INSTITUTE OF CHEMISTRY, UNIVERSITY OF SILESIA,
KATOWICE, POLAND

THE XXXVth SYMPOSIUM

CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS

MAY 30th – JUNE 1th, 2012

KATOWICE – SZCZYRK

POLAND

PROGRAM

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SESSION I WEDNESDAY, MAY 30 th, 2012

CHAIRPERSONS: D. Agbaba and B. Chankvetadze

9.25 – 9.30 am OPENING ADDRESS

9.30 – 10.00 am

1. QSAR, QSPR and QSRR in terms of 3-D-MoRSE descriptors for *in silico* screening of clofibric acid analogues

R. Kaliszan

10.00 – 10.30 am

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

J. Silberring

10.30 -11.00 am

3. The liquid chromatographic guide to the galaxy of organic impurities

A. Malenović

11.00 – 11.30 am

4. Novel Separation and Identification Techniques

J. Podgórski

1.00 pm LUNCH

SESSION II WEDNESDAY, MAY 30 th, 2012

CHAIRPERSONS: R. Kaliszan and Ž. Tešić

2.00 – 2.30 pm

5. Advances in investigation of hydrophilic and lipophilic properties of drugs

J. Jampilek

2.30 – 3.00 pm

6. Inverse Gas Chromatographic examination of potential fillers in abrasive industry

A. Voelkel

3.00 – 3.30 pm

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

Ł. Komsta

3.30 – 4.00 pm

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

J. Kołodziejek

POSTER SESSION I WEDNESDAY, MAY 30 th, 2012

CHAIRPERSONS: Ł. Komsta and J. Jampilek

4.00 – 5.30 pm (COFFEE BREAK)

6.00 pm BONFIRE

SESSION III THURSDAY, MAY 31 th, 2012

CHAIRPERSONS I. Vovk and H. Paelinck

9.30 – 10.00 am

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

B. Chankvetadze

10.00 – 10.30 am

10. Separation strategies in HPLC for the enantiomeric separation of pharmaceuticals on polysaccharide-based chiral stationary phases

A. A. Younes

10.30 – 11.00 am

11. Update of a generic chiral screening step on polysaccharide-based chiral stationary phases in supercritical fluid chromatography

D. Mangelings

11.00 – 11.30 am

12. Oligopeptidization oscillations of binary amino acid mixtures in solution

T. Kowalska

1.00 pm LUNCH

SESSION IV THURSDAY, MAY 31 th, 2012

CHAIRPERSONS: D. Milijković-Opsenica and Y. Vander Heyden

2.00 - 2.30 pm

13. Chromatography of the major dietary carotenoids

I. Vovk

2.30 – 3.00 pm

14. TLC in biochemical and pharmaceutical research

M. Waksmundzka-Hajnos

3.00 – 3.30 pm

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

Ł. Cieśla

3.30 - 4.00 pm

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

F. Andrić

POSTER SESSION II THURSDAY, MAY 31 th, 2012

CHAIRPERSONS: A. Malenović and Ł. Cieśla

4.00 – 6.00 pm (COFFEE BREAK)

6.00 pm DINNER

SESSION V FRIDAY, JUNE 1 th, 2012

CHAIRPERSONS: M. Waksmundzka-Hajnos and A. Voelkel

10.00 – 10.30 am

17. HPLC analysis of pyridinium aldoximes

H. Kalasz

10.30 – 11.00 am

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

M. Wyszomirski

11.00 – 11.30 am

19. UHPLC as a tool for purity analysis and quality control of environmental samples and polycyclic aromatic hydrocarbons

A. S. Swinarew

11.30 am CLOSING REMARKS

12.00 am LUNCH

POSTER SESSION I

1.

Determination of the critical factors in chiral supercritical fluid chromatography by use of a Plackett-Burman design

K. De Klerck, D. Mangelings, Y. Vander Heyden

2.

Fused-core stationary phases for fingerprint development of Phyllanthus and Mallotus species

G. Parewyck, Ch. Tistaert, B. Dejaegher, D. Mangelings, Y. Vander Heyden

3.

Pharmacophore-based database mining for probing fragmental drug-likeness of diketo acid analogues

A. Bąk, K. Jarzembek, V. Kozik, T. Magdziarz, J. Polanski

4.

Thin layer chromatography data in QSAR study of compounds with affinity for serotonin receptors

G. Żydek, E. Brzezińska

5.

HPLC analysis of aripiprazole and its impurities

N. Djordjevic Filijovic, B. Maricic, K. Nikolic, D. Agbaba

6.

Terpenes as biologically active constituents of medicinal plants

W. Jesionek, I. Choma, B. Majer-Dziedzic, E. Fornal

7.

A GC/MS and TLC study of the volatile fraction contained in creeping thyme (*Thymus serpyllum* L.) and common thyme (*Thymus vulgaris* L.)

M. Sajewicz, J. Rzepa, D. Staszek, K. Klauza, A. Waligóra, M. Waksmundzka-Hajnos, T. Kowalska

8.

A GC/MS and TLC study of the volatile fraction contained in rosemary (*Rosmarinus officinalis* L.), narrow-leaved lavender (*Lavandula angustifolia*), anise (*Pimpinella anisum* L.), and the fruit of clove tree (*Eugenia caryophyllata* Thunb.)

J. Rzepa, M. Sajewicz, D. Staszek, K. Dolibog, P. Marzec, M. Waksmundzka-Hajnos, T. Kowalska

9.

A TLC, HPLC/DAD, and HPLC/ELSD study of the phenolics contained in two sage species (*Salvia triloba* and *Salvia staminea*), and in two thyme species (*Thymus serpyllum* L. and *Thymus vulgaris* L.)

M. Sajewicz, D. Staszek, M. Cieślik, A. Kołaczkiewicz, M. Weloe, M. Waksmundzka-Hajnos, T. Kowalska

10.

A TLC, HPLC/DAD, and HPLC/ELSD study of the phenolics contained in two dragon's head (*Dracocephalum moldavica* L.) varieties

M. Sajewicz, D. Staszek, S. Kwiatkowski, A. Perek, M. Weloe, M. Waksmundzka-Hajnos, T. Kowalska

11.

Simultaneous multiple development HPTLC quantification of water- and oil soluble sunscreens

A.W. Sobańska, J. Pyzowski

12.

Quantification of sunscreen 2-phenylbenzimidazole-5-sulfonic acid in bathing water samples by TLC/densitometry with fluorescent detection

A.W. Sobańska, K. Derecka, J. Pyzowski

13.

Quantification of sunscreen benzophenone-4 in shampoo samples by Normal-Phase Thin Layer Chromatography/densitometry

A.W. Sobańska, K. Kałębasiak, J. Pyzowski

14.

Applications of gas chromatography to comparative study of steam co-gasification of hard coal and various energy crops focused on hydrogen-rich gas production

A. Smoliński, N. Howaniec

POSTER SESSION II

15 .

Complex-numbers representation of the retention in two-dimensional TLC

Ł. Komsta

16.

Assessment of β-lactams retention in hydrophilic interaction chromatography applying Box – Behnken design

M. Jovanović, T. Rakić, B. Jančić Stojanović, A. Malenović

17.

The study of azole antifungals retention behavior by experimetal design and artificial neural networks

A. Vemić, T. Rakić, N. Kostić, B. Jančić Stojanović, A. Malenović

18.

Determination of hyaluronidase activity by new HPCE method.

J. Matysiak, P. Dereziński, B. Urbaniak, A. Klupczyńska, Z.J. Kokot

19.

RPTLC determination of lipophilicity parameters of polydentate Schiff bases obtained from *o*-hydroxyaryl aldehydes and ketones with aromatic diamines

N. Stevanović, A.Blagus, A. Lolić, M. Natić, Ž .Tešić, R. Baošić

20.

Utilization of charge-transfer gas chromatography for analysis of formation water

P. Bielecki, D. Skraburska, W. Wasiak

21.

Rapid liquid chromatography-hybrid OrbiTrap mass spectrometry studies of polyphenols in Serbian honey

S. Kečkeš, U.M. Gašić, D.Č. Dabić, D.M. Milojković-Opsenica, M.M. Natić, Ž.Lj. Tešić

22.

Consequences of cadaverine and piperidine during the production of dry fermented sausages for the formation of *N*-nitrosopiperidine

E. De Mey, H. D. Maere, L. Dewulf, T. Kowalska, M-C. Peeters, G. Derdelinckx, H. Paelinck

23.

TLC and magneto–TLC as a method for investigation on selected d- and f-electron ion element complexes with organic ligands

A. Wronka, W. Ferenc, I. Malinowska

24.

2-Phenylpropionic acid as molecular rotor in thin-layer chromatography systems

M. Knaś, M. Sajewicz, T. Kowalska, J. Polański

25.

Polarimetric detection in high-performance liquid chromatography and its intrinsic weakness

M. Knaś, M. Sajewicz, J. Polański, T. Kowalska

26

Achiral HPLC/DAD and HPLC/ELSD applied to investigation of the oscillatory peptidization with *L*-Pro, *L*-Hyp, and *L*-Pro–*L*-Hyp

M. Sajewicz, M. Sławacka, G. Sztafińska, T. Kowalska

27.

Application of the chiral TLC to enantioseparation of *DL*-proline

M. Sajewicz, M. Matlengiewicz, M. Juziuk, M. Penkala, M. Weloe, T. Kowalska

28.

Better or faster? That is a question! Analysis of PAHs (polycyclic aromatic hydrocarbons) by UHPLC utilizing multiple detection methods

S. Golba, A.S. Swinarew, J. Gabor, Z. Grobelny, B. Swinarew, M. Szklarska

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

Roman Kaliszan‡, Paweł Wiczling,‡ Maurizio Di Tullio,† Cristina Maccallini,† Alessandra Ammazzalorso,† Letizia Giampietro,† Rosa Amoroso,† Barbara De Filippis,† Marialuigia Fantacuzzi,†

‡*Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gdańsk, Poland*

† *Department of Drug Sciences, University 'G. d'Annunzio', Chieti, Italy*

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

Marek Smoluch[1], Edward Reszke[2], Andrzej Ramsza[3], Grzegorz Schroeder[4], Jerzy Silberring[1,5]

[1]Department of Biochemistry and Neurobiology, Faculty of Materials Science and Ceramics, AGH-University of Science and Technology, Mickiewicza 30, 30-059 Krakow, Poland

[2]ERTEC-Poland, Rogowska 146/5, 54-440 Wrocław, Poland

[3]Institute of Applied Optics, Kamionkowska 18, 03-805 Warszawa, Poland

[4]Faculty of Chemistry, A. Mickiewicz University of Poznan, Grunwaldzka 6, 60-780 Poznan

[5]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Sowinskiego 5, 44-121 Gliwice, Poland

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

A. Malenović

University of Belgrade, Faculty of Pharmacy, Department of Drug Analysis, VojvodeStepe 450, 11000 Belgrade, Serbia

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

John Podgórski
Technical Sales Representative - GC/GCMS Specialist
"Shim-Pol A.M. Borzymowski"
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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

Josef Jampílek

Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic; e-mail: josef.jampilek@gmail.com

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

M. Kasperkowiak, B. Strzemiecka, A. Voelkel
Poznan University of Technology, Institute of Chemical Technology and Engineering, Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, Poland

Adam.Voelkel@put.poznan.pl

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

Ewelina Gowin and Łukasz Komsta

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Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

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

J. Kołodziejek, A. Voelkel

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Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, e-mail: joannakolo@op.pl

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

Bezhan Chankvetadze

Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, Tbilisi 0179, Georgia.

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

Ahmed A. Younes, Hasret Ates, Debby Mangelings, Yvan Vander Heyden

Department of Analytical Chemistry and Pharmaceutical Technology, Center for Pharmaceutical Research (CePhaR), Vrije Universiteit Brussel-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium

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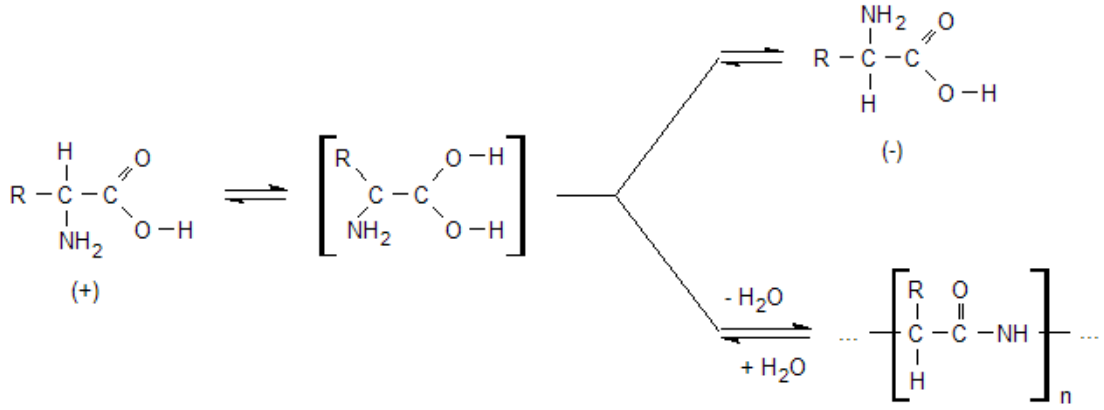
In our earlier studies [1-5], we discussed the ability of low molecular weight carboxylic acids (e.g., profen drugs, amino acids, and hydroxy acids) not only to undergo spontaneous oscillatory chiral inversion, but also spontaneous oscillatory oligopeptidization. In the case of amino acids, these two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

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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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2 - Department of Biochemistry, Institute of Soil Science and Plant Cultivation – State Research Institute, 8 Czartoryskich Street, 24-100 Puławy, Poland

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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2 - Department of Inorganic Chemistry, Medical University of Lublin, 4a Chodźki Street, 20-093 Lublin, Poland

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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[2]*Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*

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

Huba Kalász[1], Kornélia Tekes[1], Péter Szegi[1] and Kamil Musilek[2]

[1]Semmelweis University, Budapest, Hungary

[2]University of Defence, Hradec Kralove, Czech Republic

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

A.S. Swinarew[1], S. Golba[1], J. Gabor[1]
Z. Grobelny[1], B. Swinarew[2] and M. Szklarska[1]
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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.

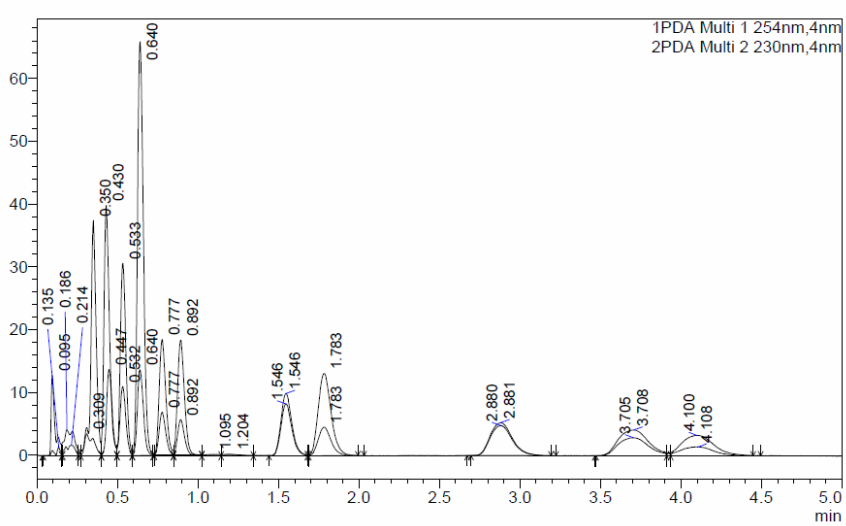

Fig. 1. Chromatogram of 15 + 1 mixture standard of PAHs. The analysed PAHs include naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr),1,2- benza[a]anthracene (BaA), chrysene (Chr), benzo[e]pyrene (BeP), benzo[e]acenaphthylene (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

POSTER SESSION I

WEDNESDAY, MAY 30 th, 2012

CHAIRPERSONS: Ł. Komsta and J. Jampilek

1.

DETERMINATION OF THE CRITICAL FACTORS IN CHIRAL SUPERCRITICAL FLUID CHROMATOGRAPHY BY USE OF A PLACKETT-BURMAN DESIGN.

Katrijn De Klerck, Debby Mangelings, Yvan Vander Heyden

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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, since high flow rates are possible, while it permits to increase the throughput capacity, without compromising the efficiency. “Green chemistry” properties are assigned to this technique, which is applicable, both at an analytical and a preparative scale. These properties in addition to the recent instrumental improvements renewed the interest in SFC.

To enable more efficient chiral method development, screening strategies are defined. These strategies start with a screening step in which the enantioselectivity of several complementary systems is evaluated. In this way a broad enantioselective range is covered. Results from previous work enabled to define a chiral screening step for SFC.

After execution of this screening step and selection of the most appropriate system, an optimization is required to achieve the desired separation. In order to define efficient optimization steps, critical influencing factors have to be identified.

In this work, a Plackett-Burman based, three-level screening design is used to determine the critical factors in chiral SFC separations. These results can then be used to complete a generic separation strategy.

2.

FUSED-CORE STATIONARY PHASES FOR FINGERPRINT DEVELOPMENT OF PHYLLANTHUS AND MALLOTUS SPECIES

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Department of Analytical Chemistry and Pharmaceutical Technology,Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Jette, Belgium.

Chromatographic fingerprint development is an accepted methodology for the identification and quality control of herbal medicinal products, which are popular to prevent or to treat diseases in many countries. The composition of herbs and their extracts is generally very complex and depends on several factors. Identification and quality control is therefore needed to avoid adverse effects as a result of adulteration or (accidental) exchange of plants. The World Health Organization (WHO) accepts chromatographic fingerprint analysis as a tool for the identification and quality control of herbal medicines because it reflects the composition of the total herbal sample or -extract. Fingerprints can also be used to model and predict given activities, e.g. antioxidant or cytotoxic activities, as a function of the fingerprints. These models can also be used to indicate in the fingerprints the peaks with potentially interesting activities. However, fingerprint development and analysis are time consuming (often 60 min runs) and the search for new approaches to speed up these processes is still ongoing. Fused-core stationary phases may present an alternative to reduce the analysis time of fingerprints without losing information. These recently introduced stationary phases consist of fused-core particles that are composed of a solid core surrounded by a porous layer. In combination with Ultra Fast Liquid Chromatography (UFLC), high resolutions, short analysis times and narrow peaks can be obtained compared to conventional stationary phases.
Fused-core in UFLC will be used to analyze different Mallotus and Phyllanthus species in order to model the antioxidant activity and to indicate interesting compounds. In a first part of this study, fingerprints will be developed for 51 Vietnamese samples. A relatively simple gradient program with different analysis times (60 min, 35 min, 22,5 min and shorter) will be tested, occasionally followed by a further optimization. Then the antioxidant activity of the samples will be determined with the Trolox Equivalent Antioxidant Capacity (TEAC) assay.
Data-analysis on the developed fingerprints will be performed in a second part of this study. In a first step, principal component analysis (PCA) will be performed to explore and reveal the data structure and to visualize occasional outliers. The antioxidant activity will be modeled using Partial Least Squares (PLS) and Orthogonal Projections to Latent Structures (O-PLS) regression methods. The obtained models for each dataset allow predicting the antioxidant activity for unknown samples and indicating the peaks in the chromatogram which potentially are responsible for the activity. All models will be compared to conclude whether the shortest fingerprints (22,5 min or shorter) still contain the same important information as the longer fingerprints (35 min and 60 min).

3.

Pharmacophore-Based Database Mining for Probing Fragmental Drug-Likeness of Diketo Acid Analogues

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The crucial objective of the computer-based techniques for the rational identification of the prospective drug candidates is the efficient and comprehensive mapping of the compound topology and/or geometry into the chemical property space. Despite the attention directed towards the development of the computational methods there is still a deficiency of the robust procedures for mining chemical space and designing molecular properties, especially in a fully user-defined mode. Furthermore, the understanding of the mutual relationships between a chemical structure, typically defined by an ensemble of the calculated descriptors, and the corresponding pharmacological activity (SAR) is still a fundamental issue in medicinal chemistry and molecular design.

While the amount of the accessible structural data has been increasing steadily it still exemplifies only a tiny fraction of all molecules in the 'infinite' chemical space (CS), representing approximately $10^{100} \div 10^{200}$ compounds. This makes a need for a proper design, filtration and/or enumeration of virtual structures, a problem often referred as 'molecular diversity'. The *in silico* transformation of the large number of compounds into corporate collections storing multidimensional data seemed to be an obvious solution. Hence, we developed the MoStBioDat environment for managing and analysing molecular and structural database information.

In our work we report the practical application of the system for the mapping of the fragmental drug-likeness topology (FDT) and the intramolecular hydrogen bonded (IHB) motifs in the diketo acid (DKA) related compounds. The DKA arrangement is a commonly observed structural feature found to be of crucial importance for HIV-1 integrase (IN) inhibitors.

A number of the structurally diverse chemical compounds with the functional DKA subunit(s) have been revealed by the combined *on line* and MoStBiodat 3D pharmacophore-guided ZINC and PubChem database screening. We used the structural data available from such screening to analyze the similarities of the compounds containing the DKA fragment. Generally, the analysis by PCA and SOM reveals four families of the compounds complying with the chemical constitution (aromatic, aliphatic) of the compounds. From practical point of view similar studies can reveal potential bioisosters of the known drugs, e.g., raltegravir/elvitegravir. In this context, it seems that *mono* halogenated aryl substructures with *para* group show the closest similarity to these compounds in contrast to the structures where aromatic ring is halogenated in both *orto*- and *para*-locations.

4.

Thin layer chromatography data in QSAR study of compounds with affinity for serotonin receptors

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A quantitative structure-activity relationship (QSAR) study has been made on 20 compounds with serotonin (5-HT) receptor affinity. A set of physicochemical parameters calculated by HyperChem 7.0 and ACDLabs 8.0 programs and chromatographic data were applied in the analysis. RP2 TLC 60F254 plates (silanized) impregnated with solutions of propionic acid, ethylbenzene, 4-ethylphenol, and propionamide (used as analogues of the key receptor amino acids) and their mixtures (denoted as S1–S7 biochromatographic models) were used in two developing phases as models of drug-5-HT receptor interaction. Correlation and multiple linear regression analysis were used to search for the best QSAR equations. The correlations obtained for the compounds studied represent their interactions with the proposed biochromatographic models. The good multivariate relationships (R^2=0.78–0.84) and leave-one/many-out cross validation procedures applied on final regression equations, demonstrated that these models have significant predictive ability (Q^2=0.57-0.70) and can be used for predicting the quantitative effect of biological activity of different compounds with 5-HT receptor affinity.

5.

HPLC analysis of aripiprazole and its impurities

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Abstract

Aripiprazole is a novel atypical antipsychotic drug used in the treatment of schizophrenia. This paper describes characterization of chromatographic behavior of aripiprazole and its nine related substances, which significantly differ in polarity. For this purpose, sensitive and reproducible method was developed and validated. The separation was performed on Phenomenex Luna® C18 column (5.0 µm particle size, 250 × 4.6 mm id) using a gradient with mobile phase A [phosphate buffer pH 3.0] and mobile phase B [acetonitrile] at the working temperature of 25°C. The buffer was 1.11g KH_2PO_4 with 1.2g sodium pentanesulfonate /L of the solution, adjusted to pH 3.0 with orthophosphoric acid. The flow rate was 1.0 mL/min. The detection was carried out at 215 nm using a diode array detector.

The proposed method is convenient and reliable for the purity control in both, raw materials and dosage forms.

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6.

TERPENES AS BIOLOGICALLY ACTIVE CONSTITUENTS OF MEDICINAL PLANTS

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[2] *Department of Veterinary Microbiology, University of Life Sciences, Lublin, Poland*
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The spread of bacterial resistance has become a major public health problem which is treated by many international organizations as a priority. Another expanding problem that affects society are free radicals which give negative influence on the processes occurring in the human body. Therefore, the increasing concern is given to an alternative medicine based on natural compounds with antimicrobial and antioxidant properties. New biologically active substances of plant origin should be less toxic and more effective than conventional ones. Plants such as: *Salvia officinalis, Thymus vulgaris* and *Menthae piperitae* are widely considered to be therapeutic. The most commonly isolated fraction from the plant are volatile terpenes [1].

Biological properties of terpenes contained in essential oils and tinctures of the above mentioned plants have been examined. In particular, the aim of our research was to establish the composition of the analyzed extracts by TLC and HPLC as well as to determine biological activity of their main components by TLC-DB (thin layer chromatography-direct bioautography) and DPPH methods [2]. In TLC-DB bacteria grow directly on a chromatogram containing separated substances. After staining with MTT dye solution, antibacterial activity is observed as white inhibition zones on purple background. The antimicrobial activity of the plant extracts against two strains of bacteria: *Escherichia coli* and *Bacillus subtilis* was investigated. The antioxidant activity of the essential oils and plant tinctures was assessed by their ability to scavenging 2,2-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The experiments pointed to both antibacterial and antioxidant activity of many components of tested extracts.

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2. Choma, I., Grzelak, E.M., Bioautography detection in thin-layer chromatography, J. Chromator. A, 1218 (2011).

7.

A GC/MS and TLC study of the volatile fraction contained in creeping thyme (*Thymus serpyllum* L.) and common thyme (*Thymus vulgaris* L.)

M. Sajewicz[1], J. Rzepa[1], D. Staszek[1], K. Klauza[1], A. Waligóra[1], M. Waksmundzka-Hajnos[2], T. Kowalska[1]

[1]*Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice, Poland*

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This study is a consecutive step in our research project devoted to fingerprinting of the volatile fraction contained in the selected sage (Salvia) species from the mint (Lamiaceae) family and presented in a series of papers (e.g., in [1-5]). In papers [1,2], we compared the performance of different techniques serving the purpose of deriving the volatile fraction prior to proper analysis by means of gas chromatography with mass spectrometric detection (GC/MS). The techniques compared were the "headspace" derivation and the two hydrodistillations carried out in the Deryng apparatus and the Clevenger apparatus. Moreover, we have performed "fingerprinting" of the volatile fraction contained in the different sage species by means of the low-temperature thin-layer chromatography (TLC) with densitometric and mass spectrometric detection, and the combined 2D technique, TLC – LC – MS [3-5].

In this study, an analogous comparison was performed for the volatile fractions derived from two other oily herbs belonging to the mint family, i.e., from creeping thyme (*Thymus serpyllum* L.) and common thyme (*Thymus vulgaris* L.). Herbal specimens originated from several different sources, i.e., from the Garden of Medicinal Plants, Faculty of Pharmacy, Medical University of Lublin and from the local market of culinary herbs (several different manufacturers). The following comparisons were performed: (i) of the volatile fraction yields depending on the different derivation techniques, prior to the "fingerprinting" by means of GC/MS, and (ii) of the "fingerprints" of the volatile fractions derived by means of different methods. Moreover, partial identification was performed of the volatile fraction components with aid of the virtual library of the mass spectra. Finally, "fingerprinting" of the volatile fraction was performed by means of the low-temperature thin-layer chromatography implemented with different detection modes. The obtained results were presented in the form of figures and tables, and upon this outcome, relevant conclusions were drawn.

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8.

A GC/MS and TLC study of the volatile fraction contained in rosemary (*Rosmarinus officinalis* L.), narrow-leaved lavender (*Lavandula angustifolia*), anise (*Pimpinella anisum* L.), and the fruit of clove tree (*Eugenia caryophyllata* Thunb.)

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[2]*Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University, Lublin, Poland*

This study is a consecutive step in our research project devoted to fingerprinting of the volatile fraction contained in the selected sage (Salvia) species from the mint (Lamiaceae) family and presented in a series of papers (e.g., in [1-5]). In papers [1,2], we compared the performance of different techniques serving the purpose of deriving the volatile fraction prior to proper analysis by means of gas chromatography with mass spectrometric detection (GC/MS). The techniques compared were the "headspace" derivation and the two hydrodistillations carried out in the Deryng apparatus and the Clevenger apparatus. Moreover, we have performed "fingerprinting" of the volatile fraction contained in the different sage species by means of the low-temperature thin-layer chromatography (TLC) with densitometric and mass spectrometric detection, and the combined 2D technique, TLC – LC – MS [3-5].

In this study, an analogous comparison was performed for the volatile fractions derived from two other oily herbs belonging to the mint family, i.e., for rosemary (*Rosmarinus officinalis* L.) and narrow-leaved lavender (*Lavandula angustifolia*), and besides, for anise (*Pimpinella anisum* L.) belonging to the Apiaceae family and for the fruit of clove tree (*Eugenia caryophyllata* Thunb.). Herbal specimens originated from several different sources, i.e., from the Garden of Medicinal Plants, Faculty of Pharmacy, Medical University of Lublin and from the local market of culinary herbs (several different manufacturers). The following comparisons were performed: (i) of the volatile fraction yields depending on the different derivation techniques, prior to the "fingerprinting" by means of GC/MS, and (ii) of the "fingerprints" of the volatile fractions derived by means of different methods. Moreover, partial identification was performed of the volatile fraction components with aid of the virtual library of the mass spectra. Finally, "fingerprinting" of the volatile fraction was performed by means of the low-temperature thin-layer chromatography implemented with different detection modes. The obtained results were presented in the form of figures and tables, and upon this outcome, relevant conclusions were drawn.

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9.

A TLC, HPLC/DAD, and HPLC/ELSD study of the phenolics contained in two sage species (*Salvia triloba* and *Salvia staminea*), and in two thyme species (*Thymus serpyllum* L. and *Thymus vulgaris* L.)

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This study is a consecutive step in our research project devoted to fingerprinting of the selectively extracted fractions of phenolic acids and flavonoids contained in the selected sage (Salvia) species from the mint (Lamiaceae) family and presented in a series of papers (e.g., in [1-7]). Respective analyses were carried out by means of liquid chromatography (basically, TLC, HPLC/DAD, and HPLC/ELSD). Due to the fact that the phenolics contained in the discussed herbs possess the well recognized anti-atherosclerotic activity as the free-radical scavengers, composition of the phenolics fraction and also quantification thereof in herbal extracts is of considerable importance for pharmacognosy. Herbs with particularly high levels of the phenolics can obtain the status of medicinal plants and an official entry in pharmacopoeia. So far, the only sage species recognized by Polish and European Pharmacopoeia is common sage (*Salvia officinalis* L.).

In this study, we compare chromatographic fingerprints for the selectively extracted fractions of phenolic acids and flavonoids derived from the four herbs belonging to the mint (Lamiaceae) family, i.e., Greek sage (*Salvia triloba*), *Salvia staminea*, creeping thyme (*Thymus serpyllum* L.), and common thyme (*Thymus vulgaris* L.). All herbal specimens originated from the Garden of Medicinal Plants, Faculty of Pharmacy, Medical University of Lublin and they are all widely recognized as culinary and decorative plants, and moreover, as medicinal herbs in various different geographical and cultural regions. Fingerprinting was performed with use of TLC, HPLC/DAD, and HPLC/ELSD. In this study, a comparison was made of (i) fingerprints valid for the phenolic acid and flavonoid fractions derived from the sage and the thyme species, and (ii) the informative value of fingerprints originating from the planar and column chromatographic techniques. On the basis of the collected experimental evidence, the relevant conclusions were drawn.

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10.

A TLC, HPLC/DAD, and HPLC/ELSD study of the phenolics contained in two dragon's head (*Dracocephalum moldavica* L.) varieties

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This study is a consecutive step in our research project devoted to fingerprinting of the selectively extracted fractions of phenolic acids and flavonoids contained in the selected sage (Salvia) species from the mint (Lamiaceae) family and presented in a series of papers (e.g., in [1-7]). Respective analyses were carried out by means of liquid chromatography (basically, TLC, HPLC/DAD, and HPLC/ELSD). Due to the fact that the phenolics contained in the discussed herbs possess the well recognized anti-atherosclerotic activity as the free-radical scavengers, composition of the phenolics fraction and also quantification thereof in herbal extracts is of considerable importance for pharmacognosy. Herbs with particularly high levels of the phenolics can obtain the status of medicinal plants and an official entry in pharmacopoeia. So far, the only sage species recognized by Polish and European Pharmacopoeia is common sage (*Salvia officinalis* L.).

In this study, we compare chromatographic fingerprints for the selectively extracted fractions of phenolic acids and flavonoids derived from the herb belonging to the mint (Lamiaceae) family, i.e., dragon's head (*Dracocephalum moldavica* L.), appearing in two varieties, with the white and blue flowers. The investigated herbal specimens originated from the Garden of Medicinal Plants, Faculty of Pharmacy, Medical University of Lublin. Dragon's head is mostly recognized as a medicinal and decorative plant, particularly rich in essential oil (composed mostly of geranyl acetate, geranial, and neral [8]). Fingerprinting was performed with use of TLC, HPLC/DAD, and HPLC/ELSD. In this study, a comparison was made of (i) fingerprints valid for the phenolic acid and flavonoid fractions derived from the two dragon head's varieties, and (ii) the informative value of fingerprints originating from the planar and column chromatographic techniques. On the basis of the collected experimental evidence, the relevant conclusions were drawn.

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11.

Simultaneous Multiple Development HPTLC Quantification of Water- and Oil Soluble Sunscreens

Anna W. Sobańska*, Jarosław Pyzowski

Abstract

A complex mixture of sunscreens of different lipophilicities was quantified for the first time by Thin Layer Chromatography followed by densitometric scanning in absorption mode. Multiple Development Normal Phase TLC was performed on silica gel 60 as stationary phase. Two mobile phases were used: A - cyclohexane-diethyl ether 5:1 (v/v) and B - ethyl acetate-ethanol-water 70:35:30 (v/v/v). After development with mobile phase A two oil soluble sunscreens: avobenzone (AVO) and octyl salicylate (OS) were analyzed at 360 and 300 nm, respectively. Subsequent development of the same plates with mobile phase B made it possible to quantify a water soluble sunscreen - phenylbenzimidazol sulfonic acid (PBS) at 300 nm. Calibration curves were non-linear. Limits of detection and quantification were: LOD (OS) 0.02 μg spot^{-1}, LOQ (OS) 0.06 μg spot^{-1}, LOD (AVO) 0.03 μg spot^{-1}, LOQ (AVO) 0.08 μg spot^{-1}, LOD (PBS) 0.02 μg spot^{-1}, LOQ (PBS) 0.06 μg spot^{-1}. The method was validated and applied to the analysis of a commercially available cosmetic product.

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12.

Quantification of sunscreen 2-phenylbenzimidazole-5-sulfonic acid in bathing water samples by TLC/densitometry with fluorescent detection

Anna W. Sobańska*, Karolina Derecka, Jarosław Pyzowski

Abstract

The water-soluble sunscreen 2-phenylbenzimidazole-5-sulfonic acid (PBS) was quantified in bathing water samples by Thin Layer Chromatography followed by densitometric scanning in fluorescent mode (cut-off filter 370 nm, analytical wavelength – 300 nm). Normal Phase TLC was performed on silica gel 60 as stationary phase. Mobile phase used was ethyl acetate-ethanol-water 70:35:30 (v/v/v). Limit of detection (LOD) was 0.0004 μg $spot^{-1}$ and limit of quantification (LOQ) – 0.001 μg $spot^{-1}$ without any sample pre-concentration. The method was validated.

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13.

Quantification of sunscreen benzophenone-4 in shampoo samples by Normal-Phase Thin Layer Chromatography/densitometry

Anna W. Sobańska*, Katarzyna Kałębasiak, Jarosław Pyzowski

Abstract

The water-soluble sunscreen benzophenone-4 (BZ-4) was quantified in shampoo samples by Thin Layer Chromatography followed by densitometric scanning in absorption mode (analytical wavelength – 285 nm). Normal Phase TLC was performed on silica gel 60 as stationary phase. Mobile phase used was ethyl acetate-ethanol-water-pH 6 phosphate buffer 72:35:30:5 (v/v/v/v). Limit of detection (LOD) was 0.03 μg $spot^{-1}$ and limit of quantification (LOQ) – 0.1 μg $spot^{-1}$. The calibration plot was non-linear. The method was applied to model shampoo samples prepared in the lab as well as to the samples of commercial products. The method was validated.

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14.

Application of gas chromatography in comparative study of steam co-gasification of hard coal and various energy crops focused on hydrogen-rich gas production

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About 87% of the renewable energy balance and over 49% of renewable electricity production in Poland in 2008 was based on solid biomass. Poland has also one of the largest in Europe area of land suitable for poplar, willow and Miscanthus cultivation (excluding forest land and land highly suitable for cereals). An increasing trend in the renewable resources share in the final energy use (9.13% in 2010 and 15.48% in 2020) as well as support given to the development of distributed energy systems and highly efficient technologies, like gasification is expected. Co-gasification of coal and energy crops is claimed to offer several advantages when compared to coal or energy crops gasification. Co-gasification gives the benefits of reliable supplies of abundant solid fuel – coal and credits resulting from utilization of renewable, zero-emission energy resource – biomass. It makes feasible energy crops gasification in larger scale and thereby with higher efficiency and with lower specific operating costs than in conventional biomass gasification plants of usually <50 MW_e.

The results of experimental comparative study on steam co-gasification of hard coal and energy crops, such as *Salix Viminalis*, *Spartina pectinata*, *Helianthus tuberosus L.*, *Sida hermaphrodita R.* and *Miscanthus X Giganteus* in a fixed bed reactor under atmospheric pressure and at the temperatures of 700, 800 and 900^{o}C are presented in the paper. The ability of coal and energy crops to undergo thermochemical transformations was determined based on their chars' reactivities. Moreover the wider view on the energy crops and hard coal chars reactivities in the process of steam co-gasification and their physical and chemical properties with a use of chemometrics methods such as the hierarchical clustering analysis and the principal component analysis is presented. The chemometric methods allow to extract the information on similarities/dissimilarities between tested samples and to identify the optimal one in terms of the highest hydrogen yield in the process of steam co-gasification with coal. Moreover, the synergy effect in the process of co-gasification consisting in an increase in the volume of hydrogen produced, when compared to the tests of coal and energy crops gasification, was investigated.

POSTER SESSION II

THURSDAY, MAY 31 th, 2012

CHAIRPERSONS: A. Malenović and Ł. Cieśla

15 .

Complex-numbers representation of the retention in two-dimensional TLC

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Main TLC coefficients such as spot position, R_F, k, R_M, ΔR_M and ΔR_F can be represented in 2D TLC as a single complex numbers, simplifying mathematical operations and their interpretation. When a spot position (x) is represented as a single complex number with one R_F as the real part and another as the imaginary part, the following generalizations can be done:

1. $\mathbf{R_F}$ can be then computed as $x/(1+1i)$, where $1+1i$ is complex position of the solvent front. The real part of R_F can be then interpreted as an average R_F value, where the imaginary part represents the difference between R_F values. The absolute value of it is the distance of the spot from starting point (origin). Module can be interpreted as the angle between diagonal and the trace of the spot.
2. ***k*** coefficient can be computed from complex R_F from default formula $(1-R_F)/R_F$. The real and imaginary parts are difficult to interpret, however absolute value is an average k value and the argument represents difference between the retention in two dimensions.
3. $\boldsymbol{R_M}$ coefficient is (as in the classic case) simply a decimal logarithm of complex k. Its real value is an average R_M and the imaginary value represents the difference.

Differences such as ΔR_M and ΔR_F have also meaningful interpretation when calculated in complex-way from the complex equivalents. The calculation can be also extended to some selectivity criteria, such as R_S coefficient.

When applying the linear regression to complex numbers with real and the same modifier concentrations, we obtain the complex extrapolated R_M with mean (averaged) R_M as the real part and extrapolated difference as the imaginary part. Analogous interpretation can be done in the case of slope. This can be useful for 2D TLC lipophilicity estimation with two modifiers of the same concentration.

16.

Assessment of β-lactams Retention in Hydrophilic Interaction Chromatography Applying Box – Behnken Design

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In this paper the retention prediction models for mixture of β-lactam antibiotics analyzed by hydrophilic interaction chromatography (HILIC) are presented. The aim of the study was to investigate the retention behavior of some organic acids including cefalosporines (cefotaxime, cefalexin, cefaclor, cefuroxime, cefuroxime axetil) and penicillines (ampicillin and amoxicillin). Retention of substances with acidic functional group in HILIC is considered to be interesting due to the lack of these investigations in literature. In the beginning of the study, classical silica columns were chosen for retention analysis. Than, preliminary study was carried out and factors with the most significant influence on retention factors were selected. The influence of these factors on retention factors were further investigated employing Box – Behnken design as a tool. On the basis of the obtained results the mathematical models were created and tested using ANOVA test and finally verified with four additional experiments. All the obtained models were adequate except of cefuroxime axetil, which showed non-retention behavior. This approach enables the presentation of chromatographic retention in many ways (three-D graphs and simple two dimensional graphical presentations). All of these gave the possibility to evaluate the impact of each factor and factor interaction on retention behavior and to predict the chromatographic retention under different conditions. The concentration of acetonitrile has shown the greatest impact on the retention factor of the analyzed compounds (directly proportional). Buffer concentration (directly proportional) and pH of the water phase (inversely proportional) had a similar but significantly less impact on the retention factor of the compounds. Furthermore, regarding the structure of the analyzed compounds, the potential retention mechanisms in HILIC were suggested.

17.

THE STUDY OF AZOLE ANTIFUNGALS RETENTION BEHAVIOR BY EXPERIMETAL DESIGN AND ARTIFICIAL NEURAL NETWORKS

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The most efficient way to provide data valuable for the evaluation of the analytes' retention behavior in liquid chromatography is the employment of certain chemometrical tools. In this study of azole antifungals' retention behavior the experimental design without and in the combination with artificial neural networks (ANNs) was applied. The investigated mixture consisted of the antifungals bifonazole, fluconazole, ketoconazole, clotrimazole, econazole and miconazole. These substances were selected based on their similar chemical structure. The experiments were performed on the chromatographic system Finnigan Surveyor Thermo Scientific. The analytical column was SunFire C18, 100 mm x 3.0 mm, 3.5 μm particle size. Flow rate was 0.75 mL min^{-1} and detection wavelength 265 nm. From the preliminary studies methanol content, pH of the mobile phase and column temperature were selected as the factors important for the further evaluation, while the content of triethylamine was set at 1%. The plan of experiments was determined by central composite design (CCD) comprised of full factorial 2^3 design, star design ($\alpha = \pm 1.7$) and four replications in central point. As the system outputs, the retention factors of all six investigated substances were chosen. The adequate models were built and from the corresponding coefficients the methanol content and pH value of the water phase were distinguished as the most influential factors. In the next step, the pattern for the analyzed system behavior was created employing ANNs. The network with highly predictive ability was obtained by network optimization. The final topology of network was 3–8–6, 12 experiments were used in a training set while the back propagation algorithm was optimal for the network training. High ability of defined network to predict the retention of the investigated azoles was confirmed by correlations higher than 0.9912 for all the analytes. Both presented approaches enabled the adequate prediction of azoles' retention behavior, as well as the extraction of the information important for the better understanding of the analyzed system.

18.

Determination of hyaluronidase activity by new HPCE method.

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Hyaluronic acid (HA), one of the most highly investigated substances in today's medicine, is a linear polysaccharide formed of disaccharide units containing N-acetylglucosamine and glucoronic acid. HA is found throughout the human body e.g. in the skin, vitreous humor and cartilage. Moreover it is a critical glucosaminoglycan in synovial joints because it is a main component of the synovial fluid. HA is used as a diagnostic factor for many diseases such as: rheumatoid arthritis, cancerous tumors and liver diseases. It is, therefore, important to quantify HA in the biological fluids and to study the profile of enzymatic digestion of this compound by hyaluronidase. There are many sources of this enzyme (including hyaluronidase from Hymenoptera venoms) and all of hyaluronidases have the ability to digest HA.

The aim of the study was to develop new capillary electrophoresis method for determination of enzymatic activity of hyaluronidase. The procedure was based on mixing of a known quantity of hyaluronic acid and an aliquot of hyaluronidase solution, followed by obtaining HPCE profiles after a known period of incubation. The activity of hyaluronidase was determined using multiple regression analysis in which sizes of the peaks of the main degradation products were used.

Studies were performed using HPCE instrument (Agilent Technologies) equipped with capillary of total length 64,5 cm, effective length 56 cm and inside diameter 75 μm. Separation was performed in the phosphate buffer (pH 8,10) in the electric field of 20 kV. Detection was performed at 220 nm.

The following steps and parameters were taken into account for the validation of the method: precision, accuracy, linearity, repeatability, LOD and LOQ. All steps of validation proved that the developed method is suitable for its intended purpose.

The developed HPCE method is characterized by short time of analysis, low volume of injected sample, a small amount of buffers used and as a result - a low cost of analysis.

The study confirmed that using HPCE it is possible to evaluate hyaluronidase activity, manifested in the creation of degradation products of hyaluronic acid. The amount and size of the peaks are dependent on time and the concentration of hyaluronidase.

19.

RPTLC DETERMINATION OF LIPOPHILICITY PARAMETERS OF POLYDENTATE SCHIFF BASES OBTAINED FROM *O*-HYDROXYARYL ALDEHIDES AND KETONES WITH AROMATIC DIAMINES

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Lipophilicity is an important physicochemical property of bioactive compounds, because it is related to the tendency of molecule to be transported through biological membranes. The lipophilicity parameters of some polydentate Schiff bases obtained from *o*-hydroxyaryl aldehides and ketones with aromatic diamines (Table) have been determined by reverse-phase thin-layer chromatography on silica gel RP-18 plates (Merck, Darmstadt, Germany).

Name		Color
1,4–bis((2-hydroksybenzylden)amino)benzen	$C_{20}H_{16}N_2O_2$	orange
[1-(2 aminofenylimino)ethyl]–phenol	$C_{14}H_{14}N_2O$	yelow
N,*N*'-bis(2-hydroksy-1-naphthaldimin)-1,2-diaminobenzene	$C_{28}H_{20}N_2O_2$	black
1,4-bis(2-hydroksyphenyl-benzylideneamino)benzene	$C_{32}H_{24}N_2O_2$	red-brown
1,3-bis(2-hydroksy-1-naphthylmethylidenamino)benzene	$C_{28}H_{20}N_2O_2$	beige
1,4-bis(2-hydroksy-1-naphthylmethylidenamino)benzene	$C_{28}H_{20}N_2O_2$	brown
1,2-bis(2-hydroksy-3-metoksibenzylidenamino)benzene	$C_{22}H_{20}N_2O_4$	orange
1,3-bis(2-hydroksy-3-metoksibenzylideamino)benzene	$C_{22}H_{20}N_2O_4$	orange-red

The mobile phases were mixtures of methanol, acetonitrile and tetrahydrofurane (as organic modifier) with water. The concentration of organic modifier in the mobile phase ranged from 50 to 80% (v/v) in 5% increments. A linear relationship, with satisfactory correlation coefficient, was obtained between R_M values and the concentration of organic modifier in the mobile phase. Regular retention behaviour was observed, i.e. retention decreased regularly with increasing concentration of organic modifier in the mobile phase. R_M^o values were obtained by extrapolation to 100 % (v/v) water in the mobile phase. Lipophilicity, C_o, was calculated as the ratio of the intercept and slope values, for each binary system. The effect of structure on chromatographically obtained lipophilicity parameters, relation between these parameters and calculated ClogP as well as effect of mobile phase on retention behaviour of the investigated compounds were discussed.

20.

UTILIZATION OF CHARGE-TRANSFER GAS CHROMATOGRAPHY FOR ANALYSIS OF FORMATION WATER

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Keywords: charge-transfer chromatography, SPME, formation water

The implementation of new law on mining wastes, forces the changes in the rules dealing with formation water, which is extracted together with oil and natural gas. A condition that must be met by the mining company before the re-congestion of formation water into the orogenic belt, is to prove the constancy of the chemical composition of this water during the mining process. Therefore, it is necessary to develop a rapid and effective method for the comparative evaluation of the formation water composition before and after the mining process. Since, the formation water includes, inter alia, petroleum hydrocarbons (eg. BTEXs, olefins), it seems reasonable to apply complexation gas chromatography to their separation. In order to reduce time-consuming sample preparation process, it was decided to utilize head-space solid-phase microextraction technique with usage of the fibre coated with PDMS. This technique enables direct sampling of the headspace phase.

In this study, chemically modified silica (particle diam.: 5 μm, pore size: 300 nm) with cyclam-$CuCl_2$ complex, was used as a stationary phase for gas chromatography. Modified silica was applied to prepare a capillary PLOT (porous-layer open tubular) column (30 m × 0.32 mm). Prepared column was utilised directly to conduct the chromatographic analyses. Earlier studies [1] have shown that the properties of such complexes are suitable for the separation of volatile olefin mixtures. This work focuses on PLOT capillary column preparation and its application to qualitative analysis of the formation water by the HS-SPME-GC technique.

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21.

Rapid liquid chromatography-hybrid OrbiTrap mass spectrometry studies of polyphenols in Serbian honey

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Polyphenolic profiles in honey samples collected from different regions of Serbia were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) coupled with hybrid mass spectrometer which combines Linear Trap Quadropole (LTQ) and OrbiTrap mass analyzer. Detection was performed in the atmospheric pressure negative heated electron spray ionization (API-HESI) mode. Honey samples were of different botanical origin: Acacia (*Robinia pseudoacacia*), Sunflower (*Helianthus annuus*), Linden (*Tilia cordata*), Basil (*Ocimum basilicum*), Buckwheat (*Fagopyrum esculentum*), Oilseed rape (*Brassica napus*), Goldenrod (*Solidago virgaurea*). The existence of numerous metabolic markers, mainly flavonoids in all honey samples was proven based on their characteristic mass spectra and fragmentation pattern. Principal component analysis (PCA), as useful multivariate statistical technique for data evaluation, was utilized to select and establish floral markers of the botanical origin of Serbian honeys.

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22.

CONSEQUENCES OF CADAVERINE AND PIPERIDINE DURING THE PRODUCTION OF DRY FERMENTED SAUSAGES FOR THE FORMATION OF *N*-NITROSOPIPERIDINE

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The formation of the carcinogenic *N*-nitrosamines (NAs) in cured meat products is in general associated to the presence of biogenic amines in combination with the use of sodium nitrite. Cadaverine, a possible precursor of the NA *N*-nitrosopiperidine (NPIP), can accumulate in the sausages during the production process. Thereafter, piperidine, the direct precursors of NPIP, can be formed by the deamination and cyclisation of cadaverine. Although it is clear that during an intense heating of cured meat products, cadaverine and piperidine can be nitrosated [1], the exact conditions for NPIP formation in dry fermented sausage remains unclear. The aim of the study is to investigate the processing parameters, i.e. pH-decline and concentration $NaNO_2$,hich can influence the formation of NPIP in the presence of cadaverine or piperidine in a dry fermented sausage model.

In the global data set, three subsets were considered: (1) blank dry fermented sausage samples, and samples fortified with (2) 500 $mg.kg^{-1}$ cadaverine, or (3) 10 $mg.kg^{-1}$ piperidine. Within each subset, two factors, i.e. concentration of $NaNO_2$ (0 or 150 $mg.kg^{-1}$) and pH-decline (to 4.8 or 5.5) were varied. During the production, samples were taken from the sausages prepared according the different recipes. Volatile *N*-nitrosamines were analyzed by gas chromatography coupled to a Thermal Energy Analyzer (GC-TEA) [2]. Biogenic amines were dabsylated and the derivatives were quantified by RP-HPLC-UV [3]. The results were statistically analyzed by means of ANOVA (PASW Statistics 18.0.0, SPSS Inc., Chicago, USA)

During the processing, the amount of cadaverine and piperidine increased in the blank samples, but no NPIP formation could be detected (< LOD=0.5 $\mu g.kg^{-1}$). When an excess of cadaverine (500 $mg.kg^{-1}$) was added, a slightly higher amount of piperidine was measured in the end product, but no *N*-nitrosamines were detected. Only in the models where 10 $mg.kg^{-1}$ of piperidine was added to the sausage dough, NPIP could be detected. but the concentrations remained under the limit of quantitation (< LOQ=1.5 $\mu g.kg^{-1}$). Alterations in pH and $NaNO_2$ gave no significant effect on the NPIP formation. The conclusion can be made that NPIP can be formed when an high concentration of piperidine is present but variation in pH or $NaNO_2$ had no influence. Finally, the addition of an excess of cadaverine could not provoke the formation of NPIP.

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23.

TLC AND MAGNETO–TLC AS A METHOD FOR INVESTIGATION ON SELECTED d- AND f-ELECTRON ION ELEMENT COMPLEXES WITH ORGANIC LIGANDS

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The chemistry of coordination compounds is an important and challenging area of modern inorganic and bio–inorganic chemistry. Variety of metallurgical processes, analytical reagents and industrial catalysts involve the use of coordination compounds. They also play important role in biological systems and medicinal chemistry. Among complexes d and 4f electron metals compounds are one of the most interesting group.

In recent decades, progress in this area was significant. Exploring their physical properties such as solubility, structural and magnetic properties. Complex compounds have been investigated using various methods, such as: IR, Raman spectroscopy and X-ray analysis. In the present work, Thin Layer Chromatography combined with the magnetic field is proposed to use as complement method for determination of properties of investigated complex compounds. The retention analysis of investigated compounds may give us some information about their affinity to different stationary phase surfaces and about the influence of the central ion or organic ligand structure on the retention of these compounds.

In present research, the retention of 10 d- and 12 of 4f-electron complexes metals with different organic ligands in RP and NP chromatographic systems was studied. Taking into account the fact, that in present times magnetic fields are different than terrestrial origin in many places, it is interesting to examine, how the magnetic field influences on the properties of investigated compounds. Therefore, the chromatograms were developed simultaneously in two identical chromatographic chambers and one of them was placed in external magnetic field with two values of vector of inductivity(0.2 and 0.4 T).

In magnetic field, retention of some complexes have been changed, what means, that magnetic field influences the properties of the analyzed compounds and their interactions with surface of the stationary phase.

Chromatographic investigations in RP systems can give us a preliminary information about biological activity of the compounds. In mentioned systems the log k_w parameter was determined for investigated compounds inside and outside of magnetic filed. Presence of magnetic field changed the log k_w values of investigated substances. This is information is very important, because some of the analyzed compounds may be in future applied in medicine and beauty care.

24.

2-PHENYLPROPIONIC ACID AS MOLECULAR ROTOR IN THIN-LAYER CHROMATOGRAPHY SYSTEMS

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In our studies we have focused on several classes of compounds, that can act as molecular propellers. Molecular and chiral rotors are molecules able to produce a variety of special effects, due to their ability for the specific rotational motion, however, these effects are not well recognized [1].
In this work we present that in the thin-layer chromatography (TLC) systems, the chiral rotors can deviate their migration route from the expected straight-line direction. Profen drugs investigating by means of TLC show lateral relocation of the analyte spots in planar chromatograms. The investigated chiral 2-phenylpropionic acid and other profens, while migrating with the solvent on the TLC plates, deviated from the straight-line route [2]. We have also showed an influence of molecular chirality of impregnates (L- and DL-arginine) on RF values and chromatographic spots' deviation. TLC systems used in these experiments were composed of the three different stationary phases. We have obtained the enantioseparation of the 2-phenylpropionic acid, selected to these studies.
Researches of chiral propellers are an important part of nanotechnology, where the application of molecular mechanisms, mimicking the real macroscopic objects, such as aircraft propellers or windmills, plays a key role. Technology based on the proposed theory of molecular propellers can be used for the profens' separation. The identification of specific properties of various chemical compounds, in this case of rotation, may allow for implementation of new nanotechnology solutions in the pharmaceutical industry. It can be used in the manufacture of drugs, development of new technology and the economy.

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25.

Polarimetric detection in high-performance liquid chromatography and its intrinsic weakness

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Polarimetric detection in HPLC might seem an of course matter, and especially in such areas as control of optical purity of drugs or fingerprinting of herbal extracts. However, relatively low popularity of polarimetric detection in HPLC is an admitted fact which certainly has the physically well founded reasons. Such reasons can be more profound than, e.g., an insufficient sensitivity of this type of detectors, when compared with PDA or ELSD.

In this paper, upon an experimental example of *R*(-)-naproxen we discuss physical phenomena (i.e., gelation of organic solvents by small organic molecules, the effect of molecular rotors, and oscillatory interconversion of chiral analytes) which might obstruct quantification of profen drugs with use of HPLC with polarimetric detection. We believe, however, that the discussed (or analogous) phenomena are of a far more general nature, which in fact hamper a widespread application of the polarimetric detection in HPLC.

26

Achiral HPLC/DAD and HPLC/ELSD applied to investigation of the oscillatory peptidization with *L*-Pro, *L*-Hyp, and *L*-Pro–*L*-Hyp

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Non-linear reactions are relatively rarely encountered, to a large extent due to considerable analytical difficulties. If a non-linear reaction occurs in a colorless solution and moreover, if it is not accompanied by any spectacular heat or electric effect (like it happens, e.g., with redox reactions), then its discovery often is a matter of chance. The chromatographic techniques can be regarded as universal and effective tools serving the discovery and monitoring of the non-linear reactions.

Ivanov et al. [1,2] were the first ones to discover the spontaneous oscillatory polycondensation of the chiral silicon derivatives with aid of TLC. In our laboratory, we managed to demonstrate the spontaneous oscillatory chiral inversion of profen drugs and the other low-molecular-weight carboxylic acids with use of the chiral TLC [3,4], and the spontaneous oscillatory peptidization of certain single amino acids (e.g., phenylglycine, [5]) and the spontaneous oscillatory oligomerization of lactic acid [6] with aid of achiral HPLC/DAD and HPLC/ELSD.

It was the aim of this study to trace the spontaneous peptidization of the two biologically important amino acids, i.e., *L*-proline (*L*-Pro) and *L*-hydroxyproline (*L*-Hyp), largely responsible for the architecture of the mammalian and human muscles, and also peptidization thereof in a binary *L*-Pro–*L*-Hyp system. The investigations were carried out with the 70% aqueous solutions of these amino acids in MeOH with aid of the non-chiral HPLC/DAD and HPLC/ELSD. In the two cases of single amino acids, the non-linear concentration changes were observed both with *L*-Pro and *L*-Hyp, although they considerably differed in terms of dynamics. Pattern of the *L*-Pro and *L*-Hyp concentration changes in the binary *L*-Pro–*L*-Hyp system allows an assumption as to the formation of the *L*-Pro- and *L*-Hyp-derived homooligopeptides, and also of the mixed *L*-Pro–*L*-Hyp heterooligopeptides.

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27.

Application of the chiral TLC to enantioseparation of *DL*-proline

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Chromatographic enantioseparation of the racemic and scalemic mixtures is a difficult task, both analytically, and on a preparative and technological scale. So far, the majority of analytical enantioseparations have been carried out by means of column chromatography (HPLC and GC), with a far less pronounced contribution from the side of TLC. Such disparity cannot be fully excused though, because (i) separation performance of planar chromatographic techniques is just enough for direct enantioseparation of a single pair of compounds; (ii) direct enantioseparation by means of planar chromatography usually can be obtained with use of relatively simple stationary phases; and (iii) such enantioseparation is usually easier to obtain and moreover, it is far less expensive than by means of instrumental techniques. In monograph [1], an overview is presented of applicability of the chiral TLC technique to direct enantioseparation with the different compound classes. It is rather obvious that for practical reasons, the most important enantioseparations are those of therapeutically and biologically active enantiomers (e.g., drugs, amino acids, and hydroxy acids). Considerable contribution to the direct enantioseparation of amino acids by means of the chiral TLC was made by Bhushan et al. [2].

In the Department of General Chemistry and Chromatography, University of Silesia, methods of direct enantioseparation by means of the chiral TLC have been developed for a longer period now. Target compounds belonged to the three classes, i.e., profen drugs (e.g., [3]), hydroxy acids (e.g., [4]), and amino acids (e.g., [5]). One approach is the complexation TLC, based on impregnation of the commercial silica gel layer with the transition metal cations (e.g., Cu(II), Co(II), Ni(II), Mn(II), or Fe(II)). Enantioseparation is obtained due to the different chelating constants of a given cation with the two antimers of a given compound. So far, this particular approach was the most widely tested upon the example of the enantioseparation of *DL*-lactic acid [6,7].

In this study, we present the results of our efforts aiming to elaborate a simple yet efficient method of the enantioseparation of *DL*-proline by means of the complexation TLC, testing a selection of the transition metal cations. This approach is particularly important due to the fact that amino acids (proline included), when dissolved in various different (aqueous and non-aqueous) solvents, tend to spontaneously peptidize and in the column techniques, one has to be skillful enough to separate the monomeric amino acid antimerss from the oligopeptides (which can be regarded as an evident drawback). In TLC, similar problems with the interference of the oligopeptides can be encounterd.

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28.

BETTER OR FASTER? THAT IS A QUESTION!

ANALYSIS OF PAHS (POLYCYCLIC AROMATIC HYDROCARBONS) BY UHPLC UTILIZING MULTIPLE DETECTION METHODS

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

Polycyclic Aromatic Hydrocarbons (PAHs) are known to be carcinogenic compounds containing several benzene rings. Government agencies, including the European Union's Scientific Committee on Food, identified PAHs as critical pollutants harmful to human health and mandated exposure limits to them. The wide variety of matrices in which these compounds are found requires various detection levels. The method commonly utilized to analyze PAHs is GC-MS, where for GC column 30 m long with an inner diameter of 0.25 mm and 0.25 μm film thickness the analysis takes about 60 minutes. An alternative method is UHPLC utilizing fluorescence detection.

In this announcement we present a method for analysis of PAHs using the Nexera UHPLC equipped with a Pinnacle DB PAH 1.9 μm, 50 x 2.1 mm column and the multiple DAD and FLD detectors enabling detection of trace-level components. Resolution was investigated using a standard mixture of 16 PAH (1 to 20 $mg \cdot L^{-1}$, acetonitrile solution). The described method allows for both accurate and fast determination of 15 + 1 PAHs in less than 5 minutes.