were treated intraperitoneally and per os by selegiline and/or by methyl-C^{14} radiolabelled selegiline and selegiline was administered also to rabbits. Time-dependence of tissue-concentrations were determined both by HPLC and liquid scintillation methods. Following isolation of tissues and appropriate clean up of the samples was developed and a validated RP-HPLC method was used applying Zorbax Rx-C18 octadecyl silica column stationary phase and a phosphate buffer (pH 3.7) with ion-pairing agent (sodium 1-decanesulfonate) and acetonitrile (organic modifier) was used as mobile phase. UV absorbance was determined at 255 nm, and amperometric detection was at 0.9 V. Radioactivity of samples was determined following solubilization with Soluene 350.

Both methods gave unanimous experimental evidence about the fast onset (a maximum at 15 minutes post-treatment) and relatively fast offset of selegiline both in rats and rabbits. The high levels of selegiline in the lacrymal gland, in the parotid gland and in the testes at the first half an hour following treatment is a striking new result.

Our results definitely suggest a rapid detoxifying intention of the animal organisms for selegiline even by hitherto not studied organs. Tissue binding, metabolism and excretion following “invasion” with such a xenobiotic agent as selegiline, needs further studies for better understanding the possible side effects/new indications of the compound. The RP-HPLC method used can be preferentially used for adequate monitoring of tissue concentrations.

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27.

Determination of Drugs Loaded to Silica Nanoparticles in Brain Tissue

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The blood–brain barrier (BBB) represents a structure with complex cellular organization that separates the brain parenchyma from the systemic circulation. The BBB is the last, critical and serious obstacle for the permeation of central nervous system (CNS) acting agents. The BBB properties result in strong selection of permeating drugs depending on their physicochemical properties, such as molecular weight, molecular volume, lipophilicity, ionisation state and/or their affinity to specific transporters (uptake/efflux transporters).

To circumvent the BBB and allow an active CNS compound to reach its target, various strategies have been developed. They can be sorted with respect to the BBB as either invasive or non-invasive such as the use of alternative routes of administration, inhibition of efflux transporters, chemical modification of drugs or encapsulation of drugs into nanocarriers (e.g., liposomes, polymeric nanoparticles and solid lipid nanoparticles).

This contribution discusses difficulties faced at determination of the concentration of drug bulk substances and drug substances in silica nanoparticles that permeated through the BBB to the brain tissues in rat brain perfusion experiments. Various extraction techniques for isolation of the drugs from the tissue and nanocarriers are discussed. The concentration of the substances in the brain was determined by means of UHPLC-DAD/HRMS LTQ Orbitrap XL.

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28.

Determination of interactions of drug and alcohol, caffeine, smoking and their clinical relevance with different bioanalytical technics

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Nowadays different types of drug interactions (drug, acid-stimulating and inhibiting drugs, food, alcohol, caffeine, smoking, drug transporter), as new discipline of pharmacokinetics, has been appreciated both in the original and generic drug development. It is proven by numerous new regulatory guidelines (FDA, EMA, WHO), as well.

The presentation summarizes, but not limited to interactions required to verify in today's modern pharmaceutical research, as well as the pharmacokinetic changes of drugs due to the effect of alcohol, caffeine and smoking. In the second part of the lecture a various mechanisms of interactions of drugs belonging to different pharmacological groups with alcohol, caffeine and smoking will be presented, as well. The enhanced effect of interactions in the case of the concomitant use of pleasure-giving materials, the different clinical effects of acute and chronic alcohol consumption and the effect of caffeine and smoking on various drug interactions of clinical relevance are also discussed. The role of cytochrome P450 detoxification isoenzymes involved in different types of interactions is also summarized.
The different bioanalytical technics with high sensitivity and selectivity of Headspace-GC, GC-MS/MS, LC-MS and LC-MS/MS (with different ion sources) applications are of high importance in the pharmaceutical drug-alcohol, -caffeine, and -smoking interaction research. Bioanalytical methods play an important role in the original, supergeneric and generic drug development.

The message of this lecture is to highlight the importance of the clinically relevant, frequently unexpected interactions of medicines with alcohol, caffeine, tobacco and drugs illustrating with several examples. The different adequate bioanalytical methods will also presented.