SESSION I

WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS:

Monika Waksmundzka-Hajnos

and Imre Klebovich

1.

LC and EC-FAPA - novel applications

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The new flowing atmospheric pressure afterglow (FAPA) ion source operates in the ambient atmosphere and has been proven to be a promising tool for direct and rapid determination of numerous compounds with little or no sample preparation. Preliminary experiments indicate good performance of the source, including stability of the signal in the negative-, and positive-ion modes and repeatability of sample injection. The FAPA has been shown to be an excellent alternative to Electron Ionization (EI) for the detection of molecules separated by gas chromatography. The foregoing experiments reveal that an *on-line* combination of electrochemistry or liquid chromatography and flowing atmospheric-pressure afterglow mass spectrometry (EC and LC/FAPA) can be a useful tool for quantitative separation and analysis of psychostimulants and their potential metabolites, and is suitable as a step towards predictive toxicology, where rapid information on toxic metabolites of the novel legal highs and designer drugs may contribute to more effective medical treatment. These combinations extend the capabilities of FAPA in predictive toxicology and forensic science. The present work also illustrates the capabilities of the FAPA source to accept flowing liquid systems, which potentially opens wider possibilities for applications not available till now (e.g. metabolomic studies). In this presentation we demonstrate, for the first time, that a liquid system can be directly introduced into this ion source.

This work was supported by the Polish National Science Center grant DEC-2012/07/N/ST4/01837, META 05/EuroNanoMed/2012 and by the U.S. Department of Energy through grant DE-FG02-98ER 14890.

2.

Methods resulting in specific enrichment of solutes

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Displacement chromatography, affinity chromatography and chromatography using molecularly imprinted polymers are excellent enrichment methods before the use of a highly selective separation method for trace compounds present in environmental and biological matrices. The enrichment step of purification can be done using either solid-phase extraction, and classical- or high-performance liquid chromatography.

Displacement chromatography is based on reversible overload of stationary phase with solutes, and the displacer forces all solutes to be released from the stationary phase. The specificity of the method is based on the proper selection of carrier (one of the mobile phases, here) and displacer (the terminal one of the mobile phases). Certain components are eluted by the carrier itself; some others totally utilize the binding/retardation capacity of the stationary phase, so they remain on it. Displacer is then given, which has higher affinity to the stationary phase than any of the bound solutes, forcing the absorbed compounds to be displaced in highly concentrated bands. The solute of our interest can be detected and isolated.

Special and selective interactions can take place between the solute to be enriched/isolated and the stationary phase. This is the case in **affinity chromatography**, where a well-defined and reversible interaction takes place, the ligand-solute is similar to the key-to-locker relation. A wide choice of stationary phases (e.g. for immune-, metal-, lectin-affinity chromatography) is commercially available, however, a special stationary phase can be prepared using costume-made activated particles.

A **molecularly imprinted polymer** stationary phase is prepared using a template bound to the polymerized matrix by complexation. A complementary and reversible binding site is formed by the removal of the template. The template is either the target solute to be purified, or its structural analogue. Usual matrices are surface water, body fluids (such as plasma, urine, milk, etc.), foods, beverages, etc. The recovery of solutes is generally high, especially when neither of its analogues but the solute itself is the template.

This project was financially supported by the grant of the OTKA T100155.

3.

Application of chromatography in analysis of microtraces – case examples

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Samples containing traces of volatile compounds, such as components of flammable liquids or active components of defence sprays as well as samples containing traces of non-volatile compounds (e.g. polymers), very often are subject of analysis by GC for forensic purposes. In the case of non-volatile traces a pyrolysis could be applied for preparation of the sample for analysis by GC. The separated compounds being identified by mass spectrometer (MS). Also, liquid chromatography is applied in sections when microtraces are analysed, e.g. for analysis of traces of explosives.

Application of various chromatographic procedures for analysis of microtraces will be presented on examples of analysis of car paint samples by Py-GC/MS technique [1], explosives by HPLC-DAD [2], fire debris by ATD-GC/MS [3] as well as irritant compounds by ATD-GC-MS and GC/MS. The application of chromatography to the analysis of the evidence samples returns data, which evidential value should be evaluated for fact finders (prosecutors, judges, policemen). Therefore, results of analyses should be presented in a form which could be comprehensible for non-specialists, but at the same time the applied method of data evaluation should express the role of a forensic expert in the administration of justice, i.e. evaluate physicochemical data (E; chromatograms) in the context of the prosecution proposition (H_p – e.g. traces of flammable liquid detected in fire debris are kerosene) and defence proposition (H_d - traces of flammable liquid detected in fire debris are diesel fuel). In practice this means that the following conditional probabilities $Pr(E|H_p)$ and $Pr(E|H_d)$ should be estimated. The ratio of these two conditional probabilities is termed the likelihood ratio (LR) [4]. Examples of application of LR approach for evaluation of evidence value of chromatograms will be also presented.

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4.

In-needle technique - the efficiency of the new extraction systems

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Sample preparation is an important analytical step because many conventional sample preparation methods are relatively complicated, time-consuming procedures and need large amount of organic solvents. *In-needle* extraction was developed as a novel sample preparation technique for chromatographic determination of organic compounds in aqueous and gaseous samples. Compared to conventional sorbent traps, *in-needle* extraction device is more convenient sample preparation tool and can provide important advantages for on-site sampling. Specially designed needle was packed with sorbent on which the analytes are retained. The extraction was made by pumping the aqueous sample into the needle extraction device. The subsequent desorption process was carried out by a flow of desorption solvent through the needle into the gas chromatograph.

The extraction properties of needles filled with commercial materials [1] and monolithic materials polymerized in the needles were investigated [2]. Such sampling systems were characterized by, e.g. breakthrough volume (which determines the maximum volume of water sample which can be introduced into the sorbent) and the sorption capacity (indicating the maximum mass of analyte that can be extracted from water samples).

Acceptable sampling conditions for direct analysis of liquid samples were selected. Experimental data collected from the series of liquid samples analysis carried out with the use of *in-needle* device have shown that the effectiveness of the system depends on various parameters. To estimate suitability of a given extraction system, a new parameter P_{IN} expressing the geometry of the system was proposed.

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SESSION II

WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS:

Huba Kalasz and Josef Jampilek

5.

ODOR AND AROMA ANALYSIS: COMBINING CHEMICAL-ANALYTICAL AND HUMAN DETECTION

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ABSTRACT

It is of utmost importance to qualitatively and quantitatively measure volatile organic compounds, as they determine the aroma quality of food and might lead to odor annoyance in ambient air. Today, a wide array of sample preparation, separation and detection techniques are available to objectively evaluate the volatile chemical profile. However, in both food and environmental studies the use of human olfactory is still of great importance to assess the complex bouquet and hedonic properties.

In this presentation an overview will be given of both instrumental as well as sensory-based strategies for the determination of odors and aromas. In particular, the application possibilities of MS-fingerprinting in combination with multivariate statistical data processing will be illustrated showing the results from two different research projects.

First, preliminary results will be discussed correlating mass spectrometric fingerprint analyses with corresponding olfactometric data in environmental odor research. The potential is demonstrated for predicting the odor concentration (expressed in European odor units per cubic meter (ouE/m³)) by means of a regression model using m/z-fragment intensities.

Secondly, results will be shown from a recently published article on the aroma properties of microalgae (Van Durme, Goiris, De Winne, De Cooman, & Muylaert, 2013). In this food-related study, correlations were made between the volatile composition and corresponding sensory attributes. Four species of marine microalgae (*Botryococcus braunii,, Rhodomonas, Tetraselmis species*, and *Nannochloropsis oculata*) and one fresh water microalga (*Chlorella vulgaris*) were investigated. Multivariate data processing revealed that microalgal samples having a seafood-like odor character contained high levels of sulfuric compounds (dimethyl disulfide, dimethyl trisulfide, and methional), diketones, α-ionone, and β-ionone. Fresh green, fruity flavors were linked with typical aldehydes such as 2,4-alkadienals and 2,4,6-alkatrienals.

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6.

Recent status of the extraction of biogenic amines from cancer samples. Is automation of this major step of an experimental workflow within our reach?

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Biogenic amines (BAs) – serotonin, tryptophan, as well as catecholamines – adrenaline, noradrenalin and dopamine are known to be associated with numerous life-threatening diseases such as neurodegenerative disorders or cancer. The assessment of their concentrations in biological fluids and tissues is helpful for early detection of on-going pathology. Various analytical methods for biogenic amines analysis from complex matrices were described including liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE) coupled to various detectors: UV-VIS, mass spectrometry (MS), fluorimetric or electrochemical detector.

Despite numerous methodologies that have been developed for biogenic amines analysis, their measurement in biological samples remains an analytical challenge. That is caused mainly by their low concentration in body fluids and the great instability (photosensitivity, easiness to oxidized) of analytes. Therefore, sample pre-concentration and clean-up are significant for the success of the whole experimental procedure.

The aim of the study was to compare and evaluate all advances in sample preparation for the BAs analysis. The greatest attention was focused on solid-phase extraction (SPE) and liquid-liquid extraction (LLE) procedures to extract and pre-concentrate BAs from biological samples. Moreover, simple protein precipitation by use of acetonitrile was taken into account as well. Finding of the appropriate, straightforward and little time-consuming sample preparation workflow was a major issue to resolve since further reliable LC and CE separations were realized.

7.

FAST BREATH ANALYSIS BY THE USE OF GCMS SUPORTED WITH NOVEL POROUS POLYMERIC MATERIALS FOR BIO APPLICATIONS

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Keywords: gas chromatography, mass spectrometry, cancer fingerprints, solid phase micro extraction.

Rapid development of chromatography in recent years is associated with search for new, bio compatible materials that can be used to produce a new type of highly selective analytical sensors for medicine.

A very large group of compounds used in this study are macrocyclic compounds (fig. 1), among others synthetic macrocyclic polyethers, discovered in 1967. That discovery involves with the development of coordination chemistry of metal ions, which are strongly and selectively complexed by selected criptands or criptand's groups. The aim of research in this area is the selection of cancer fingerprints for applications in oncology.

The possibility of new designed compounds for use in the construction of analytical tools and molecular devices requires understanding the relations between their structure and properties.

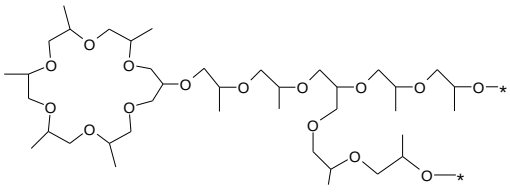

Fig. 1. Macromolecule with two branches (* means an end)

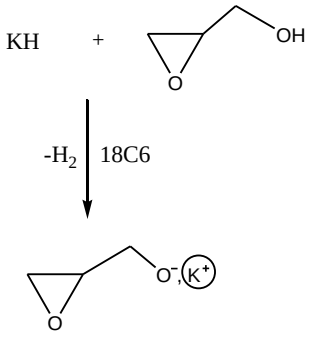

Fig. 2. Initiators synthesis

8.

Extraction of Tricyclic Antidepresants from Plasma Using Supported Liquid Extraction Plates

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Abstract

Traditional Liquid-liquid extraction (LLE) is widely used for preparation of biological fluid samples (plasma, urine) prior to LC-MS/MS analysis. LLE is labor intensive, very difficult to automate, and is therefore not well suited to high throughput bioanalytical sample preparation. Supported-liquid extraction (SLE) provides an easier to automate alternative to LLE. Problems such as emulsion
formation and automated pipetting of liquid layers are eliminated, as the two phases are never in direct contact with each other. In this short presentation the development of an automated procedure for high throughput supported-liquid extraction of three tricyclic antidepressant drugs from human plasma, using the SLE Supported-liquid Extraction Plate is presented. Analyte recovery, along with the speed and efficiency to traditional LLE are compared. Supported-liquid extraction (SLE) using the SLE plate is an easily automated technique, providing 2 x increased sample throughput compared to traditional LLE. SLE supported-liquid extraction Plates can give significantly higher analyte recoveries than traditional LLE using the same extraction conditions (sample and solvent).

SESSION III

THURSDAY, JUNE 12th, 2014

CHAIRPERSONS:

Jerzy Silberring and Hubert Paelinck

9.

BIOANALYTICS/RADIOBIOANALYTICS AND IMAGING TECHNIQUES IN DRUG METABOLISM RESEARCH: OVERWIEW OF MODERN ASPECTS AND NOVEL TECHNIQUES

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The trends of drug metabolism research significantly changed and have proven their merits over the last period, due to the *in-silico* predictions preceding the preclinical studies and the application of 3D and 4D-QSAR models. These methods enable better and more applicable prediction of ADME parameters and Cytochrom P-450 isoenzymes (CYP 3A4, 2D6, 2C9, etc.) to obtain information even for the species-dependent metabolizing properties of the original drug.

The present lecture intends to give an overview of the process and up-to date bioanalytical tools (qualitative, quantitative) of the *in vitro* and *in vivo* drug metabolism research.

In the course of drug development the labeled or multi-labeled radioactive isotopes (^{14}C and ^{3}H) pharmacokinetic/metabolism studies combined with the new generation of triple-quad techniques nowadays are essential, and for that many examples will be presented.

The new *in vitro – in vivo* Imaging Techniques (MALDI Imaging, nanoScan, PET/MRI in animal and human) will be presented.

A complex multi-step process will be illustrated from separation, purification, isolation to structure elucidation (GC-MS, LC-MS/MS, LC-NMR) of minor and major metabolites derived from animal and human biological matrices. The addition of the above systems to the off-line and on-line separation and radioactivity detection possibilities of GC-RD, HPLC-RD, and OPLC (OPLC-DAR/PIT, OPLC-RD, OPLC-DAD-RD, OPLC-DAD-RD-MS/MS) resulted in a new, flexible and rapid high-performance complex solution in metabolism research. Pharmacokinetic and metabolism informations of different species, contributing to registration, are also summarized.

A complex multi-step process will be illustrated from separation, purification, isolation to structure elucidation (the high sensitive and highly selective hyphenated techniques – LC/Tripe Quadrupole-Jet Stream-ESI-MS, APCI, APDI, Q-TOF/Q-TOF, Ion Trap and GC/MS-MS, LC-NMR, etc.) of minor and major metabolites/active and toxic metabolites derived from animal and human biological matrices.

10.

EFFECT-DIRECTED ANALYSIS: BIOAUTOGRAPHY – PRINCIPLE OF THE METHOD AND APPLICATONS.

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Effect-directed analysis (EDA) is usually used in environmental analysis, food control and drug discovery [1]. The method provides information on biologically relevant substances enabling searching them in very complex matrices. EDA connects bio- or toxicity-assays with instrumental analysis. The targeted substances having given biological properties are isolated, identified and quantified. In most cases separation/fractionation is needed to find a proper substance responsible for the biological effect. The ideal separation method to be linked with biological detection is thin-layer chromatography (TLC).

Thin-layer chromatography – direct bioautography (TLC-DB) can be applied to the analysis of various antimicrobial agents in body fluids, pharmaceutical preparations, environmental and food samples. The principle or this EDA method is that both separation and microbiological detection are performed directly on the same TLC plate. A developed (HP)TLC plate is dipped in a suspension of microorganisms growing in a proper broth, incubated and visualized with tetrazolium salts. Substances possessing antibacterial properties form cream-white growth inhibition zones against a purple background of a (HP)TLC plate [2,3].

Two bioautographic assays to be used after TLC separation were developed, optimized and fully validated. One of them is based on Gram negative bacteria, *Escherichia coli* another one on Gram positive bacteria, *Bacillus subtilis.* These tests were used successfully to determine antibiotics at their MRL (maximum residue level) in milk. Other applications cover screening antibacterial properties of herbal drugs, plant extracts and their constituents as well as estimating quality of sample preparation procedures [4].

References:

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11.

Liquid-chromatography mass spectrometry for food authenticity assessment

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Serbia belongs to the group of middle-developed agricultural countries with large possibilities in production of high-quality food. Excellent quality of soil, water as well as moderate continental climate provide good basis for this sort of production. Main export products might be honey, milk, wine, vegetables and berry fruits. In order to have a successful export it is extremely important to make an appropriate brand. That means that each product must be chemically characterized primarily to the presence of important phytochemicals, with defined geographical and botanical origin and also unequivocally recognized as a natural product. These analyses are very demanding and complex, the most demanding of them being the techniques of liquid-mass analysis.

High performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS) has the most important place in phytochemical determination. Polyphenolic substances represent the most important class of phytochemicals found in food. By examination of their presence as well as of relative relations between separate components it is possible to obtain important data for assessment of quallity, as well as botanical and geographical origin of food. For these complex analysis the best method shown is ultra highperformance liquid chromatography coupled with hybrid mass spectometer which combines linear trap quadropole and Orbitrap mass analyzer (UHPLC– LTQ OrbiTrap MS).

Highly efficient liquid chromatography connected to mass detector for determination of relations between stable isotops is very successeful for food authenticity assessment. This method combines chrotographic technique with stable isotop determination. Variations detected in isotopic representation of stable isotops of hydrogen, carbon, nitrogen and oxygen are of great interest for food authenticity studies. Most chemical elements have stable isotops that might be useful in this purpose. All plants are divided into 3 types by way of photosyntesis: C_3 (Calvin-Benson photosyntetic cycle), C_4 (Hatch-Slack cycle) and CAM (Crassulacean cycle) plants. Each photosyntetic way discriminates in a different manner harder carbon isotop (^{13}C), found in the atmoshere. In that case C_3 and C_4 demonstrate different ranges ^{13}C, $\delta^{13}C$ value and so in C_4 plants that value varies from -11 to -14 ‰ while in C_3 plants it varies from -23 to -30 ‰. In this manner relative quantities of C_3 i C_4 ingredients used from their production can be determined. By using this method it is possible to determine counterfeit honey and wine. In addition to that, stabile isotops provide us with other important information such as: isotopes H - climate indicator, geographical origin, botanical origin; isotopes O – geographical origin; isotopes C – climate indicator, ways of nutrition, geographical origin; isotopes N – organic food / agricultural practice; isotopes S – geological indicator.

12.
Planar chromatography-direct bioautography - an effective tool for bio-monitoring

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In the fight against various human, animal and plant diseases there is an increasing demand for new bioactive agents, especially antimicrobials, because of the emergence and spread of bacterial resistance to commonly used antibiotics. The plant kingdom possessing an arsenal of secondary metabolites represents an underestimated source of bioactive substances; however obtaining pure active compounds can be time-consuming and expensive, especially when bioactivity is studied only after the isolation process that usually incorporates extraction, fractionation and purification steps. Therefore, to save time and money, the strategy of the isolation process has been changed in the last decade to focus on the isolation of only the components having the desired activity. The bioassay-guided analyses are essential for this purpose, as they lead only the active fractions along the process and discard the uninteresting ones. These procedures require continuous bio-monitoring as guidance, for which planar chromatography hyphenated with bioassays is especially suited, fulfilling such requirements as being high-throughput, rapid, relatively simple and reliable. Its additional advantage is a possibility to analyse single components in various matrices, thus eliminating synergistic and antagonistic effects. The further combination of this system with spectroscopic and spectrometric techniques enables the characterization of the bioactive substances.
In this study we demonstrate through examples the applicability of planar chromatography-direct bioautography as a rapid, easy-to-use tool for monitoring the components having antibacterial effect against the Gram-negative pepper pathogen *Xanthomonas vesicatoria*, the luminescence gene-tagged *Arabidopsis* pathogen *Pseudomonas syringae* pv. *maculicola*, luminescent marine *Vibrio fischeri* bacteria and the Gram positive soil bacterium *Bacillus subtilis*.

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SESSION IV

THURSDAY, JUNE 12th, 2014

CHAIRPERSONS:

Živoslav Tešić and Łukasz Komsta

13.

Preparative Layer Chromatography (PLC) and its use in phytochemistry

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Preparative Layer Chromatography is dedicated to the separation of natural or synthetic mixtures in an enlarged scale for various purposes, mainly for isolation of pure compounds or fractions for further investigations. Because one has to introduce a sample as large as possible to the layer and work in overloaded conditions, several problems fundamental for PLC appear. There is a decrease of resolution owing to the band broadening effect and, as a result, a necessity to optimize system selectivity, the way of application of the large samples to the layer, the way of delivery of the eluent to obtain satisfactory yields of the process and high purity of isolated compounds and /or fractions. Composition of eluent is also important to avoid the non-volatile components and derivatisation especially by use of non-destructive reagents. Moreover, problems remain characteristic for analytical TLC such, as the general elution problem, separation of closely related compounds etc.

The following points should be taken into the account: sampling of a mixture solutions – a kind of overloading (volume or mass) and the sampling mode – a way of introducing large volumes to the adsorbent layer, in view of an impact on resolution of the neighboring bands and possibilities of overloading.

The use of special development modes (such, as multiple development techniques and multidimensional separations) can increase the separation yields and bring satisfactory results.

The goals of preparative layer chromatography (apart from isolation of the pure compounds) are the following ones: an important step of sample preparation, an on-line purification of crude plant extracts, and optimization of column preparative separations.

The use of PLC for the separation of plant extracts containing coumarins and furanocoumarins, taxoids, and alkaloids will be discussed.

14.

CHROMATOGRAPHIC METHODS FOR ANALYSIS OF TRITERPENOIDS AND PHYTOSTEROLS IN PLANT EPICUTICULAR WAXES

Irena Vovk[1,2], Katerina Naumoska[1], Zoran Kitanovski[1], Alen Albreht[1,2], Breda Simonovska[1] Mitja Martelanc[1], Karmen Kapp[3], Heikki Vuorela[3]

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Triterpenoids represent a large class of secondary metabolites, which are widely distributed in nature. Their structures with different functional groups (sometimes some of them derivatized to esters, glycosides etc.) and the lack of chromophores represent a big challenge in their analysis. Although several beneficial effects of various triterpenoids for human health indicate their importance in the diet, there is almost no data about the content of triterpenoids in vegetables and about their intake by everyday diet.

The aim of our work was to investigate triterpenoids and phytosterols in epicuticular waxes of various vegetables and several Slovenian apple cultivars. Among vegetables we investigated cabbage, eggplant, zucchini, tomato, red pepper, mangold, spinach, lettuce white-colored radicchio di Castelfranco, raddichio Leonardo, white cabbage, red cabbage, savoy cabbage and parsley. Screening of the extracts before and after hydrolysis was performed by silica gel and reversed-phase (C18 RP) thin-layer chromatography (TLC), RP-TLC-MS/MS^2 and C18 RP high-performance liquid chromatography (HPLC) with UV and mass spectrometric (MS) detection using atmospheric pressure chemical ionization (APCI) and gas chromatography (GC). For all the extracts TLC screening on silica gel (separation of triterpenoids with different functionality) and on C18 RP (separation of isomers) was performed before the HPLC-UV and HPLC-MS analysis. Different developing solvents were used for the separation of triterpenolic acids and neutral triterpenoids on the C18 RP HPTLC plates. For the RP-TLC-MS/MS^2 confirmation of the presence of the triterpenoids and phytosterols in the extracts we created mass spectra library. Additionally, all the extracts were analyzed by the new HPLC-UV-APCI-MS/MS^2 and GC-FID methods. Critical evaluation of the results obtained by all the methods confirmed the presence of some of the studied triterpenols (α-amyrin, β-amyrin, lupeol), esterified triterpenol (lupeol acetate) triterpenolic acids (ursolic,

oleanolic), lupenone and phytosterols (β-sitosterol, stigmasterol) in the plant extracts, while the presence of cycloartenol, cycloartenol acetate and betulinic acid was not confirmed.

15.

Bioactive Natural Compounds in Vegetables: *Scorzonera hispanica* L. (black salsify) and Apiaceae Vegetables Carrot, Celery, Fennel, Parsley, and Parsnip

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Scorzonera hispanica L. commonly known as black salsify is a perennial herb of the Asteraceae family. The roots of *S. hispanica* have been traditionally used to enhance digestion and perspiration and as a diuretic agent; currently, roots of *S. hispanica* are widely cultivated in Western Europe, especially in Belgium and in the Netherlands as vegetables. Little is known about the chemical composition of *S. hispanica*; compound classes known from subaerial parts of *S. hispanica* include sesquiterpenoids and lignans. There are no studies on the chemical composition of aerial parts of black salsify yet. An in depth investigation of the chemical composition of both subaerial and aerial parts of *S. hispanica* and the development of the first validated HPLC-DAD-CAD method for the quantification of major phenolic constituents in extracts will be presented. Important results include the isolation of a number of closely related bisabolane derivatives and a group of oxo-octadecadienoic acid derivatives from subaerial parts of *S. hispanica* as well as caffeoyl quinic acid derivatives and flavonoids (mainly quercetin derivatives) from aerial parts. Moreover, the anti-microbial and cytotoxic activities of the bisabolane derivatives from *S. hispanica* will be discussed.

Polyacetylenes falcarinol, falcarindiol, panaxydiol, and 8-*O*-methylfalcarindiol were isolated from root celery (*Apium graveolens* L.). Extracts of roots and bulbs, respectively, of carrots, celery, fennel, parsley, and parsnip were investigated for their content of polyacetylenes by HPLC-DAD. All species contained polyacetylenes, although carrots and fennel only in minor amounts. Additionally, the cytotoxicity of the polyacetylenes against five different cell lines was evaluated using the annexin V-PI assay. Falcarinol proved to be the most active compound. Possible chemo-preventive impacts of food polyacetylenes are discussed in the context of the so far unexplained paradox that high contents of natural carotenes in blood correlate with a low incidence of several types of cancer, while carotenes taken as food supplements do not have a positive effect. As carrots are the major source of food carotenes in Europe and North America but also the only important source of falcarinol, polyacetylenes and not carotenes might be responsible for the beneficial health effects of carrot consumption.

16.

Novel techniques of chromatography coupled with tandem mass spectrometry as a universal tool for analysis of organic compounds.

Paweł Stalica
"Shim-Pol A.M Borzymowski" E. Borzymowska-Reszka, A. Reszka sp.j.

Novel analytical techniques require speed, accuracy, precision, and most of all selectivity and specificity. Increasing demand for a lot of information coming from a single analysis are high expectations for instrument vendors. This can be assured by coupling separation techniques such as liquid or gas chromatography with mass spectrometric detectors. Such combination is widely used in almost all kinds of laboratories. High resolution power of chromatographs and a variety of detection and determination modes used in detector make coupled techniques the most advanced and fastest developing.

In the presentation newest instruments from SHIMADZU in terms of gas chromatography – tandem mass spectrometry (GC-MS/MS) and liquid chromatography – tandem mass spectrometry (LC-MS/MS) will be shown. Properly equipped instruments can deliver lots of information from the sample from the qualitative and quantitative point of view. A single instrument can work in different modes after few clicks in software. By this Shimadzu mean injection techniques for all sample kinds and more than one detector in one instrument. Powerful databases assure fast identification, and special wizards help in developing quantitative methods. The complexity of the instruments is made simple by one LabSolution software platform for all chromatography modes.

SESSION V

FRIDAY, JUNE 13th, 2014

CHAIRPERSONS:

Vovk and Christian Zidorn

17.

Determination of phenolic acids composition in *Lemna minor* L. by LC-ESI-MS/MS

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INTRODUCTION. Natural products are cheap and claimed to be safe. They are also suitable raw material for production of new synthetic agents. Many of them are renowned for their numerous medicinal uses. Creation of effective pharmaceuticals is one of the priorities of the Ukrainian and Polish scientific centers, industry and healthcare institutions. Drugs of natural origin have a particular application in prevention and therapy of several diseases, e.g. atherosclerosis and cancers. Searching for plants containing biologically active substances for the production of new drugs could be facilitated by application of scientific approaches. In this respect, identification of plant raw materials with pharmacologically important constituents is a promising direction. Duckweed (*Lemna minor* L.) is a small, free floating aquatic plant belonging to Lemnaceae family. According to literature data *L. minor* is use in folk medicine for the treatment of dyspepsia, rheumatism, leucodermy, gout, diabetes, vitiligo, as well as cancer. The phytochemical composition of duckweed is rich in a wide range of compounds. Amongst the biologically active ingredients, a number of phenolic constituents, with the prominent presence of phenolic acids can be found. Therefore, the aim of this study was to investigate the phenolic acid content in *L. minor* using LC-MS technique.

MATERIAL AND METHODS. *L. minor* L. (duckweed) plants were collected during July-August 2012-2013 in Vereschytsia River near Yavorivski National Park (Western Ukraine). The plant material was shade-dried at room temperature. The extraction was performed using 80% ethanol and accelerated solvent extraction system. Extract was then lyophilized. Phenolic acids content was determined by reversed-phase high-performance liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS/MS). For this purpose an Agilent 1200 Series HPLC system connected to AB Sciex 3200 QTRAP mass spectrometer was used. Chromatographic separations were carried out at 25°C, on a Zorbax SB-C18 column (2.1 x 50 mm, 1.8-μm particle size; Agilent).

RESULTS. As a result, eight phenolic acids in *L. minor* extract were identified. The most important constituents were ferulic, protocatechuic, sinapic, rosmarinic, 4-OH-benzoic, caffeic, vanilic, p-coumaric. Moreover, trace amounts of gentisic acid were found.

CONCLUSION. This study provides the first report on phenolic acids content in *Lemna minor* L. Many of the revealed compounds possess multidirectional biological activity, e.g. caffeic acid derivatives such as rosmarinic acid have a therapeutic potential in treatment or prevention of bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischaemic heart disease, cataract, cancer and poor sperm motility; protocatechuic acid has been reported to induce apoptosis of human leukemia cells, as well as malignant HSG1 cells taken from human oral cavities. Therefore, duckweed with its constituents is the potential source of new drugs and demonstration of the presence of

biological active compounds in the plant material may contribute to the support of its use in traditional medicine.

18.

Investigation of Cholic Acid Derivatives as Transdermal Permeation Enhancers

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The development in the field of pharmaceutical dosage forms results in discovery of additional highly sophisticated drug delivery systems that allow maintaining constant level of active substance in organism. Transdermal administration of drugs represents an excellent alternative to conventional pharmaceutical dosage forms. However, transdermal drug delivery often faces the problem of insufficient or no permeation of active pharmaceutical substances through the skin.

To solve this critical issue various approaches for overcoming the skin barrier were developed. These approaches can be classified as chemical (modification of drugs, using transdermal chemical permeation enhancers) or physical (modification of drug particles size to nanosize, physical enhancement techniques). Transdermal permeation enhancers are special pharmaceutical excipients that interact with skin components to increase permeation of drugs to blood circulation after topical application. Numerous compounds of different chemical structures were evaluated as permeation enhancers and several possible mechanisms of action of enhancers have been hypothesized, but exact mechanisms have not been elucidated.

The lecture deals with evaluation of permeation of the model drug theophylline through the full-thickness pig ear skin (*Sus scrofa* f. *domestica*) and the effect of potential permeation enhancers. The enhancement activity of 39 acyloxy derivatives of 5β-cholan-24-oic acid from propyleneglycol/water (1:1) donor vehicles was studied using static Franz diffusion cells. The amount of permeated theophylline was determined using an Agilent 1200 series HPLC system, equipped with a DAD detector. A Waters Symmetry C8 chromatographic column was used. The retention time of theophylline was approx. 5 min. The relationships between lipophilicity, solubility, polar surface area, molar volume and enhancement effect are discussed in this contribution.

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19.

Model studies on the antioxidant activity of common terpenoid constituents of essential oils by means of the 2,2-diphenyl-1-picryhydrazyl method

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Oxygen-breathing organisms, including humans, are prone to deleterious effects of elevated amount of free radicals, i.e. reactive oxygen species (ROS). During evolution organisms have developed different enzymatic and non-enzymatic systems for the safe dissipation of ROS. Secondary plant metabolites may be helpful in preventing the development of diseases caused by oxidative stress. Essential oils have been found to possess free radical scavenging activity, in some cases comparable to the activity of plant polyphenols, commonly recognized as potent antioxidants. Structure-activity relationships have been recognized well for polyphenols' direct antioxidant properties. The amount and position of aryl hydroxyl groups determinate the activity of plant polyphenols. Essential oils are rich in monoterpenes, which do not possess aryl hydroxyl groups, yet exert free radical scavenging activity. The aim of this research was to indicate structural elements responsible for radical scavenging activity of monoterpenes towards nitrogen radical: $DPPH^{\cdot}$. It was shown for the first time, that conjugated double bonds are responsible for monoterpenes' antioxidant activity. It was proved that blocking the double bonds, by the formation of complexes with silver (I) ions, results in losing free radical scavenging activity by monoterpenes. The reaction between monoterpenes, with conjugated double bonds, and $DPPH^{\cdot}$ results in formation of resonance-stabilized structures. The stabilization is associated with the charge delocalization over the whole molecule, possible due to the presence of conjugation of π bonds. Probable reaction mechanism between monoterpenes and $DPPH^{\cdot}$ was proposed. Moreover it was shown that the activity of monoterpoenes strongly depends on the polarity of solvent used in the study. Some monoterpenes were also found to be as potent as thymol or eugenol, which are usually seen as responsible for potent free radical scavenging activity of some essential oils.