SESSION I WEDNESDAY, MAY 30 th, 2012

CHAIRPERSONS: D. Agbaba and B. Chankvetadze

1.

QSAR, QSPR and QSRR in Terms of 3-D-MoRSE Descriptors for *in silico* Screening of Clofibric Acid Analogues

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A series of 27 analogues of clofibric acid, mostly heteroarylalkanoic derivatives, have been analyzed by a novel high-throughput reversed-phase HPLC method employing combined gradient of eluent's pH and organic modifier content. The such determined hydrophobicity (lipophilicity) parameters, log k_w, and acidity constants, pK_a, were subjected to multiple regression analysis to get a QSRR (Quantitative Structure-Retention Relationships) and a QSPR (Quantitative Structure-Property Relationships) equation, respectively, describing these pharmacokinetics-determining physicochemical parameters in terms of the calculation chemistry derived structural descriptors. The previously determined *in vitro* log EC_{50} values – transactivation activity towards PPARα (human Peroxisome Proliferator-Activated Receptor α) – have also been described in a QSAR (Quantitative Structure-Activity Relationships) equation in terms of the 3-D-MoRSE descriptors (3D-Molecule Representation of Structures based on Electron diffraction descriptors). The QSAR model derived can serve for an *a priori* prediction of bioactivity *in vitro* of any designed analogue, whereas the QSRR and the QSPR models can be used to evaluate lipophilicity and acidity, respectively, of the compounds, and hence to rationally guide selection of structures of proper pharmacokinetics.

2.

A novel Flowing Atmospheric Pressure Afterglow (FAPA) ion source for direct analysis of organic compounds

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A novel, atmospheric pressure Flowing Atmospheric Pressure Afterglow (FAPA) source for mass spectrometry has been developed. The source operates at ambient pressure and can be used for direct analysis of organic compounds as a soft ionization technique. No or limited fragmentation is observed. FAPA was mounted on the Esquire ion trap instrument after removal of standard source. Both positive and negative ion modes can be applied and all features of multiple fragmentation could be assessed. Helium at atmospheric pressure was used as a discharge gas. The angles between FAPA, sampling region, and inlet to MS in the pin-to-capillary arrangement were optimized for the highest sensitivity of analyses. Sample application is possible in several ways, including direct screening of solid compounds (e.g. tablets), deposition on a glass slide (solution or after drying out), or on paper napkin (paper chromatography), and after nebulization. An advantage of direct analyses without any sample preparation is a major feature of FAPA source. The analytical capabilities of the source were evaluated including narcotics, deodorants, and homemade legal highs. The novel source can serve for rapid analysis and identification of harmful substances that comprise a health hazard.

This work was partially supported by the grants from the Ministry of Higher Education, No. NN 204 02 86 36 and NN 204 30 48 37.

3.

The Liquid Chromatographic Guide to the Galaxy of Organic Impurities

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Travelling through the galaxy of organic impurities may be full of surprises and unexpected situations. Having on mind the small quantities and understanding their origin, we face the fact that the additional problem is always hidden somewhere in the chemical structures of these substances. Either they resemble the parent drug and the analysis is quite weighted by this fact or the polarity is completely reversed which arises other difficulties. The situation gets more complicated in drugs comprised of two or more active substances as the number of impurities, and consequently the analytes, increases significantly. In the drug products a completely new surrounding for active substance is provided and we might confront the drug-excipient compatibility issue.

The most common technique for monitoring the impurities in drug substances and drug products is HPLC with UV detection. Depending on the chemical structures of the parent drug and the present or emerging impurities, the selection may be done from the wide variety of HPLC methods. The analyst's experience is of utmost importance as it facilitates and speeds up the overall process. The classical RP-HPLC is the soundest and probably the safest choice. In the case of substances with basic characteristics, certain modifications should be done. Nowadays, as the most acceptable alternative to ion-pair addition, the chromatography based on chaotropic effects is offered. However, for the analysis of polar and basic compounds HILIC would be preferable in some situations, regardless the complexity of retention mechanisms involved in the separation that might bring additional problems. For the analysis of complex matrices, like suppositories, creams or ointments, the surfactant modified systems have demonstrated many advantages. Namely, micellar and microemulsion liquid chromatography are facilitating the sample preparation enormously as they solubilize the hydrophobic part of drug carrier. Also, this modification of mobile phase might be quite useful if someone wants to avoid tedious lengthy gradient elution.

4.

Novel Separation and Identification Techniques

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Today's analytical laboratory provides analytical chemists with unique challenges both in the separation and identification of organic compounds. Compounds which are of most interest are often the most difficult to work with such as stereoisomers or those found in difficult matrices.

Techniques such as multi-dimensional (MDGC), comprehensive (GC x GC) or hybrid (LC x GC) chromatography can be useful to lessen the amount of time and effort needed to solve some of the most challenging analytical problems. In addition, using dual-line systems in standard GCMS allows for the separation and identification of components which would be difficult to separate on one column in one injection. In a dual-line system, two columns of different polarities can be installed directly into an MS detector allowing for a quick separation and identification of isomers.

These novel and cutting-edge techniques will be presented along with specific examples associated with petrochemical, pharmaceutical, environmental and food science applications.

SESSION II WEDNESDAY, MAY 30 th, 2012

CHAIRPERSONS: R. Kaliszan and Ž. Tešić

5.

Advances in Investigation of Hydrophilic and Lipophilic Properties of Drugs

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Determination of physico-chemical parameters of biologically active compounds has become more important with an age of rational thinking in drug design. One of the major prerequisites for pharmacological screening and drug development is the prediction of absorption, e.g. the transport of a molecule through cellular membranes. Lipophilicity is a property that has a major effect on ADME/Tox properties, because drugs cross biological membranes through the passive transport, which strongly depends on their balanced hydrophilic and lipophilic properties. Lipophilicity has been studied and applied as an important drug property for decades. It was usually measured by octanol/water partition coefficients (log *P*) of molecules since the pioneering work of Hansch, Fujita and Leo. Log *P* is the logarithm of the partition coefficient in a biphasic system, defined as the ratio of a compound concentration in phase 1 and in phase 2. The log *P* is determined for the uncharged species of the drug. Note that it may exist preferably in the ionic or zwitterionic form(s). Different lipophilicity descriptors such as log *P*, log *D*, log k_w, R_M, etc. can be used for description and prediction of structure-activity relations. Experimentally expressed hydrophilic and lipophilic properties take into account configuration specificity and intramolecular and/or intermolecular interactions of molecules.

It has long been recognised that the retention of a compound in reversed-phase liquid chromatography is governed by its hydrophilicity or lipophilicity, and thus shows correlation with an octanol–water partition coefficient. RP-HPLC methods have become popular and widely used for lipophilicity measurement. HPLC provides an excellent platform for computer controlled automated measurements with computerised data acquisition for a large number of research compounds. The other advantages in the use of the HPLC retention data (log *k*) for lipophilicity determination are as follows: there is no need for concentration determination and method validation; small impurities are separated from the main component; small amounts of material are needed for measurements; and the measurements can be completely automated.

This lecture deals with advances in the method of lipophilicity determination, *i.e.* effect of stationary and mobile phase selection, isocratic or gradient conditions, etc.

6.

Inverse Gas Chromatographic Examination of Potential Fillers in Abrasive Industry

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Filler is an important component of any abrasive tool. It is usually inorganic compound that performs various functions during production and exploitation. It collects the heat and prevents the melting of resin, improves the mechanical properties of the final products and reduces their production costs. Standard fillers in abrasive articles most often emite hazardous compounds. Pyrite (FeS_2) and lithopone ($ZnS+BaSO_4$) decompose to dangerous sulphur, cryolite (Na_3AlF_6) decomposes with the emission of fluorine. This was the main reason of searching for new proecological fillers that are stable during work of grinding tool. The aluminosilicates (perlites and zeolites) were chosen as potential new generation of fillers in abrasive industry.

The surface properties of commercial and new fillers were investigated by Inverse Gas Chromatography (IGC). In this method the examined material is placed in the chromatographic column and its properties are determined basing on retention behavior of suitable test compounds. Acid-base, specific and dispersive properties of the surface were studied by means of IGC. Several parameters such as: γ_s^d , γ_s^{sp} , γ_s^+ , γ_s^- , K_A and K_D were determined.

Physicochemical investigation of the surfaces of fillers were accompanied by the determination of the magnitude of interactions of the examined materials with fragrance compounds (terpenes).

The obtained data were analyzed using chemometric methods. Principal component analysis (PCA) is useful method for classification of IGC data. PCA was applied for selection of the best fillers and parameters carrying information significant for completed characterization of the fillers. Cluster analysis allowed to group set of all parameters and all investigated fillers into several distinct clusters.

This work was supported by N N209 108939 project.

7.

A closer look on the meaning of "extrapolated retention" and "average retention" concepts

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The extrapolation of retention coefficients is a standard procedure in liquid and planar chromatography, mainly in the lipophilicity estimation. However, the extrapolation of retention (expressed as log k or R_M) to zero concentration of a modifier can be done in many ways. Several equations can be considered and even simple linear regression can be done in several variants, for example weighted and robust. The question about the choice of extrapolation technique is a significant problem, as in many cases the dependence is not exactly linear, but sligthly concave or piecewise-linear.

Therefore, the aim of the presentation is to clarify advantages and disadvantages of different techniques: classical linear regression, weighted linear regression, robust regression using M-estimators, least quantile of squares, least trimmed squares in the context of extrapolation of "almost linear retention". The results are compared on 35 model compounds TLC dataset. The best correlation of lipophilicity with extrapolated R_{M0} values was obtained in the case of weighted regression on 1/x values and robust M-estimator techniques, which slightly outperform the classical retention. The other techniques gave worse results (or comparable results only on selected modifiers). Polynomial (quadratic and cubical) regression resulted with large extrapolated values, weakly correlated with lipophilicity.

Another problem is averaging the extrapolated retention from several modifiers to use averaged retention as a lipophilicity measure. It can be done by averaging R_F values, averaging k values, averaging R_M values or regressing of all data in one time. Each approach is mathematically different – for example averaging of R_M values is equal to computing geometric mean from k values. A comparison of these approaches is also worthy of investigation. Surprisingly, averaging of R_F values between modifiers gave better correlation with lipophilicity than averaging k or R_M values.

8.

Application of Inverse Gas Chromatography in the characterization of pharmaceutical hybrid materials

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Hybrid materials are becoming more popular because of the possibility of connection organic, inorganic materials and even biological molecules. The biggest advantage of these materials is possibility favorably combine dissimilar properties of compound to obtain new properties not accessible otherwise. Some of them have ability to entrap functional molecules and they are used to prepare functional materials applicable in catalysis, electrochemical, biomaterials or pharmacy. Such materials can therefore applied to drug delivery system.

Development and introduction into practice of the new excipient – hybrid matrix is associated with a series of experiments including physicochemical tests. Inverse Gas Chromatography (IGC) is one of the methods used to study the physicochemical properties of complex multicomponent systems. In this method an investigated material is placed in a column and than is characterized using volatile probes of known properties (test solutes), which are carried by a mobile phase. It allows the determination of the following parameters:

- $\chi_{23}^{'}$ Flory-Huggins parameter expressing the strength of interactions between the components of the hybrid material and active agent,
- γ_S^D the dispersive component of the free surface energy allows the determination of activity of the examined material,
- K_A and K_D parameters correspond to the ability of the examined material to act as electron acceptor/donor, respectively – the acidity and basicity of the surface.

This work was supported by 32/045/12 DS PB PUT project.

SESSION III THURSDAY, MAY 31 th, 2012

CHAIRPERSONS I. Vovk and H. Paelinck

9.

Recent studies on enantiomer separation mechanisms in aqueous and non-aqueous capillary electrophoresis

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Capillary electrophoresis (CE) represents one of the major techniques not only for analytical scale enantioseparations but is also a powerful tool for a better understanding of the fine mechanisms of enantioselective intermolecular recognition. The major advantages of CE from the viewpoint of enantioselective molecular recognition studies are the following: 1. CE allows very fast screening of selector-selectand pairs. 2. The high peak efficiency in CE permits to observe enantioselective features in selector-selectand interactions which are invisible by other techniques. 3. A small thermodynamic selectivity of recognition can be transformed into a high separation factor in CE. 4. CE is very flexible technique for adjustment of enantioseparation.

The major disadvantage of CE for studies of non-covalent intermolecular interactions is that this technique does not provide any direct information regarding the structure of intermolecular diastereomeric associates. The experiments based on the nuclear Overhauser effect (NOE) in nuclear magnetic resonance (NMR) spectroscopy complement CE from this viewpoint very well. In addition, NMR-spectroscopy is very useful technique for determination of stoichiometry and enantioselective binding constants of selector-selectand associates. However, NMR spectroscopy fails when mixed complexes are formed between a selector and selectand. Mass spectrometry (MS) may appear useful in this case. This presentation summarizes our recent studies on the combined application of CE, NMR and MS methodologies to mechanistic studies of enantioselective selector-selectand interaction in the liquid phase. The methodology is illustrated with the examples including interaction of chiral drugs such as ketoconazole and terconazole, propranolol, ephedrine, norephedrine, ketoprofen, and talinolol with various cyclodextrins in aqueous and non-aqueous media.

10.

Separation Strategies in HPLC for the Enantiomeric Separation of Pharmaceuticals on Polysaccharide-Based Chiral Stationary Phases

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Chirality is the property of stereoisomers that form non-superimposable mirror images. These mirror images are called enantiomers. Enantiomers of a given drug molecule might exhibit different potency and/or toxicity when entering a chiral environment, such as living systems. They can be absorbed, distributed, metabolized, and excreted differently. Therefore, many pharmaceuticals are nowadays distributed as pure single enantiomer, to produce the desirable effects and decrease the risk of side-effects or toxicity. In most cases, chiral separations rather than time-consuming and expensive chiral syntheses are used to obtain the pure drug enantiomers. Therefore, chiral separations have become an important part of the drug discovery and development process not only for preparative purposes but also analytically. Several techniques are applied for chiral separations, such as gas chromatography (GC), liquid chromatography (HPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE) and capillary electrochromatography (CEC). The current study evaluates the enantioselectivity of six recently commercialized polysaccharide-based selectors, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, Lux Amylose-2 and Sepapak-5, and of three classic, Chiralpak AD-RH, Chiralcel OD-RH and Chiralcel OJ-RH, at previously defined normal-phase (NP), reversed-phase (RP) and polar organic solvents chromatography (POSC) generic screening conditions (as part of a separation strategy). A set of 58 pharmaceutical compounds was used. The results on both sets of CSPs and also the three HPLC modes were complementary. Moreover, the three HPLC modes were also complementary. Sets containing both new and classic CSPs were proposed to update the screening step in the three modes. This update showed enantioresolution for 55/58 (90%) compounds at NP conditions, for 48/58 (83%) at POSC conditions and for 51/58 (88%) at RP conditions. Cumulatively, the three modes were able to separate 57/58 (98%) compounds. Naproxen was the only compound that could not be resolved in any of the three modes.

11.

UPDATE OF A GENERIC CHIRAL SCREENING STEP ON POLYSACCHARIDE-BASED CHIRAL STATIONARY PHASES IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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As the number of chiral drugs launched onto the market is yearly increasing, the need for fast and performant enantioseparation methods with minimum costs and preferably with low environmental impact is becoming more compelling. In this context, supercritical fluid chromatography (SFC) has great potential as chiral separation technique, since high flow rates can be used without compromising the efficiency. As a result column equilibration- and analysis times are reduced, enabling a higher throughput. “Green chemistry” properties are also assigned to SFC.
To enable efficient chiral method development, generic separation strategies are defined. These broadly applicable strategies start with a screening step in which a limited number of experiments are proposed, with the aim of selecting a chromatographic system with the appropriate enantioselectivity. The strategy also includes optimization steps to achieve the desired separation and alternative conditions are proposed when no separation was yet achieved.
This work focusses on the definition of a generic screening step for SFC. For this purpose, a test set of 59 pharmaceutical racemates was screened with 96 chromatographic systems with the intention to evaluate their enantioselective resolving capacity. More specifically, six non-chlorinated and six chlorinated polysaccharide-based columns are evaluated in combination with four methanol- and four isopropanol-containing CO_2-based mobile phases.
The similarity and complementarity of these different systems is determined to enable defining a fast and efficient screening step for SFC.

12.

Oligopeptidization oscillations of binary amino acid mixtures in solution

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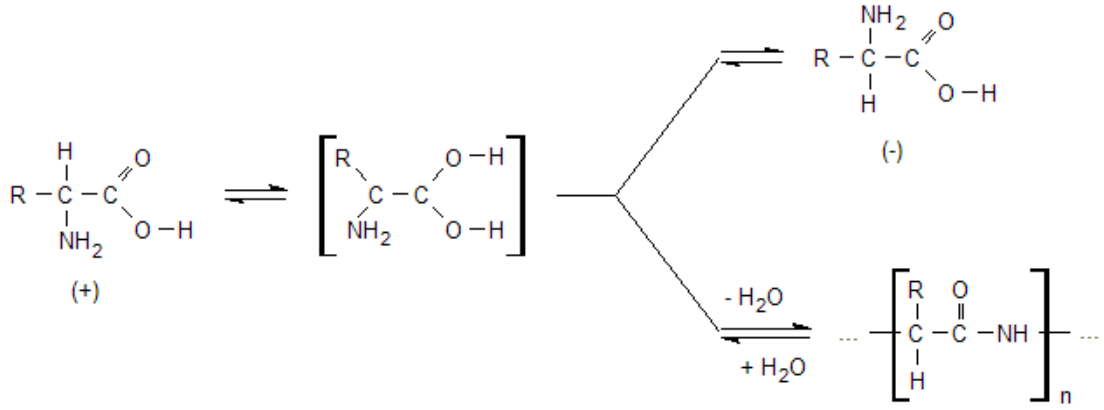
In our earlier studies [1-5], we discussed the ability of low molecular weight carboxylic acids (e.g., profen drugs, amino acids, and hydroxy acids) not only to undergo spontaneous oscillatory chiral inversion, but also spontaneous oscillatory oligopeptidization. In the case of amino acids, these two processes running in parallel can be illustrated with the following scheme:



The main tools in our investigations on the dynamics of oscillatory oligopeptidization with single amino acids dissolved in the aqueous and non-aqueous solvents were HPLC with DAD and ELSD detectors, LC/MS, and ^{1}H NMR and ^{13}C NMR spectroscopy. Theoretical models were proposed to illustrate the mechanisms of the oscillatory chiral inversion and the oscillatory oligomerization.

In this study, we present the results of our most recent investigations of spontaneous oscillatory oligopeptidization in binary *L*-Phg–*L*-Phe and *L*-Pro–*L*-Hyp mixtures dissolved in aqueous and non-aqueous solvents. All amino acids we investigated are important building blocks in protein systems. We demonstrate that although the peptidization dynamics of each individual amino acid may be different, in binary mixtures they are able to spontaneously produce mixed oligopeptides (as shown with use of HPLC, LC/MS, and NMR). A possible mechanism of mixed oligopeptidization is also proposed.

References

[1] M. Sajewicz, M. Gontarska, D. Kronenbach, T. Kowalska, Acta Chromatogr., **21**, 151-160 (2009)
[2] M. Sajewicz, M. Matlengiewicz, D. Kronenbach, M. Gontarska, T. Kowalska, Acta Chromatogr., **21**, 259-271 (2009)
[3] M. Sajewicz, M. Matlengiewicz, M. Leda, M. Gontarska, D. Kronenbach, T. Kowalska, I.R. Epstein, J. Phys. Org. Chem., **23**, 1066-1073 (2010)
[4] M. Sajewicz, M. Gontarska, D. Kronenbach, M. Leda, T. Kowalska, I.R. Epstein, J. Syst. Chem., **1**:7 (2010); DOI:10.1186/1759-2208-1-7
[5] M. Sajewicz, M. Dolnik, D. Kronenbach, M. Gontarska, T. Kowalska, I.R. Epstein, J. Phys. Chem. A, **115**, 14331-14339 (2011)

SESSION IV THURSDAY, MAY 31 th, 2012

CHAIRPERSONS: D. Milijković-Opsenica

and Y. Vander Heyden

13.

CHROMATOGRAPHY OF THE MAJOR DIETARY CAROTENOIDS

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Carotenoids are an important group of about 700 natural pigments. Some of them are part of the everyday human diet, where β-carotene, α-carotene, β-crpytoxanthin, lycopene, lutein, zeaxanthin, and astaxanthin are the most abundant and show different health protective effects (e.g. preventing vitamin A deficiency, antioxidant and immune-enhancing activity, cancer prevention). Therefore, apart from those extracted from different natural sources, synthetically produced carotenoids became a big world business: projection is 1.3 billion $ till 2017.
Chemically, carotenoids belong to highly unsaturated terpenoid compounds and as such are not very stable, especially if exposed to oxygen, heat, light and acids. Special care must be taken into account during their analysis to avoid errors. Different chromatographic techniques are successfully used for their separation and quantification in a wide range of concentrations in samples, from plasma to vegetables, fruits and some of them also food supplements. Nowadays, HPLC methods coupled to PDA and MS detector prevail, however TLC sometimes offers additional useful information about the analytes and represents an alternative and complementary technique. Different layers and numerous development solvents were applied for the TLC separations of carotenoids in the past. Besides the limited separation capacity, stability of carotenoids on the TLC plate represented the main drawback compared to HPLC.
The aim of our work was to investigate the major dietary carotenoids in plants, foods and food supplements. Sample test solutions prepared by optimised extraction or saponification were analysed by new TLC and HPLC methods. A substantial improvement of stability of carotenoids on the RP C18 HPTLC plates enabled confirmation of identity by in situ visible spectra and TLC-MS, as well as densitometric quantitation in ng range. Introduction of triethylammonium acetate buffer (pH 7) as a constituent of the mobile phases in the HPLC separations of carotenoids performed on C30 and additionally C18 core-shell columns resulted in enhanced peak areas and lower RSD of peak areas. The advantage of using triethylammonium acetate buffer instead of triethylamine in mobile phases is better peak symmetry and lower back pressure. Besides the prevailing, usually all-*trans* compounds, a number of geometric isomers were separated and identified by visible spectra using PDA detector.

14.

TLC in biochemical and pharmaceutical research

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Thin-layer chromatography plays an important role in screening complex samples of natural origin, for the presence of compounds with desired activity (effect directed analysis). It has been applied to detect substances with antibacterial, antifungal, antioxidant properties, as well as the inhibitors of several enzymes. The results of the latest researches have shown these tests can be used not only for the preliminary studies, but also to obtain quantitative data or to study structure-activity relationships.

TLC is also applied to determine lipophilicity of natural and synthetic compounds, candidates for new drugs. Such tests are an important part of preclinical studies aimed at fishing out the compounds with the best properties.

Biochemists apply thin-layer chromatography to study for example the lipids content in different samples of animal origin.

In this presentation the latest developments in the aforementioned fields will be discussed and future trends will be also outlined.

15.

TLC coupled with biodetection for studying antioxidant structure-activity relationships of polyphenols

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TLC-DPPH$^{\cdot}$ test has become an important test for screening plant samples for the presence of compounds with free radical scavenging activity. However it is usually recognized as a preliminary examination that have to be followed by other methods to confirm the results or to obtain quantitative data. Most recently TLC-DPPH$^{\cdot}$ method coupled with image processing has been applied to quantitatively measure direct antioxidant properties of compounds found in complex samples. Our studies have also confirmed the influence of adsorbent type, used in the test, on the observed results. An approach to standardize TLC-DPPH$^{\cdot}$ test for assessing free radical scavenging properties of polyphenols has also been undertaken. TLC-DPPH$^{\cdot}$ test with image processing have been also applied to study the influence of polyphenols' structure on their free radical scavenging properties. Selected flavonoid glycosides acylated with hydroxycinnamic acids were used in the study to check their free radical scavenging properties in comparison to corresponding nonacylated forms and aglycones. It has been discovered that acylation increases the observed free radical scavenging properties when compared to corresponding nonacylated forms. The compounds possessing ferulic acid moiety in their structures were characterized with the strongest free radical scavenging properties when compared to other examined substances. TLC based test has been found suitable to study antioxidant structure-activity relationships of polyphenols.

16.

Screening of polyphenolic profile of Serbian propolis by UPLC-LTQ-Orbitrap Mass Spectrometry

Petar Ristivojević[1], Filip Andrić[1], Jelena Trifkoviæ[1], Ljubiša Stanisavljević[2], Živoslav Tešić[1], Dušanka Milojković-Opsenica[1]

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Propolis (bees glue) is resinous natural substance collected from plant buds and exudates of certain trees and plant. Due to its anti-inflammatory, immunostimulatory, antiviral, antifungal, anti-inflammatory, anticancer, antioxidant and antibacterial activity, propolis has been used in Serbian folk medicine from ancient times. This bee product is composed of resin (consisted of flavonoids and phenolic acids and regarded as the polyphenolic fraction), wax, essential oils, pollen, and various organic compounds. The composition of propolis depends on time, vegetation, and the area of collection.

Biological activity of propolis mainly depends on flavonoids and phenolic acids content. In this study we determined polyphenolic composition of 56 samples of propolis collected from different regions of Serbia. Separations were performed on RP-18 column. The mobile phase was consisted of (A) water + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid. The mass spectrometer was operated in negative mode. MS spectra were acquired by full range acquisition covering m/z 100–900. For fragmentation study, a data dependant scan was performed by deploying the collision induced dissociation (CID).

More than thirty six phenolic compounds were identified and quantified in propolis samples according to the corresponding spectral characteristics: mass spectra, exact mass, characteristic fragmentation.

Acknowledgment: This research was supported by The Ministry of Education and Science of Serbia, Grant No. 172017 and by FP7 RegPot project "Reinforcement of the FCUB towards becoming a centre of excellence in the region of WB for molecular biotechnology and food research" (FCUB-ERA GA No. 256716).

SESSION V FRIDAY, JUNE 1 th, 2012

CHAIRPERSONS: M. Waksmundzka-Hajnos and A. Voelkel

17.

HPLC Analysis of Pyridinium Aldoximes

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HPLC analysis of various bis-pyridinium mono-aldoxime (BPMA) antidotes for organophosphate poisoning makes possible to follow their pharmacokinetics in various body fluids and tissues.

Sample clean-up was done using precipitation of proteins by perchloric acid at a temperature near 0 ^{o}C (to minimize degradation). BPMA concentration in serum, cerebrospinal fluid, brain, eyes and fluid in oral cavity was quantitatively analyzed by ion-pairing reversed-phase chromatography on C18 silica column using aqueous mobile phases. Ultraviolet detection was used to monitor serum and oral cavity fluid concentration of BPMA (K027, K048 and K203) in the range of 0.1 through 150 μg/mL. Determination of BPMAs in cerebrospinal fluid and brain was monitored by electrochemical detection from 0.015 through 4 μg/mL range. Calibration curves were constructed using spiked samples.

Serum level of K203 followed zero order kinetics. Drug level in cerebrospinal fluid and brain showed a definite delay as a consequence of hindered penetration through the blood-brain barrier.

Methodological aspects of HPLC of certain polar organic compounds and conclusions on the possible distribution mechanisms in the body will be presented.

This work was sponsored by the Hungarian National Grant Agency, OTKA K100155.

18.

Molecular modelling of a template substitute and monomers used in molecular imprinting for aflatoxin B1 micro-HPLC analysis

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The contamination of food and drinking water with toxic substances is one of serious problems in modern world. Mycotoxins are very important group of food-contaminating compounds due to their acute toxicity, carcinogenic, and mutagenic action. The main mycotoxins, aflatoxins, are present in high concentration in corn, peanuts, and cotton but there are also other aflatoxin-contaminated products like almonds, figs, and milk. Currently, for quantitative testing of multiple samples mainly radioimmunoassay and enzyme-linked immunosorbent assays are used. The specific recognition of a certain analyte may be achieved in a chromatographic system by molecularly imprinted polymers (MIPs) which are attractive synthetic materials mimicking the highly specific receptor properties of antibodies.

Because of aflatoxin toxicity and cost, we decided to find their substitutes which would give similar effect of specific interaction between an analyte and a stationary phase during imprinting process. In this note, we report the results of our studies during which we found an equivalent molecule of aflatoxin B1. 5,7-dimethoxycoumarin was found to be a structural analogue for aflatoxin B1 and to serve as its substitute in a grafting solution for the molecularly imprinted polymer synthesis. We also present simulations of interactions between aflatoxin or its substitute and selected monomers of a grafting solution. We discovered that both methacrylic acid and allylamine are functional monomers which could provide a similar binding towards aflatoxin B1 and 5,7-dimethoxycoumarin. The monomers were selected for preparation of MIP for the aflatoxin B1 HPLC quantification method.

We would like to express our gratitude to the Polish Ministry of Science and Higher Education for financing this research with a grant no N N 204012038.

Reference

Wyszomirski M., Prus W., *Mol. Sim.,* 2012, accepted to press, doi: 10.1080/08927022.2012.667876

19.

UHPLC AS A TOOL FOR PURITY ANALYSIS AND QUALITY CONTROL OF ENVIRONMENTAL SAMPLES AND POLYCYCLIC AROMATIC HYDROCARBONS

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Keywords: liquid chromatography, gas chromatography, environmental samples, quality control.

Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbon molecules containing two or more aromatic rings. Some PAHs, such as benzo[a]pyrene, are classified as carcinogens; PAHs are commonly found in the environment as a result of partially burned organic materials, such as petroleum, plastics, rubber, lubricants and wood. In addition to environmental concerns, there are concerns about PAHs in food, especially in grilled meats.

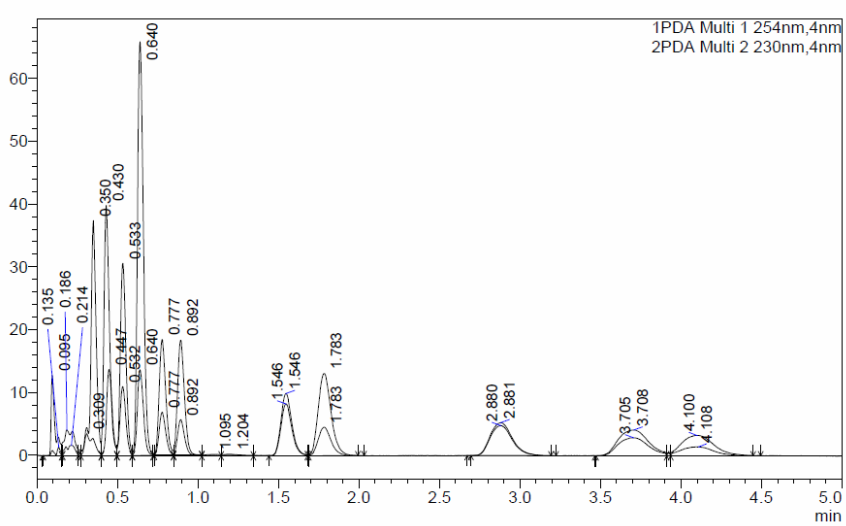

Fig. 1. Chromatogram of 15 + 1 mixture standard of PAHs. The analysed PAHs include naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr),1,2- benza[a]anthracene (BaA), chrysene (Chr), benzo[e]pyrene (BeP), benzo[e]acenaphthylene (BeA), benzo[k]fluoranthene (BkF), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (Bghi)P and indeno[1,2,3- cd]pyrene (InP).

The work put on aim to prepare and develop short and high resolution method for organic samples analysis by the use of UHPLC and compare it with other different techniques for validation. Presented application will introduce a short and efficient method to measure and identify of 15 + 1 PAHs at low concentration by the use of UHPLC, GC/MS and MALDI-TOF