SESSION I

WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS:

Monika Waksmundzka-Hajnos

and Roman Kaliszan

1.

About some unusual findings in HPLC separation of enantiomers with polysaccharide-based chiral stationary phases

Bezhan Chankvetadze

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This presentation summarizes our recent findings in the field of enantioseparation using polysaccharide-based chiral columns in high-performance liquid chromatography (HPLC). In particular, the reversal of enantiomer elution order of chiral analytes depending on the temperature, composition, nature and concentration of minor additives of the mobile phase, as well as unusual increase of the retention and separation selectivity with increasing analysis temperature and enantioselective peak focusing phenomena will be discussed. Novel series of polysaccharide-based chiral stationary phases (CSP) were used for HPLC separation of enantiomers under normal-phase, reversed-phase and polar organic mobile phase conditions. Chiral analytes studied involved FMOC-amino acids [1], chiral drugs such as dihydropyridine [2,3] and arylpropionic acid [4] derivatives, β-blockers [5], imidazole and triazole derivatives [6], sulphoxides [7], chiral epoxides [8] and cyclopropane derivatives with multiple centers of chirality, etc. Possible mechanisms of observed phenomena will be discussed.

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2.

Capillary liquid chromatography

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Capillary LC is one of the most powerful analytical tools available for scientists. Its unique analytical features are associated with numerous technical issues that may cause operation of such systems to be troublesome. Other approaches, e.g. LC/MS and multidimensional capillary chromatography linked to MS, become popular and efficient methods, leading to a better sensitivity and higher throughput. On the other hand, as many columns, connections, and valves are involved, methodological problems described here are multiplied. Operation of a nanoLC system is often subjected to far more methodological obstacles than an "ordinary", analytical HPLC.

Although capillary LC coupled to the mass spectrometer combines most of the important features of both HPLC and MS, it also combines or multiplies their problems. Between the sample and successful final result, there is an analytical instrumentation able to lead almost every scientist to desperation. The aim of this lecture is to provide a brief overview of LC/MS development and practical recommendations on system applications.

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3.

Recent Results on Food Chemistry Using nano-Liquid Chromatography

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Nano-liquid chromatography (nano-LC) is a modern analytical chromatographic technique offering high efficiency, high resolution, reduced peak dilution, short analysis time and use of minute volumes of mobile phases. These mentioned features belong to the use of the same stationary phases employed in conventional HPLC and to the nano-flow involved in the chromatographic process. The application of low flow rates is very important for i) easy coupling with mass spectrometry (MS) electrospray and ii) lowering the waste of dangerous organic solvents. Just for its features, nano-LC has been applied in several fields such as proteomics, metabolomics, forensic, drug and food chemistry.

Aim of this presentation is to briefly introduce the main features of nano-LC considering the main advantages over conventional LC techniques. This will be done reporting some of our recent results obtained with this technique utilizing both UV and MS detectors. Among them, the analysis of wines, fruit juices, olive oils, tea will be shown.

Attention will also be paid to the method optimization giving some examples of selection of the appropriate stationary phase and mobile phases, preparation of capillary columns taking also in mind the recent proposed core-shell particles. Obviously limitations of the miniaturized technique will also be briefly reported.

4.

BIOACTIVE COMPOUNDS OBTAINED BY STRUCTURAL MANIPULATION OF NATURAL PRODUCTS

M.A. Castro,* J.M. Miguel del Corral, P.A. García, A.P. Hernández

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There are hundreds of thousands of secondary metabolites that have been isolated from natural sources. Many of them showed bioactive properties and it is well known that they became lead compounds for drug design. There are many others that are inactive natural products, although they can be considered as potential starting materials for designed and synthesizing new bioactive molecules.

Among the bioactive natural compounds, our research group has been involved in the chemomodulation and chemoinduction of bioactivity in cyclolignans, a family of natural products that includes drugs in clinical use such as podophyllotoxin (antiviral) and etoposide or etopophos (anticancer). We have prepared a large number of cyclolignans, and among them, it is worth to mention the podophyllic aldehyde that showed an interesting selectivity against HT-29, becoming our lead compound for further modifications.[1,2]

Among the inactive metabolites, our research group has put his attention on easily available terpenoids such as myrcene and communic acids and we have performed several chemical transformations[3-5] leading to series of derivatives, with structure of terpenylquinone, that shown a very interesting cytotoxic properties with GI_{50} values at the μM level or below.

The experience with these two families of natural products prompted us to design and synthetize a new family of hybrids between a quinone fragment and a podophyllic aldehyde derivative, named lignoquinones, joined through different aliphatic or aromatic linkers.

An overview of the research performed by our group in this field will be presented.

Acknowledgements: Financial support came from Conserjería de Educación de la Junta de Castilla y León (SA028A10-2) and The University of Salamanca (Ayudas de apoyo a la investigación para: P.A. García, 2011).

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SESSION II

WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS:

Danica Agbaba

and Bezhan Chankvetadze

5.

Quantitative Structure-Retention Relationships (QSRR) in Proteomics and Metabolomics

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At the molecular level, processes at the basis of drug action are physicochemical interactions, which do not involve formation of the new or breaking of the existing covalent bonds within the interacting molecules. Analogous intermolecular interactions are assumed to determine chromatographic retention. Therefore, chromatographic retention data of structurally defined analytes - usually after chemometric processing - can be used to model their activity in biological systems. The basic research strategy consists in analysis of Quantitative Structure-Retention Relationships (QSRR).
Example QSRR will be demonstrated as predicting in a reliable manner the physicochemical properties of xenobiotics, which are considered to be decisive for their pharmacokinetics and pharmacodynamics. Emphasis will be put on the combination of QSRR with mass spectrometry to help to identify bioanalytes in proteomics and metabolomics. QSRR models will be discussed for the prediction of retention of peptides and hence, for verification of their correct identification, based on our proposed semiempirical structural descriptor based on determination of retention of only 7 out of 20 existing natural amino acids. Another QSRR model will be presented, which has been proposed to help identify bioanalytes resulting from doping in sport. The QSRR models derived have generally been based on structural descriptors of hypothetical compounds, generated solely by the calculation chemistry methods.

6.

High dimensional nested analysis of variance of HPLC-DAD fingerprints to assess the effect of production season, quality grade and steam pasteurization on the phenolic composition of fermented rooibos herbal tea

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Recently, the use of chromatographic fingerprints for quality control of herbal products and the determination of the effect of various factors on the composition taking into account minor or/and unidentified constituents has been advocated [1].

In this work, an extended methodology [2,3] based on analysis of variance-simultaneous component analysis, ASCA [4,5], which allows for a significance evaluation of the effects of production season, quality grade and post-production processing (steam pasteurization) on the phenolic content of a rooibos infusion prepared at 'cup-of-tea' strength [6] using chromatographic fingerprints is proposed. Specifically, a four-way analysis of variance where the experimental design involves nesting in one of the three crossed factors is considered. Furthermore, a scheme for the approximate permutation testing of the fixed and random effects in agreement with the expected variance components is proposed.

With the proposed methodology [2], it was possible to come to the conclusion that all of the factors had a significant effect on the phenolic content of rooibos infusion at 'cup-of-tea' strength. The grade A (highest quality) infusion contained a higher content of almost all of the phenolic compounds than the infusion from lower quality plant material. Ferulic acid can be used as indicator of the quality of rooibos tea as its content generally decreases with increasing tea quality. The content of the majority of phenolic compounds decreases in a rooibos infusion at 'cup-of-tea' strength prepared from steam pasteurized plant material.

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7.

The best thermal conditions for SFC separation

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ABSTRACT

The retention factors and the efficiency of SFC columns eluted with neat CO_2 or CO_2 - Methanol 95/5 v/v at 323 K were studied with columns placed in different thermal environments. A stainless steel column operated in a convective air bath exhibited severe efficiency losses when its outlet pressure was dropped below 120 bar. The efficiency of the same column enclosed in a shell made of foam insulation was restored at low outlet pressures and it yielded fast, efficient separations at outlet pressures down to 100 bar, and the retention factors decreased due to the adiabatic cooling of the mobile phase. For both these cases, the height equivalent to a theoretical plate (HETP) showed an abnormal dependence on the mobile phase flow rate when the outlet pressure was close to the critical pressure. At low flow rates the HETP increased first with increasing flow rate, and then decreased before increasing again at higher flow rates. With increasing outlet pressure, the dependence of the HETP on the flow rate gradually reverted to a typical van Deemter behavior. The effect was most pronounced when the column was in the convective air bath.

The efficiency of the column SFC was also tested for column worked in still air conditions. For outlet pressures lower than 150 bar, this conditions proved to be the best – the column efficiency was highest and abnormal van Deemter was not observed.

The analysis of the column work in above mentioned thermal environment were mathematically modeled. We obtained at least the good qualitative agreement between experiment and theory.

8.

Chemometric analysis of chromatographic retention data

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During investigation of chromatographic retention, obtained retention indices can be arranged as a two dimensional matrix, where rows correspond to compounds and columns to chromatographic systems. There is also a possibility to arrange many retention datasets as multidimensional tensors, for example “compound/modifier/concentration” (a 3D cube).

Chemometric methods, such as Principal Component Analysis (PCA) or Parallel Factor Analysis (PARAFAC) can be used for advanced insight into such data. PCA decomposes such matrix to several independent (orthogonal) trends, allowing to analyze intercorrelation and see some invisible dependencies between compounds and/or chromatographic systems.

The first trend is most often the average retention (explaining most of the variance), whereas several subsequent ones are independent trends in retention differences. In the case of PARAFAC, retention can be modelled as a product of three (or more) independent coefficients (contributions).

In most cases, retention can be modelled as two, three or four independent trends only, explaining almost whole variance. Subsequent principal components contain then noise or some irrelevant small variations.

The presentation will provide a summary and examples of HPLC and TLC analysis of retention datasets by PCA and PARAFAC in classical, micellar and salting-out conditions.

SESSION III

THURSDAY, JUNE 6th, 2013

CHAIRPERSONS:

Irena Vovk and Živoslav Tešić

9.

Selected Problems in Chromatographic Separation of Ionizable Drugs

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A majority of drugs there are ionizable compounds, because they exhibit activity towards appropriate receptors. The system of the first choice is RP HPLC with alkyl-bonded stationary phases and aqueous eluents. In such systems organic electrolytes exist in two forms: in form of ion and in form of undissociated molecule. Both forms interact in different manner with chromatographic system components which causes two interfering peaks on the chromatogram. In practice one obtain single tailing peak of bad symmetry and high width. The most difficult situation is in case of basic analytes and alkyl–bonded phases on silica matrix, because then apart from hydrophobic interaction ion-exchange forces between surface residual silanols and bases cations occur, causing extremely wide and asymmetric peaks. The methods for the analysis of ionizable compounds are different. Most simple method is the use of appropriate buffers as mobile phase components. Those of low pH cause inversion of dissociation of acidic analytes as well as residual surface silanols, those of high pH cause inversion of bases' dissociation. Thus, in acids' analysis one has used buffers of low pH or acidic additives (mostly acetic, formic acids) and in case of bases' separation one has used buffers at low or at high pH. It is, however, limited by stationary phases' stability at conditions of extreme pH. Often basic additives to the aqueous mobile phase are also used playing the role of silanol blockers and/or dissociation-inverse agents, most often short-chain amines (diethyl amine DEA) or aqueous ammonia. The effect of silanol blocker concentration is a resultant of blocking of silanols causing decrease of retention for basic analytes and/or inversion of bases' dissociation which causes increase of their retention. However, effect on the peak symmetry and system efficiency is always positive – peaks of bases are symmetric and narrow. Sometimes positive effects are obtained by the use of ion-pair systems. In such situation cationic reagents (amines) are used in analysis of acids and anionic (alkyl sulphonates or alkylphosphates) in analysis of bases. There are several theories about mechanisms of ion-pair formation. The simplest is the theory of formation of uncharged ion-pair in eluent and interaction of it with alkyl (or other nonpolar) groups on the adsorbent surface. The most real is the theory of forming of adsorbed film of ion-pair reagent with ion-exchange properties. The weakness of the IP-RP method is long time of system equilibration and difficulties of the use of those systems in routine analyses. However, one can optimize the analytes' retention by the change of ion-pair reagent kind and concentration, modifier kind and concentration as well as pH of mobile phase.

Normal-phase systems with the polar adsorbent and nonpolar eluent are often applied in analysis of ionizable compounds by TLC. Also in this case ion-suppressant additives to non-aqueous eluent such as organic acids (formic acid, acetic acid) or bases (short chain amines or aqueous ammonia) are often applied.

10.

Optimized RP-HPLC method to determine mCPBG from biological matrices

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Introduction: Meta-chlorophenylbiguanide (mCPBG) is a $5HT_3$ receptor agonist widely used both in *in vitro* and *in vivo* studies. However neither data on its blood-brain barrier (BBB) penetration nor bioanalytical methods are published to determine it. Our aim was to develop a selective and sensitive RP-HPLC method to determine the BBB penetration of mCPBG following intraperitoneal (i.p.) administration to rats and determine the dose-dependence of the drug levels in the blood, brain and cerebrospinal fluid (CSF) .

Materials and methods: The chromatographic system consisted from a Jasco pump (PU1580) equipped with a DG-2080-54 four-line degasser, an AS 2057 Plus Automatic injector connected to an Intro (Antec, Leyden, Zoeterwoude,Netherlands) amperometric/electrochemical detector. Samples were analyzed by reversed phase high-performance liquid chromatography on a Poroshell 120 EC-C18 (150mm x 2.1 mm, 2.7 µm) stationary phase using a Zorbax Eclipse Plus-C18 12.5mm x 2.1 mm, 5 µm precolumn. Samples were injected directly using a 10 µl loop and separation was carried out at 35 °C, at Eox 1.15 V. The mobile phase contained 50 mmol/L citric acid, 2.5 mmol/L octane sulfonic acid and 22 v/v% acetonitrile. The pH was adjusted to 3.0 with 10N NaOH.The flow rate of the mobile phase was 0.2 mL/min.. Chromatograms were electronically stored and evaluated using a Borwin 1.50 chromatographic software (JMBS, Le Fontanil, France). Calibration curves were established using seven dilutions from a 10µg/mL stock solution prepared with 0.8 M perchloric acid (PCA) in the range of 3 – 300 ng/mL, in triplicate. The linearity was evaluated by least-squares regression analysis. Groups of male Wistar rats were injected i.p. with 1, 3, 10 and 30 µmol/200g doses of the compound, then following 20 min the animals were sacrificed under anesthesia according to the animal ethical codex of Semmelweis University Budapest, Hungary (permission number of local authorities:22.1/609/001/2010). Blood and CSF were taken, and brain was dissected. The rat brain was homogenized, samples of brain homogenate, blood and CSF were subjected to clean-up using precipitation by 0.8 M PCA and centrifuged at 14,000 rpm at 4 ^{o}C for 20 min. The supernatants gained were used for HPLC analysis. Samples were kept at – 80 ºC before their analysis and were thawed just before injection onto the column.

11.

Selection of adequate detection for chromatographic analysis of drugs and metabolites from biological matrices

Kalász, H.

(Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary)

Drugs and metabolites are frequently determined from biological matrices. The analysis may be performed in several distinct steps, such as clean-up, derivatizations, chromatographic separation, detection. Choice of detection method depends on several factors, such as the concentration of drug in the target sample, sensitivity of the monitoring system, background noise, simplicity of the method, etc.

Analyses are generally done to determine drug level in the blood, brain, cerebrospinal fluid, liver, urine, etc. The generally used detection methods include monitoring the signal of ultraviolet/visible absorbance, fluorescence, amperometry, conductivity, radioactivity, light scattering, mass spectrometry. Each one of these detection modes requires specific structural element(s) of the target compound.

Simplicity of UV monitoring, sensitivity of amperometry, high specificity and sensitivity of mass spectrometry are the reason of their use in drug level monitoring.

Separation examples will demonstrate their advantages and shortcomings for analyses of phenylalkylamines, pyridinium aldoximes, opiates and small molecular size (fragment) metabolites.

This project has been supported by the Hungarian National Granting Agency (OTKA 100155).

12.

NEW APPLICATIONS OF SULFONES IN SYNTHESIS AND ORGANOCATALYSIS.

Mari Fé Flores, Javier Peña, N. M. Garrido, David Diez*

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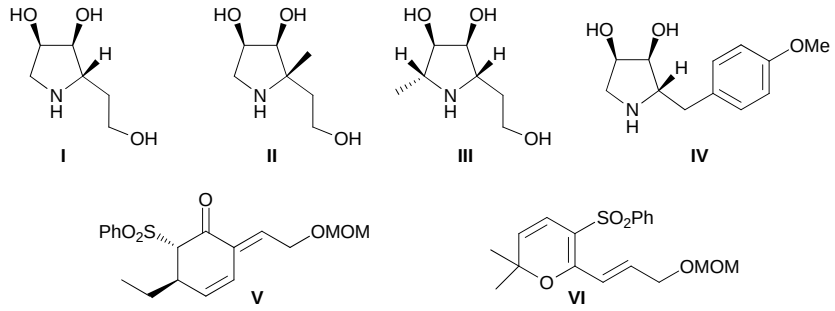
The sulfone group is one of the more versatile functionalities in organic chemistry, for its physical and chemical properties. It has been used for the stabilization of anions in α position and as leaving group in the synthesis of many of the most demanding and sophisticated complex molecules.[1] In our group we have been interested in the development of new chemistry and applications with the sulfone group. It has been possible to use vinyl sulfones for 1,3-dipolar cycloaddition with nitrones and to study the reactivity of the isoxazolidines obtained for the synthesis of C-branched,[2] highly substituted chiral pyrrolidines[3] and iminosugars glycosidases inhibitors[4] (**I-IV**). Recently the sulfone group is one of the latest groups to be incorporated into the panoply of organic functionalities used in organocatalysis.



In this area in constant evolution that has interested in the last years to many organic chemists we have been able using the sulfone chemistry to synthesise chiral cyclohexenones and 2*H*-pyrans (**V-VI**), by a domino reaction using tandem catalysis and by a L-proline-catalysed domino Knoevenagel/6π-heterocyclization respectively.

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13.

INNOVATIVE SOLUTIONS OF SHIMADZU IN CHROMATOGRAPHY AND MASS SPECTROMETRY

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Since 1875, **Shimadzu** is pursuing leading-edge science and technologies in analytical and measuring instruments including chromatographs and mass spectrometers.

Shimadzu HPLC/UHPLC systems demonstrate high reliability, with outstanding performance, such as ultra-low carryover and exceptional area reproducibility, and superior data quality. In addition, they are carefully designed for broad applicability and easy operation. The very good example of high-tech solution in Shimadzu is the Perfinity iDP for protein analysis based on HPLC system.

iDP is a 3 column system that automates protein digestion using immobilized trypsin, desalting and reverse phase separation prior to ESI-MS. iDP provides users with *QUALITY, SPEED & VALUE*:

- Variation $\leq$ 10%
- Protein digestion in as little as 1 minute
- At least 50% cost reduction compared to competing technologies.

Since our first GC was introduced in 1956, **Shimadzu** has been developing **innovative** Gas Chromatography **solutions**. Newly introduced Advanced Flow Technology products provide enhanced separation capability and reduced analysis times to increase productivity. A series of dedicated devices allows users to precisely control flow switching, backflushing and other operations with excellent repeatability.

The completely new Tracera GC System is now ready to solve current trace analysis needs. This system utilizes the new Barrier Discharge Ionization Detector technology coupled with a GC-2010 Plus capillary gas chromatograph to create a GC system that makes it possible to reveal trace components that are difficult to see by other GC detectors.

Conventional analytical techniques require a system configuration with multiple detection schemes to analyze for permanent gases and light hydrocarbons. The use of a methanizer and FID is often required to detect ppm levels of CO and CO_2. However, the BID replaces all of this hardware and allows for the highly sensitive detection of mixtures of inorganic gases and light hydrocarbons.

FID is a great choice for hydrocarbons due to its selectivity for the C-H bond. However, it exhibits a poor response to compounds with other functional groups such as: carbonyl, carboxyl, the hydroxyl group (-OH), aldehydes, or halogens. In contrast, the BID achieves superior sensitivity for such compounds, with less variation in relative response.

Chemists need to measure chemical substances quickly and accurately, but sample pretreatment and interference from complex matrices remain a problem. A solution to these challenges has arrived. The solution is the GCMS-TQ8030 Triple Quadrupole Gas Chromatograph Mass Spectrometer, which provides the speed (ASSP™ permits high-speed scanning at 20,000 u per second), accuracy, and easy operation scientists want.

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SESSION IV

THURSDAY, JUNE 6th, 2013

CHAIRPERSONS:

Huba Kalasz and Hubert Pealinck

14.

High Performance Liquid Chromatography as an Indispensable Tool in Plant Chemosystematics and Chemical Ecology

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Plant extracts are highly complex mixtures of plant primary (e.g. amino acids and sugars) and secondary metabolites (e.g. flavonoids and phenolic acids). One of the classical applications of high performance liquid chromatography (HPLC) in phytochemistry is the identity and quality control of plant derived medicinal products. However, HPLC is also employed to address more fundamental scientific questions such as the variation of secondary metabolites under varying ecological conditions (chemical ecology) and the applicability of secondary plant metabolites as markers in botanical systematics (plant chemosystematics).

In the examples outlined below, particularly complex chromatographic conditions were needed to identify and quantify the decisive compounds to perform meaningful chemosystematic and chemical ecological studies, respectively.

In a recent chemosystematic study, caffeoyl glucaric acid derivatives formerly only known from the genus *Leontopodium* (Edelweiss) were systematically searched for in Austrian members of the related genus *Gnaphalium*. The study revealed that some members of the genus *Gnaphalium* not only represent an easier accessible additional source of the anti-oxidant phenolics at hand but that some additional related compounds exist in *Gnaphalium* which had not been detected in *Leontopodium* yet.

Altitudinal effects on secondary metabolites were investigated in *Arnica montana*. The ratio of *ortho*-dihydroxy- to other flavonoids, the total amount of caffeic acid derivatives, and the radical scavenging potential of extracts obtained from flowering heads increased with the altitude of the growing site. Initially, these results were interpreted as reactions of plants in higher altitudes to elevated UV-B radiation in these sites but results from climate chamber experiments revealed that a decrease of the temperature caused the shift of secondary metabolite profiles. Thus, altitudinal variation in plant phenolics is at least partially caused by lower temperatures in high altitude sites and not (exclusively) by enhanced UV-B radiation.

15.

CHROMATOGRAPHY OF FLAVANOLS, PROCYANIDINS AND METHYLXANTHINES IN PLANT EXTRACTS AND CHOCOLATE

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Flavanols and their dimer procyanidins as well as methylxanthines are secondary metabolites which represent plants first barrier against various pests and diseases. Several biological activities have been reported for these compounds. The described effects of flavanols on human health are mostly positive, while apart positive effects of methylxanthines on the central nervous system (e.g. increased attention, physical performance and muscular recovery) their negative effects on human health are also known (e.g. high doses of caffeine can cause shivering, wakefulness, heart beating and even delirium) [1]. It is well known that compounds from these groups are present in our diet. Among the processed food of plant origin cocoa powder and dark chocolate contain the highest amounts of procyanidins, which according to the epidemiological studies have a protective role in cardiovascular diseases and type II diabetes [2, 3].

The aim of our work was to develop analytical methodology to study flavanols, procyanidins and methylxanthines in chocolate samples, plant extracts and food supplements. TLC on silica gel and cellulose sorbents with different developing solvents and DMACA detection reagent was used for screening and quantitative determination of selected flavanols and procyanidins as well as for optimisation of solid phase extraction procedure on polymeric reversed phase cartridge. Our goal was to develop the fastest HPLC methods for the separation of flavanols, procyanidins and methylxanthines in one run. Therefore, we applied two C18 core-shell columns of different producers. Mobile phases based on acetonitrile-water or methanol-water with addition of acetic or formic acid were tested and the flow rate and the temperature were optimised. The baseline separation of five flavanols (epigallocatechin, catechin, epicatechin, epicatehin gallate), three procyanidins (procyanidin B1, B2 and A2), three methylxanthines (theobromine, theophylline and caffeine) was achieved by gradient elution in the shortest run time ever published. Both HPLC methods were validated, tested by use of the baking chocolate SRM 2384, NIST (National Institute of Standards and Technology) and applied to the analysis of the real samples.

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16.

Effect-directed isolation of plant antibacterials

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The rising incidence of antimicrobial resistant microorganisms means an increasing risk in the human and animal health as well as in agricultural. Therefore, there is a continuous challenge to find new chemicals, which can efficiently kill/inhibit the pathogens. To seek new perspective antimicrobials the plant kingdom is offered as an untapped reservoir containing the most diverse substances.

Searching for bioactive components of complex matrices requires appropriate bioassay monitoring the desired activity (e.g. antifungal, antibacterial), as well as various techniques for isolation and identification.

In our laboratory we focus on the antibacterial components sourced mainly from plant extracts. For screening the extracts for the presence of antibacterial activities the high-throughput thin layer chromatography - direct bioautography is applied. The test organisms are Gram negative pepper pathogen *Xanthomonas vesicatoria*, the luminescence gene-tagged *Arabidopsis* pathogen *Pseudomonas maculicola*, luminescent marine *Vibrio fischeri* bacteria and the Gram positive soil bacterium *Bacillus subtilis*. This step helps to choose the prospected extracts containing effective components as well as it assures that we isolate only the active components, which are important. After gravimetric column or flash chromatographic sample preparation the active compounds can be isolated by means of preparative TLC or OPLC. The OPLC is preferable providing a better separation and the possibility of in-situ further clean-up of the applied sample as well as the on-line fraction collection. The fractionated components with confirmed effect are identified and further in vitro (BioArena) and in vivo (green house) investigations can be done about their mechanism.

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17.

Importance of a further development of chromatographic methods for a more adequate quality control of medicinal plants and better understanding of their applications

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When considering quality control of medicinal plants, spectroscopic determinations are still often methods of choice encountered in different Pharmacopoeia. Hereby, results are mostly expressed as "equivalents" of a specific compound which may be one of the active ingredients but can also represent a "marker" compound. The problem with this approach is not only that all active compounds are considered to have a similar extinction coefficient. Also, all of them are presumed to exert a similar/equivalent activity that is only influenced by the relative proportion in which they are present in the total mixture.

Complementary to quality control is the importance of chromatographic techniques for a further better understanding of the activities and applications of plant based preparations. It is commonly known that the additional value of phytotherapeutics is based on the interactions taking place between different compounds. As well synergistic as antagonistic effects may optimize total effect exerted by these preparations. In the majority of the cases (if not all) not all compounds responsible for the total effect have been identified. An improved knowledge about this "totum effect" can only be obtained when relating results found on base of (pre)clinical studies to the exact chemical composition of the extract used in the corresponding study.

The importance of this approach will further be demonstrated by two examples. The first one includes the use of the hydroxyanthracene containing plant species belonging to the *Cassia* and *Rheum* genus. Preparations derived from several species are used for their stool promoting activities. In general, quality control is based on the requirement of a minimal content of total hydroxyanthracenes, this independently of the specific species used as plant material. However, specific hydroxyanthracene composition not only varies between the different species but is also strongly influenced by culture conditions. Hereby, different hydroxyanthracenes exert their effect by differently affecting several mechanisms responsible for the stool promoting effect. Use of the "total hydroxyanthracene equivalent" as quality parameter is therefore inadequate for predicting a specific activity. A more reliable idea will only be obtained by considering the exact hydroxyanthracene composition.

The second example involves the use of different *Artemisia* species for the preparation of antimalarial medicines. This application is only partly understood as activity is mostly attributed to the presence of artemisinine. In the past, also infusions derived from *A. annua* proved to exert an anti-*Plasmodium* effect though they only contained low artemisinine contents. The same was noticed when applying other *Artemisia* species characterized by absence or very low contents of the presumed active compound. Again, the question arises about the importance of other compounds also present in the different extracts. And here also, a better understanding and improved standardization will only be obtained when directly relating the observed effects with a (more) complete chemical profile of the extracts used in the different trials.

SESSION V

FRIDAY, JUNE 7th, 2013

CHAIRPERSONS:

Teresa Kowalska

and Krzysztof Kaczmarski

18.

On the mechanism of DPPH· reaction with free radical scavengers in solvents and on the surface of TLC plates

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Free radicals are believed to be responsible, at least partially, for the development of pathological processes leading to the occurrence of many human illnesses, e.g.: cancer, neurodegenerative diseases, arthrosclerosis and many others. Therefore there is a growing need to search for potent free radical scavengers that may be potentially used to prevent human organism form the development of these ailments. Spectrophotometric and chromatographic techniques have been commonly used to screen the samples for the presence of direct antioxidants. Majority of them make use of a stable free radical DPPH· (2,2-diphenyl-1-picrylhydrazyl), that is reduced to hydrazine in the presence of free radical scavenger. Several mechanisms have been proposed for the reaction between phenolic direct antioxidants and DPPH· in different solvents. However there are limited studies concerning the probable reaction mechanism between non-phenolic antioxidants (e.g.: terpenes) and DPPH·. To the best of our knowledge there are also no data in scientific literature concerning the influence of adsorbent on the results obtained in TLC-DPPH· test. Our latest research results will be presented focusing on the probable reaction mechanism between terpenes and DPPH·. The influence of adsorbent surface on the results observed in TLC-DPPH· test will also be discussed.

19.

Pharmaceutical Nanoparticles and Methods of Their Characterization

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Development in the field of pharmaceutical administration has resulted in the discovery of highly sophisticated drug delivery systems that allow for the maintenance of a constant drug level in an organism. Contrary to these revolutionary biopharmaceutical results, over the last ten years the number of poorly bioavailable drugs has steadily increased. Progressive ways for increasing oral bioavailability are a technique of nanoparticles preparation or a technique of nanoparticle drug delivery. The application of these techniques allows many pharmacological agents to reach the site of action. Nanotechnology allows insoluble compounds to be attached or encapsulated in highly soluble nanoparticles, offering the potential to expand the number of drugs introduced into clinical trials.

A wide range of techniques have been developed for preparation of nanomaterials. Synthetic methods for nanoparticles are typically grouped into two categories: *i*) top-down (generally dispergation processes), and *ii*) bottom-up (generally precipitation processes). A number of various materials are used for preparation of nanoparticle carriers. Liposomes, solid lipid particles, dendrimers, micellar and polymeric particles, capsules, spheres, shells and crystals are the most frequent types of nanoparticles. The most common analytical techniques such as transmission electron microscopy, atomic force microscopy, X-ray diffraction, UV-VIS spectrometry, dynamic light scattering, laser Doppler electrophoresis, interactive force apparatus or hydrodynamic chromatography can be used for characterization of nanoparticles. The content of drug substance in nanoparticles can be determined by chromatographic or spectral methods.

This lecture deals with pharmaceutical nanoparticles, especially techniques of their analytical characterization. Also the most significant properties and the most important applications of pharmaceutical nanoparticles are briefly mentioned.

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20.

NEW FILLERS FOR DENTAL COMPOSITES – EXAMINATION BY MEANS OF IGC

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Numerous modifications in the composition of dental composites and glass-ionomer cements have been made. We were looking for the improvement of the physical and chemical properties, colour stability, reduction of the polymerization shrinkage and the improvement of adhesive properties of new dental composites. The composition of the polymer matrix was constant. A difference in the chemical composition consists in the use of different kinds of fillers. Moreover, the efforts to introduce the maximum amount of the filler into the polymer matrix were undertaken.

The surface activity of the new materials was also examined with the use of the inverse gas chromatography (IGC). Moreover, a part of the obtained samples was stored in an artificial saliva of 6.8 – 7.8 pH for a period of 7 days and afterwards their surface activity was also examined by means of the IGC.

Humid carrier gas was applied during IGC experiments. It has a significant influence on the values of the dispersive component of the surface free energy γ_S^d, characteristic to the surface layer of dental composites, as well as on the values of other parameters such as: K_A, K_D and S_C. Dental composites storage in the artificial saliva for a period of 7 days has also an impact on the value of the aforementioned parameters.

21.

Study of thermally-induced reaction products of chemically bonded RP-18 stationary phase

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Study of the silica-based chemically bonded stationary phases by means of Raman spectroscopy, IR and UV absorption spectrometry, differential scanning calorimetry, HPLC and GC-MS examination of extracts from the thermally treated samples provided indirect evidence of the nature of transformations taking place in the stationary phases at increased temperature. The latter methods concern the transformation products arising by spontaneous cleavage from silica matrix, i.e. extractable with dichloromethane or hexane only. The absence of aromatic compounds from the extracts investigated suggests that aromatic products remain chemically bonded, so they are not cleaved from the solid system, and hence they are not extracted with the solvent used. Thus, the identified non-aromatic products of thermal modification of the adsorbent are evidently the by-products of the main process.
In continuation of the research on the thermally induced chemical transformation of the silica-based chemically bonded stationary phases (C18), the oxidative cleavage of the silicon-carbon bonds with hydrogen peroxide and potassium fluoride was utilized, followed by the GC-MS study of the resulting products. In this reaction respective hydroxy-derivatives arising from covalently bonded organic ligands are the expected products. These investigations allowed determination of the probable structures of certain thermal modification products as the various different alkyl derivatives of the phenylsilane ligands. Apart from aromatic compounds, the products with unsaturated bonds and carbonyl functionalities were found in the analyzed extracts. The analysis of the GC-MS chromatograms reveals that under the applied working conditions, the investigated process runs with relatively low yields.