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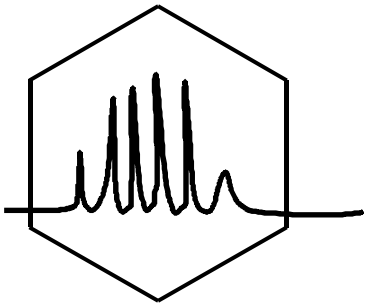

THE XXXIVth SYMPOSIUM

CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS

JUNE 8th – 10th, 2011

KATOWICE – SZCZYRK

POLAND

PROGRAM

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SESSION I WEDNESDAY, JUNE 8 th, 2011

CHAIRPERSONS: D. Agbaba and R. Kaliszan

9.25 – 9.30 am OPENING ADDRESS

9.30 – 10.00 am

1. New effects with new chiral stationary phases for liquid-phase enantioseparation techniques

B. Chankvetadze

10.00 – 10.30 am

2. Application of monolithic chromatography in drug discovery and development

Y. Vander Heyden

10.30 -11.00 am

3. The impact of stationary phase backbone on HILIC selectivity

W. Lindner, W. Bicker, G. Schuster

SESSION II WEDNESDAY, JUNE 8 th, 2011

CHAIRPERSONS: M. Waksmundzka-Hajnos and H. Kalasz

11.30 – 12.00 am

4. QSRR: Extrathermodynamic *vs.* thermodynamic modeling of chromatographic retention

R. Kaliszan

12.00 – 12.30 am

5. Analysis of the chromatographic column efficiency packed with the totally and superficially porous particles and their separation power

K. Kaczmarski

12.30 – 1.00 pm

6. Molecular rotors in thin-layer chromatography

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

1.30 pm LUNCH

POSTER SESSION I WEDNESDAY, JUNE 8 th, 2011

CHAIRPERSONS: T. Kowalska and Ž. Tešić

3.00 – 5.00 pm (COFFEE BREAK)

6.00 pm BONFIRE

SESSION III THURSDAY, JUNE 9 th, 2011

CHAIRPERSONS: W. Lindner and A. Voelkel

9.30 – 10.00 am

7. Thin-layer chromatography with biodetection to screen plant extracts for the presence of potential drugs

M. Waksmundzka-Hajnos, Ł. Cieśla

10.00 – 10.30 am

8. Investigation of vegetable triterpenoids and phytosterols by chromatography and mass spectrometry

I. Vovk, M. Martelanc, B. Simonovska

10.30 – 11.00 am

9. Hyphenated HPTLC for fast analysis of bee's products

E. S. Chernetsova, G. E. Morlock

SESSION IV THURSDAY, JUNE 9 th, 2011

CHAIRPERSONS: B. Chankvetadze and Y. Vander Heyden

11.30 -12.00 am

10. Update of generic chiral separation strategies for pharmaceutical compounds using chromatographic and electrophoretic techniques

D. Mangelings, H. Ates, K. De Klerck, A. Hendrickx, A. Younes, Y. Vander Heyden

12.00 -0.30 pm

11. HPLC monitoring of blood-brain-barrier penetration of certain polar drugs

H. Kalász, K. Tekes, P. Szegi, K. Kuca

0.30 – 1.00 pm

12. Tracking of the food-drug interactions including alcohol-drug interaction with different bioanalytical methods

I. Klebovich

1.30 pm LUNCH

POSTER SESSION II THURSDAY, JUNE 9 th, 2011

CHAIRPERSONS: D. Mangelings and Ł. Komsta

3.00 – 5.00 pm (COFFEE BREAK)

6.00 pm DINNER

SESSION V FRIDAY, JUNE 10 th, 2011

CHAIRPERSONS: I. Vovk and K. Kaczmarski

10.00 – 10.30 am

13. Disadvantages of the current methods of selectivity evaluation in TLC analysis

M. Kobyłka, Ł. Komsta

10.30 – 11.00 am

14. A validated reversed phase hplc method for simultaneous determination of aspirin and clopidogrel and their related substances in combined dosage forms

G. Kahsay, A. Van Schepdael, E. Adams

11.00 – 11.30 am

15. TLC-DPPH test revisited

Ł. Cieśla, J. Kryszeń, A. Stochmal, M. Waksmundzka-Hajnos

11.30 am CLOSING REMARKS

11.30 -12.00 am COFFEE BREAK

POSTER SESSION I

1.

Mapping fragmental drug-likeness in the MoStBioDat environment: Intramolecular hydrogen bonding motifs in β-ketoenols

A. Bak, T. Magdziarz, A. Kurczyk, J. Polański

2.

Chemometric models for efficient predictions of chromatographic behavior of ziprasidone and its impurities

M. Pavlovic, K. Nikolic, A. Smoliński, D. Agbaba

3.

Knowledge discovery in molecular and structural chemical databases

A. Kurczyk, A. Bąk, T. Magdziarz, J. Polański

4.

The influence of heat effect on the peak profiles in overload conditions

J. Kostka, W. Zapała, K. Kaczmarski

5.

Chiral separation in normal-phase liquid chromatography: Enantioselectivity of recent polysaccharide-based selectors. Part II. Enantioselectivity at optimization conditions

A. A. Younes, D. Mangelings, Y. Vander Heyden

6.

Evaluation of reduced test sets for the development of separation strategies for chiral drug compounds

H. Ates, B. Desmedt, Y. Vander Heyden

7.

Updating a screening strategy for chiral separations in supercritical fluid chromatography with new chlorinated polysaccharide-based selectors

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

8.

The influence of sample matrix composition and injected sample volume on the electrophoretic behaviour of small non-enveloped viruses

I. Oita, H. Halewyck, B. Thys, B. Rombaut, Y. Vander Heyden

9.

Inverse gas chromatography in the examination of modern organic-inorganic hybrid materials

J. Kołodziejek, Ż. Olszak, A. Voelkel

10.

The HPLC study of aqueous and non-aqueous solutions of phenylalanine and phenylglycine

M. Sajewicz, M. Gontarska, N. Kuśmierz, M. Możdżeń, A. Prudnik, T. Kowalska

11.

Quality control of *citri reticulatae pericarpium*: exploratory analysis and discrimination

Ch. Tistaert, A. Szandrach, L. Thierry, B. Dejaegher, Y. Vander Heyden

12.

Study of condensation oscillations with *L*-lactic acid in pure acetonitrile and aqueous ethanol

M. Sajewicz, D. Kronenbach, M. Gontarska, T. Kowalska

13.

The HPLC and optical tracing of the molecular level inhomogeneity with the aqueous ethanol solution of *S*(+)-naproxen

M. Sajewicz, M. Gontarska, A. Rudzka, M. Sosenko, T. Kowalska

POSTER SESSION II

14.

Stability study of acetylsalicylic acid in solutions by the use of UHPLC/ESI-Q-TOF method

R. Skibiński, Ł. Komsta

15.

Photostability Study of Alopres® tablets

V. Marinkovic, I. Savic, Ivan Savic, K. Nikolic, P. Sibinovic, D. Agbaba

16.

Solid phase extraction for the determination of biogenic amines in dry fermented sausages

E. De Mey, G. Drabik-Markiewicz, M-C. Peeters, G. Derdelinckx, H. Paelinck, T. Kowalska

17.

Determination of the soil-water, octanol-water, and air-water partition coefficients for the twelve benzodiazepines by the means of the reversed-phase thin-layer chromatography

F. Andrić, P. Ristivojević, J. Trifković, Ž. Tešić, D. M. Milojković-Opsenica

18.

Degradation of C.I. Reactive Black 5 using water falling film dielectric barrier discharge. An investigation of carboxylic intermediates by IC

M. Natić, B. Dojčinović, T. Tosti, D. Milojković-Opsenica, Ž Tešić, D. Manojlović, G. Roglić

19.

Revisiting thin layer chromatography as a lipophilicity determination tool. Part II. Is silica gel a reliable adsorbent for lipophilicity investigation?

E. Gowin, Ł. Komsta

20.

Densitometric RP TLC-DPPH method for quantitative evaluation of free radical scavenging activity of N,N`-bis(acetylacetone)ethylenediimine and corresponding copper(II) complex

N. Stevanović, D. Perušković, M. Aleksić, M. Natić, Ž. Tešić, R. Baošić

21.

Application of gas chromatography in experiments on steam gasification and co-gasification of coal and biomass

N. Howaniec, A. Smoliński

22.

Application of gas chromatography in experiments of underground lignite gasification to hydrogen-rich gas

A. Smoliński, K. Stańczyk

23.

The HPLC analysis of the selected extract fractions derived from a variety of the sage (*Salvia*) species

M. Sajewicz, D. Staszek, E. Recmanik, K. Sobczyk, M. Waksmundzka-Hajnos, T. Kowalska

24.

Thin-layer chromatography with micellar mobile phase of aromatic biogenic amines in magnetic field

M. Studziński, K. Stępnik, I. Malinowska

25.

Comparison of GC/MS, GC/ECD and HPLC/DAD techniques in determination of PBDEs in water samples.

I. Fulara, J. Wypych, M. Witoszek, A. Swinarew

26.

Dehydratation and analyssis of 18-Crown-6 used for anionic polymerization of propylene oxide

A.S. Swinarew, B. Nowakowski, M. Szklarska, B. Swinarew, J. Gabor

A. Stolarzewicz, I. Fulara, J. Jurek

27.

New CZE-DAD and LC-DAD methods for honeybee venom analysis and standardization of the product.

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

28.

Using gas chromatography to control the functioning of polytrioxane production installation

A. Łodyga, P. Tyński, M. Kozioł, D. Minda-Data, Z. Majerczyk

29.

Application of GC analysis in the control of trioxane synthesis using ion-exchange resins as catalysts

A. Łodyga, P. Tyński, D. Minda-Data, M. Kozioł, Z. Majerczyk

30.

Determination of volatile components and phenolic acids in *Satureja montana* by GC/MS

J. Rzepa, T. Baj, P. Gorczyca, M. Włodarek, K. Głowniak, M. Waksmundzka-Hajnos, T. Kowalska

SESSION I WEDNESDAY, JUNE 8 th, 2011

CHAIRPERSONS: D. Agbaba and R. Kaliszan

SESSION II WEDNESDAY, JUNE 8 th, 2011

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SESSION III THURSDAY, JUNE 9 th, 2011

CHAIRPERSONS: W. Lindner and A. Voelkel

SESSION IV THURSDAY, JUNE 9 th, 2011

CHAIRPERSONS: B. Chankvetadze and Y. Vander Heyden

SESSION V FRIDAY, JUNE 10 th, 2011

CHAIRPERSONS: I. Vovk and K. Kaczmarski

1.

New effects with new chiral stationary phases for liquid-phase enantioseparation techniques

B. Chankvetadze

Department of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Tbilisi 0179, Georgia

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In this presentation the results of our recent studies on development of novel chiral stationary phases (CSP) for enantioseparations using high-performance liquid chromatography (HPLC), capillary liquid chromatography (CLC) and capillary electrochromatography (CEC) are summarized. In the first part of the presentation the emphasis will be made on novel phenylcarbamate derivatives of cellulose and amylose as useful CSPs for analytical and preparative scale enantioseparations. These novel materials are applicable for HPLC enantioseparations in combination with normal phase-, polar organic mobile phase and reversed-phase eluents, as well as for SFC enantioseparations at higher pressure. In the second part of the presentation the screening results on 5 commercially available representatives of this series of chiral columns will be discussed with major emphasis on the complementary properties of various chiral selectors and mobile phases. The effect of fine tuning of the properties of these materials and separation conditions on resolution of enantiomers of various compounds will be discussed in detail using the examples of challenging separations. In the final part of the presentation newly observed reversal of the enantiomer elution order of some chiral drugs and amino acid derivatives by variation of separation temperature and composition of the mobile phase will be discussed [1].

[1] L. Chankvetadze, N. Ghibradze, M. Karchkhadze, L. Peng, T. Farkas, B. Chankvetadze, *J. Chromatogr. A*, submitted.

2.

Application of monolithic chromatography in drug discovery and development

Yvan Vander Heyden

Department of Analytical Chemistry and Pharmaceutical Technology (FABI), Center for Pharmaceutical Research (CePhaR), Free University Brussels (VUB), Laarbeeklaan 103, 1090 Brussels, Belgium

Monolithic columns have a continuous separation bed which is prepared by in situ polymerization inside the column tubing. The stationary phase itself has a sponge-like structure with numerous flow-through pores. Due to the presence of these pores, relatively low back pressures are obtained when these columns are operated at higher flow rates. Therefore, monoliths allow working at higher linear velocities compared to classical particle-packed columns. Hence, higher efficiencies can be achieved using longer or coupled columns.

The introduction of monolithic supports as stationary phases in liquid chromatography started around the beginning of the 1990's. At that time, polymer-based monoliths were the dominating type of monolithic stationary phases. The introduction of commercially available silica-based monoliths followed about ten years later. Since then, the applications of monolithic columns extended to different fields of separation science, among which drug development.

In this presentation, applications in drug development are discussed using monolithic supports with liquid chromatographic techniques, such as high-performance liquid chromatography, capillary liquid chromatography and capillary electrochromatography. A distinction is made regarding applications on polymeric monoliths on the one hand, and on inorganic monoliths on the other.

3.

The impact of stationary phase backbone on HILIC selectivity

W. Lindner, W. Bicker and G. Schuster

Institute of Analytical Chemistry, University of Vienna, Austria.

In recent years the research on and the application of HILIC (hydrophilic interaction chromatography) regained popularity as it provides methodologies to separate and analyze highly polar compounds which can hardly be retained on reversed phase LC columns. Generally speaking, the retention mechanisms of HILIC is dominated by partition of the analytes between a water rich (very polar) hydro-organic layer adsorbed on a polar sorbent surface and a (less polar) organic rich stagnant layer in the pores and thus of the mobile phase. However, in reality there are besides partition often also direct adsorption related increments responsible for retention of the analytes. These are mainly driven by electrostatic interactions encompassing ion pair formation, hydrogen bonding and polar interactions, respectively, depending on the chemical structure of the polar analytes and the polar surface of the "adsorbents". Most of the time plain silica or polar modified silica are used as "adsorbents". With other words, the observed HILIC retention mechanism refers inherently to mixed modal retention characteristics which are difficult to de-convolute into the individual parts responsible for the overall observed retention and selectivity parameters. Integral to this discussion will also be the contribution of the mobile phase characterized by the polar solvents, the buffer salts and the pH thus driving the composition of the water and buffer salt rich stationary phase layer.

Given this situation it becomes imperative that besides the mobile phase composition also via the covalently modified adsorbents the HILIC type selectivity will be tuneable. The polar stationary phase backbone can be of so-called neutral, acidic, basic or zwitterionic nature.

In this contribution we will describe and discuss novel silica based and polar modified HILIC type phases in terms of their unique selectivity pattern. These phases are overall of (i) neutral, (ii) weakly basic, or (iii) weakly acidic character. In addition they all will contain polar functional groups suitable for (multiple) hydrogen bonding. Based on a broad and systematically selected set of test analytes we will try to set up a protocol to characterize HILIC phases and to discuss retention models.

4.

QSRR: Extrathermodynamic *vs.* thermodynamic modeling of chromatographic retention

R. Kaliszan

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland

The retention prediction performance was tested of the well thermodynamically founded solvophobic theory of Csaba Horváth of the reversed-phase HPLC in comparison to the extrathermodynamic, statistically derived Quantitative Structure-Retention Relationships (QSRR). The model derived when observing the rules of classical thermodynamics appeared to be clearly interpretable in physicochemical terms but of limited retention prediction ability. An improvement was attained when applying an extrathermodynamically derived correction to that model based on thermodynamic hermeneutics. The simple QSRR model, relaying on chemical intuition and employing analyte structural descriptors from calculation chemistry, produced similar retention predictions as the combined thermodynamic/extrathermodynamic model. Both the thermodynamic and the QSRR models accounted well for abilities of analytes to participate in nonspecific, dispersive intermolecular interactions. Less reliable appeared descriptors of analyte polarity. The approach proposed can be further developed to search for appropriate descriptors of polarity which would allow a better prediction of physicochemical and/or biological properties of chemical compounds, and hence, would help to rationally design substances of requested quality.

5.

Analysis of the chromatographic column efficiency packed with the totally and superficially porous particles and their separation power

K. Kaczmarski

Department of Chemical and Process Engineering, Rzeszów University of Technology, 35-959, Rzeszów, Poland

In recent years the chromatographic columns technology was evolving in the direction of the use of sub-2μm adsorbent particles. The adsorbent particles can be totally porous or have solid central part and active outer layer (shell particles). The reduction of the size of adsorbent causes the decrease of mass transfer resistances and increases the column efficiency. Further reduction of mass transfer resistances is attained with introduction of superficially porous particles by reduction of diffusion path to active shell.

The performance of chromatographic columns, filled with different adsorbent, is conveniently compared on the basis of the values of their HETP. The theoretical equation of the HETP for columns packed with spherical particles, shell particles and for monolith column can be developed by application of the moment analysis to elution peaks, calculated with the General Rate model. This equation can be developed for linear adsorbtion isotherm or first order adsorption-desorption kinetic.

The more important measure of a chromatographic separation power is the resolution of the components. The resolution of the components can also be evaluated with the help of the moment analysis.

From analysis of HETP equations, for infinite adsorption-desorption kinetic, follows that the highest column efficiency can be obtained for shell thickness decreasing to zero. However, it does not mean that the maximum resolution of the components is also obtained for shell thickness decreasing to zero.

The aim of this work was to investigate the effect of the shell thickness on the column efficiency and the components resolution for infinite and finite adsorption-desorption rate. The optimal solid core radius was calculated to achieve minimum HETP or maximum resolution in different process conditions.

6.

Molecular rotors in thin layer chromatography

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

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

While investigating chiral profens by means of TLC, some of us have serendipitously discovered an effect of lateral relocation of the analyte spots in planar chromatograms (LR-TLC) [1-4]. The investigated profens migrating upward with the solvent on the vertical TLC plates, deviated from the expected straight-line route. The preferential left or right-handedness of the choice interactions with a TLC plate could not be understood on the basis of the known chromatographic theories. Here we will discuss a hypothesis that rotoring ability can explain the LR-TLC effect.

Molecular and chiral rotors (CRs) are molecules able to produce a variety of special effects, due to their ability for the specific rotational motion, although these effects are not well recognized. Although lateral relocations (LR) have been theoretically predicted for the fluxes of specifically rotating molecules [5], such phenomena have never before been observed in the experiments. We will discuss in this presentation how this model can explain the LR-TLC effect.

Literature:

[1] M. Sajewicz, G. Grygierczyk, M. Gontarska, T. Kowalska, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 2185.
[2] R. Bhushan, J. Martens, in: T. Kowalska, J. Sherma (Eds.), Thin Layer Chromatography in Chiral Separations and Analysis, Chromatographic Science Series Volume 98, CRC Press, Boca Raton, 2007, pp. 323.
[3] T. Kowalska, M. Sajewicz, in: T, Kowalska, J. Sherma (Eds.), Thin Layer Chromatography in Chiral Separations and Analysis, Chromatographic Science Series Volume 98, CRC Press, Boca Raton, 2007, pp. 231.
[4] M. Sajewicz, M. Gontarska, A. Dąbrowa, T. Kowalska, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 2369.
[5] Y. Pomeau, Phys. Lett. A 39 (1971) 143.

7.

Thin-layer chromatography with biodetection to screen plant extracts for the presence of potential drugs

Monika Waksmundzka-Hajnos and Łukasz Cieśla

Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland
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Numerous plant extracts as well as compounds isolated from them have been investigated for their potential use in various diseases. For example in the case of Alzheimer disease drugs belonging to a class of acetylcholinesterase (AChE) inhibitors are currently used to alleviate the symptoms of this ailment. However considerable body of evidence indicates that oxidative stress plays an important role in the development and progress of age-related neurodegenerative diseases. The continuous generation of reactive oxygen species (ROS) in living cells leads to cumulative damage to cellular organelle and finally to age-related pathology and other diseases such as: cancer, inflammation, asthma etc. Thus there is a need to screen different natural samples for the presence of antioxidants that may protect against the development of the aforementioned ailments. The important part of biological detection is connected with screening of plant samples for the presence of antibacterial and antifungal compounds.

Thin-layer chromatography coupled with biodetetction gives the possibility to screen plant extracts for the presence of AChE inhibitors, glucosidase inhibitors, antioxidants, free radical scavengers and antibacterial and antifungal constituents. In this presentation the possibility of using planar chromatography for the search of new substances with potential to be used as drugs in different diseases is discussed. Practical problems encountered while performing such analyses is addressed as well as some solutions are proposed. The future perspectives for the method development are outlined and discussed.

8.

Investigation of vegetable triterpenoids and phytosterols by chromatography and mass spectrometry

I. Vovk [1,2], M. Martelanc[1,3], B. Simonovska[1]

[1] *National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia*

[2] *EN-FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia*

[3] *Centre of Excellence for Polymer Chemistry and Technology, Tehnološki park 24, SI-1000 Ljubljana, Slovenia*

Triterpenoids are secondary metabolites widely distributed in nature. Structures enriched with different functional groups, sometimes some of them derivatised to esters, glycosides etc. render a huge number of over 20.000 compounds belonging to this group. For many of them biological activity was proved. Reported beneficial effects of various triterpenoids for human health indicate the importance of this group of compounds in the diet. However, little is known about the content of triterpenoids in vegetables and about the triterpenoids intake by everyday diet. Determination of triterpenoids in plant extracts is rather difficult, since they contain a vast amount of various triterpenoid compounds, which differ in skeleton structure and polarity because various functional groups can be attached to the ring system. Furthermore, the presence of isomeric triterpenoids in plant epicuticular waxes and the fact that triterpenoids lack chromophores render the determination of triterpenoids even more difficult. Therefore, suitable modern analytical methods are highly desired.

The aim of our work was to investigate triterpenoids in epicuticular waxes of various vegetables like cabbage (*Brassica oleracea* L.), pepper (*Capsicum annum* L.), lettuce (*Lactuca sativa* L.), chicory (*Cichorium intybus var. foliosum*) and parsley (*Petroselinum crispum* L.). Screening of the vegetable surface extracts before and after hydrolysis was performed by silica gel and reversed-phase (C18 RP) thin-layer chromatography (TLC), TLC-MS and C18 RP high-performance liquid chromatography (HPLC) with UV and mass spectrometric (MS) detection using atmospheric pressure chemical ionization (APCI). TLC screening of triterpenoids on silica gel HPTLC plates revealed that all samples contained a triterpenol fraction. Lettuce and chicory extracts contained also esterified triterpenols, oleanolic and ursolic acid. Sterol fraction was present in all samples in trace amounts. The RP 18 layer gave much more accurate insight in the qualitative composition of isomeric triterpenoids in vegetable surface extracts, since some isomeric triterpenols (α-amyrin, β-amyrin, lupeol) and esterified triterpenols were separated. Glycosylated or/and esterified triterpenols were also present in the extracts. In order to detect them, sample preparation included basic hydrolysis (saponification) for detection of esters and acid hydrolysis together with saponification for detection of both types of derivatives.

9.

Hyphenated HPTLC for fast analysis of bee's products

E. S. Chernetsova[1,2*], G. E. Morlock[1]

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[2]*On leave from Russian Research Center "Kurchatov Institute"*
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People's Friendship University of Russia
Miklukho-Maklaya st. 6, 117198 Moscow, Russia

New hyphenated HPTLC approaches for fast analysis of bee's products (honey and propolis) were suggested for the first time.

The quantitation of 5-hydroxymethylfurfural (HMF) in honey is now possible using HPTLC with just 5 minutes migration time, and analyzing up to 24 samples simultaneously. The detection is performed by absorbance measurement at 290 nm. Other possible detection modes include fluorescence measurement after post-chromatographic derivatization and mass spectrometric detection. The reliability of the suggested approach was evaluated using 10 samples of honey with known quantities of HMF and it proved to be at least at the same degree suitable for honey analysis as the conventional methods, including HPLC and Winkler method. HPTLC-ESI/MS coupling can be used as an additional tool, when it is necessary to confirm the results of prior quantitation by HPTLC/UV. Due to the lower sensitivity, HPTLC-DART/MS could be recommended just for the initial screening performed, applying large volumes of honey solution to the HPTLC plate.

The other hyphenated approach was applied for analysis of a large number of propolis extracts, collected during several years in different regions of Germany. A new HPTLC-UV/Vis/FLD-MS method was developed based on the flavonoid profile and a recognizable pattern of biomarkers was detected after selective derivatization. Mass spectrometry employing electrospray ionization or 'direct analysis in real time' (DART) ionization was used for confirmation and verification of the identity of the biomarkers found.

This work was financially supported by the Ministry of Education and Science of the Russian Federation, partially within a program "The development of a scientific potential of a higher school (2009-2011)" (projects #2.2.2.3/9055 and #2.2.2.3/15112), and partially with a grant of the Council at the President of Russia (MK-594.2010.3).

10.

TLC-DPPH TEST REVISITED

Ł. Cieśla[1], J. Kryszeń[1], A. Stochmal[2], M. Waksmundzka-Hajnos[1]

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Reactive oxygen species (ROS) are constantly produced under physiological conditions in human organism. Apart from their role in intracellular signaling they may react with DNA, proteins and lipids causing the change of their biological functions. It has been proved that ROS may be involved in the progress of many ailments, e.g.: atherosclerosis, Alzheimer's and Parkinson's diseases, asthma, cancer and still others. Antioxidants and free radical scavengers are currently the subject of an intensive research interest, as they may protect cellular organelle from damages caused by oxidative stress. In vitro screening is the primary selective tool for finding potential antioxidants and free radical scavengers. One of the techniques commonly applied for the screening of plant extracts for the presence of antiradical compounds is the TLC-DPPH test. After development under the optimized conditions a chromatographic plate is sprayed with a solution of a stable radical – 1,1-diphenyl-2-picrylhydrazyl (DPPH). Intense purple color of DPPH changes into pale yellow in a presence of a substance able to donate hydrogen atom or electron. The majority of the reported TLC-DPPH tests have been performed on silica gel layers, as normal phase systems usually enable satisfying resolution of polyphenolic compounds characterized with antiradical potential. In the experiments performed in our department we have observed that the results of the test are influenced by several factors. Free radical scavenging properties of polyhenolics are influenced by the chemical nature of the adsorbent used. The antiradical potential of the investigated phenolic acids and flavonoids was strengthened on silica and alumina and weakened on polar bonded stationary phases (DIOL- and CN-silica). The results also differed when methanol, used to dissolve DPPH, was changed to acetone or acetonitrile. Thus there is an urgent need to elaborate a standard TLC-DPPH procedure in order to obtain reliable results in the search for potent free radical scavengers.

11.

HPLC monitoring of blood-brain-barrier penetration of certain polar drugs

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Introduction: Lipophilic compounds can easily penetrate the blood-brain-barrier (BBB), however carriers may transfer not only hydrophilic, but even polar organic compounds from the blood to the central nervous system. Our aim was to study the BBB penetration of hydrophilic compounds determining the drug levels in the blood, brain and cerebrospinal fluid (CSF) using reversed-phase chromatography.

Materials and methods: Various bis-pyridinium mono-aldoximes (K-compounds) were synthesized having a variety of different alkyl bridges between the two pyridinium parts as published earlier. Compounds such as pralidoxime, K-027, K-048 and K-203 were studied. Rats were injected by different doses of the compounds, then the animals were sacrificed under anesthesia according to the animal ethical codex of Semmelweis University Budapest, Hungary (permission number of local authorities:22.1/609/001/2010). Blood and CSF were taken, and brain was dissected. The rat brain was homogenized, samples of brain homogenate, blood and CSF were subjected to clean-up using precipitation by perchloric acid and centrifuged at 14,000 rpm at 4 ^{o}C for 20 min. Following the centrifugation pH of the supernatants was adjusted to 2. The samples were subjected to reversed-phase HPLC on Zorbax Rx-C18 stationary phase.

Results: Brain homogenate and CSF samples contain low level of pyridinium aldoximes, therefore the use of electrochemical detector was required to detect the submicrogram/g (0.01 through 1 μg/g) level of pyridinium aldoximes. The corresponding blood samples contain higher level of pyridinium aldoximes, in the range of 1 through 200 microgram, so UV detection at 276 nm could be used. Moreover, the background peaks originated from either the brain or CSF samples required high concentration of ion-pairing agent (0.25% of octanesulfonic acid sodium), while blood samples could be chromatographed using 0.1% of the same ion-pairing agent. To work with the same HPLC method, all separations were performed using mobile phase containing 0.25% octanesulfonic acid sodium

Conclusions: Carefully adjusted ion-pairing agent concentration in the mobile phase helped us to avoid peak-overlapping of the samples of biological origin. The quantitative determination showed that BBB penetration of these highly polar compounds show dose-dependence in the respect of blood-brain and blood-CSF ratios.

12.

Tracking of the food-drug interactions including alcohol-drug interaction with different bioanalytical methods

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Food can alter the bioavailability of drugs either by direct physical or chemical interaction or by the physiological response. The physiological response to food intake, in particular gastric acid secretion, may reduce the bioavailability of certain drugs. Since the bioavailability and clinical effect of most drugs are correlated, the bioavailability is an important pharmacokinetic effect parameter. Such interactions are frequently caused by chelation with components in food or dairy products (ciprofloxacin and norfloxacin). For drugs belonging to the BCS (Biopharmaceutical Classification System) Class I (highly soluble, highly permeable) that rapidly dissolve from immediate release solid oral drug products, bioequivalence under fed conditions has been postulated. Alcohol can profoundly influence both drug metabolism and nutritional status.

The sensitivity and selectivity of HPTLC/OPLC-MS, Headspace-GC, GC-MS, LC-MS and LC-MS/MS applications are of high importance in the pharmaceutical food-drug and food-alcohol interaction research. Bioanalytical methods play an important role in the original and generic drug development. In the recent years it has come to the centre of attention that food intake exerts a complex influence on the biological availability of certain drugs. A number of *in vivo* studies have been published, however, only a few *in vitro* data are known. Fed and fasted conditions can be simulated *in vitro* with dissolution tests using dissolution media with appropriate food components according to the Ph. Eur. 5.

The primary aim of the present lecture is give a comprehensive view about the tracking possibilities of food-drug and alcohol-drug interactions with bioanalytical methods. Another purpose is to illustrate the possible implementation of novel simulated in-vitro method for the analysis of food-drug interactions with several examples. The presented method enables good estimation of IVIVC concerning the prediction of the type and mechanism of food interaction.

13.

Update of generic chiral separation strategies for pharmaceutical compounds using chromatographic and electrophoretic techniques

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Pharmaceutical drug compounds are often chiral, which can imply that different pharmacological and pharmacokinetic activities of the enantiomers are seen in the human body. Therefore, regulatory authorities demand that, if possible, a single enantiomer drug is developed, only containing the therapeutically active enantiomer. In the registration procedure of a drug molecule, methods for the separation and quantification of the enantiomers must be presented. However, in early drug development, industry mostly prefers to synthesize a racemate and to separate this afterwards. In this stage, a fast screening for separation conditions is already performed to reduce the method development time at later stages. Therefore, generic screening and optimization strategies can be very useful at this stage of drug development. A fast screening experiment gives an idea about the enantioselectivity, and the optimization steps can be used afterwards to enhance the obtained separation. The defined strategies are generic, meaning that they are applicable on large sets of structurally diverse molecules. It was seen that polysaccharide-based chiral stationary phases (CSP) were well suited to define such strategies, as they show a broad enantioselectivity range. Different strategies were already developed in normal-phase liquid chromatography [1,2], polar organic solvent chromatography [3], reversed-phase liquid chromatography [2,4] and capillary electrochromatography [5]. For supercritical fluid chromatography, only a screening step was defined [6].
A new challenge concerns the evaluation of newly introduced CSP with other types of polysaccharide selectors for their applicability in generic analysis. In a first step, the applicability of the screening step on the newly introduced CSP is evaluated for all considered techniques. When the enantioselectivity of the new CSP is better than the older ones, the screening step was altered by replacement of some CSP by other ones to achieve a higher success rate than before.

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14.

Disadvantages of the current methods of selectivity evaluation in TLC analysis

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The selectivity of the TLC system is a very important topic in quantitative analysis. The analyst must prove that the analyte is determined without influence from any other compounds. In the case of thin layer chromatography, a spot of analyzed substance cannot be contaminated by another one.

Currently, the most common practice of selectivity validation is to measure the spectrum of the spot by a densitometer. The spectrum can be compared between the analyzed spot and reference spot of pure standard; additionally spectra of different parts (start, middle and end) of the spot are often compared. The Pearson's correlation coefficient is most often used as a similarity measure, together with visual inspection.

Up to date, noone investigated the distribution of correlation coefficient between real spectra at different conditions. There is no reference data, what is a probability to achieve a particular correlation between spectra of different compounds (or the same compound contaminated by spectrum of another one). Our preliminary simulation showed that there is a high risk to obtain very high spectral correlations even if contamination is very high. Therefore, we have performed comprehensive simulation of correlation distribution, done on 170 UV spectra of real drug-like molecules. We have simulated:

[6] Correlation distribution between pure spectra of compounds

[7] Correlation distribution between spectrum of compound and the same spectrum contaminated by another random spectrum in many different ratios

[8] Influence of noise addition and different levels to experiments 1 and 2

[9] Correlation distribution between the same spectra when a random noise is added at different levels, together with experiments on real noisy spectra.

The distribution of all experiments overlap significantly in many cases. This led to conclusion, that current methods cannot be treated as a reliable tool for spot impurity detection and the use of such practice should be suppressed. Instead, a peak purity approaches should be developed in analogous manner to HPLC, which will be the subject of our further work.

15.

A validated reversed phase hplc method for simultaneous determination of aspirin and clopidogrel and their related substances in combined dosage forms

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The analysis of a pharmaceutical substance and its impurities to the desired level remains an analytical challenge. In a formulation, it is even more difficult due to possible chemical interactions of the ingredients. Impurity control is a continuing concern of regulatory agencies and pharmaceutical industries. This calls for the development of sensitive and selective analytical methods for the quality control of drugs' safety and efficacy. In this study, a LC method was developed and validated for the simultaneous determination of aspirin and clopidogrel and their related substances in combined dosage forms. For the analysis, we started from the Pharmeuropa monograph for clopidogrel hydrogen sulfate. Three different columns have been tested for the separation of the specified and unspecified impurities of both APIs from the principal peaks. The Luna column (150 x 4.6 mm, 3 µm) was used for further method development and optimization. The proposed method was validated based on the ICH guidelines. The validation results revealed that the method is specific, linear, sensitive, accurate and precise for the determination of aspirin and clopidogrel and their related substances in dosage forms. It is shown that separation of the impurities of both APIs, if present, was satisfactory with the optimized chromatographic conditions. The method was applied for the analysis of 11 commercial batches of clopidogrel and aspirin combination dosage forms (tablets and capsules) for their content and related substances. The developed LC method was also found to be suitable for simultaneous assay determination of aspirin and clopidogrel in pharmaceutical formulations in the presence of their potential impurities. As there is no official method for this purpose, the LC method can be applied for routine quality control of the APIs and their related substances in combined dosage forms.

Keywords:
Aspirin; Clopidogrel; Liquid chromatography; Impurities; Method development

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POSTER SESSION I WEDNESDAY, JUNE 8 th, 2011

CHAIRPERSONS: T. Kowalska and Ž. Tešić

1.

Mapping fragmental drug-likeness in the MoStBioDat environment: intramolecular hydrogen bonding motifs in β-Ketoenols

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The general knowledge of hydrogen bonding effects is extremely important in chemistry, in particular, in medicinal chemistry and drug design, where molecular recognition and binding between a small molecule and a target receptor is decided by short-range noncovalent interactions. Intramolecular hydrogen bonding in β-ketoenol, which is a tautomeric form of β-diketones, is an interesting example of the equilibrium increasing the stability of the enolized form significantly. The enolizing motif (O=C–C=C–OH) is a structural moiety that is fairly common in chemically and structurally diverse sets of molecules that has recently been identified as a promising pharmacophoric pattern, e.g. indicating a potent anti-HIV-integrase activity.

This bidentate, oxygen-based difunctional building (sub)block might be involved in a variety of molecular effects involving binding divalent metal ions, hydrogen-bond acceptors (HBA) and hydrogen-bond donors (HBD). The metal chelating ability of the system might play a crucial role in the antiviral activity against HIV, blocking the integration step. Moreover, the spatial arrangement of the carbonyl and hydroxyl groups seems to determine the capability of β-ketoenol derivatives to recognize the surrounding environment by forming inter- and intra-molecular hydrogen bonds (IHB) determining the antiviral activity of the compounds.

In recent years chemoinformatics has seen an explosion in available molecular information resources. At the same time the investigations of the hydrogen bonding motifs provide a challenging problem. Although databases are frequently *on line* and supported by a searching capability, this option is not always useful if we are to precisely define the complex molecular fragments or substructures to be screened. Thus, we have recently developed a novel molecular and structural database managing system (MoStBioDat), which is available as a public domain package designed as a dual purpose storage/extraction platform that maintains high-standards of data integrity and reliability and provides software-based solutions for massive *in silico* protocols.

In our last work we report the practical application of the system for a systematic survey of the intramolecular hydrogen bonded (IHB) motifs in β-ketoenol derivatives. The virtual 3D data derived from the ZINC and PubChem repositories have been compared to the experimentally determined CSD results that acted as a ‘benchmark’ database. Differences specific for each database were discovered, which indicated inaccuracies in the simulated data.

2.

Chemometric models for efficient predictions of chromatographic behavior of ziprasidone and its impurities

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The retention behavior of ziprasidone and its five main impurities was investigated by use of the RP–HPLC. The optimal chromatographic conditions (gradient elution mode with mobile phase consists of water phase - 1% TEA in 0.05M potassium dihydrogen phosphate solution, pH adjusted to 2.5 by orthophosphoric acid, and organic phase – acetonitrile, working temperature of 25°C, UV detection at 250 nm, the flow rate of 1.5 ml/min and run time of 20 minutes) were applied to examine 20 different reversed-phase columns for their selectivity and efficiency towards the ziprasidone and its five impurities. Influences of the different stationary phases on the retention parameters and selection of the most suitable columns were performed by means of principal component analysis. The same elution order was observed for the different components with most of the columns examined. Most of these columns did not allow separation of critical pair ziprasidone and impurity II. However, separation of this pair was achieved on three columns, and the optimal column (Waters Spherisorb® ODS 1, (4.6mm×250 mm, particle size 5.0 μm)) was selected. Finally, relationship between molecular parameters and chromatographic behaviour of the ziprasidone and its five impurities was examined.

3.

Knowledge discovery in molecular and structural chemical databases

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Knowledge Discovery process, also known as Knowledge Discovery in Databases (KDD), is defined as the nontrivial extraction of implicit, previously unknown, and potentially useful information from data.[1] It is the most distinguished branch of *data mining* and provides accurate detailed data description. Furthermore, there is an urgent need for invention and development of computational approaches to extract reliable helpful information from all kind of databases, especially the rapidly growing molecular and structural chemical data resources.

Successful modeling work is relative to the availability of essential structural and experimental data, and yet a number of molecular databases for available organic compounds, screening compounds, medicinal agents (drugs), as well as databases with ADMET properties and physico-chemical properties are publicly available and can be used in drug design, e.g. PubChem Compound, ZINC, ChemDB, ChemBank, ChEMBL and DrugBank databases contain ca. 32, 13, 4.1, 1.2, 0.76 and 0.04 mln compounds, respectively.

Here we report an application of a novel and unique molecular and structural database managing system, MoStBioDat[2] for the massive *in silico* protocols parallely analyzing small molecule ligand and protein data. In this study, a compilation of various publicly available databases of small molecules has been analyzed to locate all possible occurrences of the intramolecular hydrogen bonded motifs in catechols.[3] The comparison of the experimentally determined structural data to those that are simulated using virtual structural data indicated a high uncertainty of the topology of this system for *in silico* simulations using data coming from different sources.

What is more, mining small molecule databases relevant to drug discovery could be also a fruitful method for classifying chemical compounds as being druglike and/or leadlike. In some case it is feasible to identify common molecular fragments, so-called *privileged motifs*, which ease ligand binding to an individual receptor or particular receptor family.[4] As a result, privileged scaffolds might be successfully applied in drug discovery process, e.g. as core structures for synthesis[5] and optimal starting points for the library design.[6] Although privileged substructures are intended to be target class-specific it has been shown that this separated molecular subunits also appeared in compounds active against other target families.[7] Furthermore, a single separated structural subunit time and again could be present in thousands substances including various natural products exhibiting miscellaneous pharmacological activities. Frequency of occurrences of that kind generic druglike molecular fragment among drug populations and bioactive compounds ensembles could be a valuable index of privileged structures estimation. By screening databases we can estimate the population of privileged (sub)structural motifs[8] or investigate the evolution of organic chemistry which has a well-defined, modular architecture.[9] This forced us to perform comprehensive exploration of azanaphthalene polypharmacology.

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4.

The influence of heat effect on the peak profiles in overload conditions

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The trend in modern chromatography, still tends towards the achievement of high efficiency and shorter analysis times. To achieve the high column efficiency and the short analysis times, the use of columns packed with sub-2μm particles, are necessary. However, the requirement of the shortest time of analysis enforces the application of high mobile phase velocity, which causes a large value of head pressure to overcome the viscous forces. For 1.7 [μm] particle diameter used in Very High Pressure Liquid Chromatography (VHPLC), the required pressure can be more than 1000 bar. In columns, operating at high mobile phase velocities, under high pressure gradients, a large amount of heat due to the viscous friction of the eluent percolating through the column bed, is produced. The heat generation, cause the formation of an axial and a radial temperature gradient, which follows in a loss of column efficiency. In many papers, the influence of heat generated by viscous friction on the column efficiency was analysed for linear isotherm equation, but not for nonlinear.

We have measured the retention time of butyric acid for three different case of heat transport: the natural convection, the air thermostated column and the column thermostated in a water bath. The Dionex RSLC Polar Advantage II (PA2) 100mm x 2.1mm column packed with 2.2 [μm] totally porous particle was used. The aim of this work was to present the results obtain in overload conditions.

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5.

Chiral separation in normal-phase liquid chromatography: Enantioselectivity of recent polysaccharide-based selectors Part II. Enantioselectivity at optimization conditions

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Abstract

Enantiomers of a chiral drug molecule may show significant differences in their pharmacological, pharmacokinetic or toxicological effect in biological systems [1]. Therefore, enantiomeric separation of chiral compounds is a critical challenge in drug discovery and development. Separation of enantiomers can be achieved using different chromatographic techniques such as gas chromatography (GC), liquid chromatography (LC), supercritical fluid chromatography (SFC), and electromigration techniques. To avoid time-consuming trial-and-error approaches for developing a chiral separation method, generic strategies, consisting of a screening and optimization stages, have been developed. Earlier, a set of pharmaceuticals with different chemical structures has been used to evaluate the enantioselectivity of four recently commercialized polysaccharide-based chiral stationary phases, Lux Cellulose-1, Lux Cellulose-2, Lux Amylose-2 and Lux Cellulose-4 and of three earlier commercialized columns, Chiralpak AD-H, Chiralcel OD-H and Chiralcel OJ-H, using the screening conditions of an existing generic separation strategy in normal-phase liquid chromatography (NPLC) [2]. In the current study, the applicability of previously developed optimization steps [3] on those columns was examined using 48 drugs (74 optimization cases). The resolution, peak shape and the analysis time were nicely improved after the application of the original optimization, for 49/74 cases (66%), baseline resolution was observed. The introduction of some modifications to the original optimization increased the number from 49 to 62 cases, i.e. from 66% to 84%. Finally, an updated generic separation strategy in NPLC was proposed.

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6.

Evaluation of reduced test sets for the development of separation strategies for chiral drug compounds

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During the past decades, a lot of emphasis is put on chiral analysis in pharmaceutical research. Chiral lead compounds have to be analysed in such a way that both, the therapeutically active enantiomer (eutomer) as well as the other (distomer) are identified, quantified and well characterised since in biological environment eutomer and distomer behave differently with possible harmful effects of the distomer as a consequence.
Therefore, the development of chiral separation strategies gained a lot of attention during the past decades. These strategies are used in different stages of chiral drug development. The chiral recognition mechanisms are still not always completely elucidated and there are no universal selectors available to obtain separation between all eutomers and distomers. The defined strategies might be updated when new selectors, claimed to have a broader enantioselectivity than previous ones, are marketed. These updates as well as the development of such strategies are time consuming, especially when large test sets are used.
The goal of this study is to define smaller test sets and verify whether it is still possible to select the most enantioselective systems with these sets. For the selection of the reduced test sets the molecules were described by molecular descriptors. These descriptors 'translate' the chemical information of a molecule into mathematical information (numbers). This will allow selecting a predefined percentage of the original test set by running a selection algorithm (Kennard and Stone) on the data.
The results showed that even with a decrease of 70% of the original test set (19 instead of 62 compounds), the as most enantioselective systems selected ones, turned out to be mainly the same as those obtained with the large test set.

7.

Updating a screening strategy for chiral separations in supercritical fluid chromatography with new chlorinated polysaccharide-based selectors

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Chiral separations are an extensively studied topic, especially in pharmaceutical analysis as drug enantiomers potentially exhibit different properties in the human body.
High-pressure liquid chromatography in RPLC, NPLC and POSC modes remain the most widely used techniques for chiral separations in the pharmaceutical industry. However, drawbacks are related to these techniques, such as the rather long analysis times that limit the throughput and/or the high consumption of toxic and flammable solvents.
Therefore, supercritical fluid chromatography (SFC) has gained interest as an alternative. SFC offers the benefit that higher flow rates can be used in comparison with conventional HPLC, thus reducing column-equilibration- and analysis times and enabling a higher throughput. Additionally, SFC methods have a lower consumption of organic solvents and can thus be considered more environmental friendly.
To enable fast chiral method development, generic chiral separation strategies are defined. A first step in these strategies is a screening step. The aim of these screenings is to quickly determine whether an (acceptable) separation of a certain racemate can be achieved on a given chromatographic system. A generic screening step for SFC has been defined earlier by Maftouh et al [1]. Recently, new chiral stationary phases (CSP) have been introduced containing chlorinated polysaccharide-derivatives as chiral selectors. These new CSP have proven to display an even broader enantioselectivity in NPLC and POSC than those used in the initially earlier defined screening steps.
In this study, the previously defined SFC screening conditions were applied on new chlorinated polysaccharide-derivatives. New mobile phases, containing both a basic and an acidic additive, are tested with the aim of selecting the most generic enantioselective screening conditions. The global result of this study is on updated generic screening step, with a broader enantioselectivity and a faster analysis. Hereby a higher success rate for chiral separations is achieved.

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8.

The influence of sample matrix composition and injected sample volume on the electrophoretic behaviour of small non-enveloped viruses

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For the past three years the authors have been involved in the development of capillary electrophoresis (CE) methods for poliovirus (PV) identification and separation from subviral particles in various types of samples. Based on the PV concentration and viral purity of the sample, the samples were injected in plugs of 1%, 5% and 12% effective capillary length to obtain an optimal separation and an acceptable signal level. Various samples, with diverse matrix composition or purities were successfully separated, but a certain matrix influence was observed.

The present study thouroughly investigates the electrophoretic behavior of poliovirus injected as plugs of 1%, 5% and 12% effective capillary length, respectively, to understand the CE limits and benefits when separating virus suspensions. When samples are injected as 5% and 12% plugs, the signal is enhanced. However, the complexity of the sample did not allow a straightforward identification of the signal enhancing mechanism. The effect of sample matrix, temperature, buffer or SDS concentration were carefully considered.

The matrix composition proved to be especially important for the signal increase and virus stability. The sample matrix had only a limited effect on both PV and electroosomotic flow (EOF) mobility. When larger sample plugs were injected, the PV mobility slowly increased. A decrease with 4°C of the separation temperature or the doubling of the BGE concentration decreased EOF almost ten times more than increasing the injected sample from 1% to 12% plug. However, extremely diluted samples injected as 12% plugs induced a serious EOF increase. In case of PV, the decrease in the separation temperature with 6°C reduced the PV mobility with 30%.

The results provide a better understanding of CE separation of non-enveloped viruses, opening the way to further application, such as stability indication, detection of subviral particles or the study of the interactions between poliovirus and nanobodies, RNA or cellular proteins.

9.

Inverse gas chromatography in the examination of modern organic-inorganic hybrid materials

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In the last years, research on a new class of compounds such as hybrid materials are becoming very popular. Thanks to the ability to control materials properties within the wide range hybrids can be applied in various fields as nanoelectronics, biomaterials, pharmacy and many others.

Inverse Gas Chromatography (IGC) is the method allowing to examine the physicochemical properties of various materials including multicomponet hybrid systems. IGC is an extension of conventional gas chromatography. In this method the examined material is placed in the chromatographic column and its properties are concluded basing on retention behavior of carefully selected test compounds. Acid-base and dispersive properties of the surface may be studied by means of IGC.

The experiments carried out allowed to examine the surface properties of individual components of the hybrids, as well as these for two-and three-component systems. This enabled the evaluation of the mutual impact magnitude of the hybrid material components.

10.

The HPLC study of aqueous and non-aqueous solutions of phenylalanine and phenylglycine

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Among chiral compounds investigated within the framework of our research project (profens, amino acids, hydroxy acids), phenylalanine plays a particular role due to its recognized biological importance, whereas phenylglycine is an important substrate to produce drugs and cosmetics.

In our earlier studies [1-4], we described the results of investigating the remarkable phenomenon of spontaneous *in vitro* oscillatory chiral conversion of the selected optically pure amino acids (e.g., *L*-phenylalanine, *L*-alanine, *L*-tyrosine, *L*- and *D*-phenylglicine), when dissolved in the abiotic aqueous and non-aqueous media, and then aged for certain periods of time at ambient temperature, in the stoppered glass vials. These investigations were carried out with aid of the chiral thin-layer chromatography and polarimetry. Later, we managed to experimentally prove that the spontaneous *in vitro* oscillatory chiral conversion of *L*- and *D*-phenylglycine is accompanied by the spontaneous oscillatory polycondensation of these compounds and our polycondensation results originated from the non-chiral high-performance liquid chromatography [5].

High-performance liquid chromatography with the diode array detection (HPLC-DAD) is a reliable and accurate enough tool to monitor and quantify oscillatory reactions in purely organic and colorless solutions, basically with the UV-absorbing analytes. In this study, we employed HPLC with the two different detectors (diode array (DAD) and evaporative light scattering (ELSD)) to present the chromatographic results of chemical transformation with *L*-phenylalanine and *L*-phenylglycine, when dissolved in 70% aqueous ethanol and in pure dichlomethane, and then stored for certain periods of time in the stoppered glass vials at ambient temperature.

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11.

Quality control of *citri reticulatae pericarpium*: exploratory analysis and discrimination

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Traditional medicine (TM) is becoming a more popular approach to treat or prevent diseases in many countries, including our Western society, making it a lucrative business worth billions of dollars. The main problem in this booming industry is the lack of quality control, needed to ensure the products identity and quality, and the patient's safety.

An example of TM is the dried peel from the mature fruits of *Citrus reticulatae Blanco* (PCR) and its cultivars. This herbal product, also called *Chen Pi*, is often used in traditional Chinese medicine to eliminate phlegm and strengthen the spleen. While the Chinese Health Department only requires *Citri reticulatae* pericarpium samples to contain at least 3.5% of hesperidine, most citrus species meet this criterion. Other problems include the existence of '*mixed peels*' and '*coupled herbs*': i.e. contamination with other citrus species and other herbal parts of the same plant species, respectively.

To detect adulterations and contaminations, an HPLC methodology was developed for the fingerprint analysis of citrus samples. The data set was recorded in three different stages considering: (1) samples obtained as *Citrus reticulatae* pericarpium, (2) potential mixed peels and coupled herbs samples, and (3) dried peels of other citrus species. In a first data analysis step, an exploratory analysis was performed on the developed data sets using Principal Component Analysis. For the samples bought as *Citrus reticulatae* pericarpium, the PC1-PC2 score plot revealed two clusters based on the sample preparation procedure: a cluster of samples obtained in ground form and extracted as they were and a cluster of samples obtained as pieces of peel and extracted after grinding ourselves. To eliminate the variation between the fingerprints not caused by differences in species, the sample preparation procedure was re-optimized and the new PC1-PC2 score plot did not separate both groups anymore.

Once the HPLC optimizations were performed and the method validated, discrimination between the authentic PCR samples and all other samples was performed by probabilistic Discriminant Partial Least Squares. The established model was able to differentiate between both classes with a high reliability for each sample. Furthermore, evaluation of the score and loading plots of the model indicated nobiletin, tangeretin, naringin and hesperidin as important markers for the quality control of *Citrus reticulatae*

12.

Study of condensation oscillations with *L*-lactic acid in pure acetonitrile and aqueous ethanol

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Spontaneous nonlinear chemical processes that occur in the Nature still seem not to be adequately explored. On the other hand, these very processes can play crucial role in various different metabolic and evolutionary pathways. Tracing oscillatory reactions in purely organic and colorless solutions is a challenging experimental task. High-performance liquid chromatography with the diode array detection (HPLC-DAD) is a reliable and accurate enough tool to monitor and quantify such phenomena, basically with the UV-absorbing analytes. A bottleneck of the chromatographic analysis is, however, the time needed for a single analytical run, which makes continuous measurements of the concentration changes (needed for the kinetic assessment of the investigated reactions) virtually impossible, even if an autosampling device is under the hand. One way to circumvent this acute inconvenience is to obtain the shortest possible single analytical run, in that way getting a series of quantitative results bearing a semi-continuous importance.

Among chiral compounds investigated within the framework of our research project, *L*-lactic acid plays a particular role due to its known biological importance, but there has been no experimental evidence prior to our own research [1,2] on its ability to undergo a spontaneous oscillatory *in vitro* chiral conversion. One reason is that lactic acid is poorly retained in the HPLC systems [3] and in that way it causes considerable analytical problems. To solve these problems, an alternative enantioseparation method had to be developed [4] and it is probably noteworthy that one thin-layer chromatographic method is also available [5].

In this study, we present high-performance liquid chromatographic and mass spectroscopic results on chemical transformation of *L*-lactic acid, when dissolved both in 70% aqueous ethanol and in pure acetonitrile, and then stored for certain periods of time in the stoppered glass vials at an ambient temperature. In order to gain a better insight in the nature of the investigated processes, in our experiments we employed HPLC with the three different detectors (diode array (DAD), evaporative light scattering (ELSD), and mass spectrometric (MS) detector).

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13.

The HPLC and optical tracing of the molecular level inhomogeneity with the aqueous ethanol solution of *S*(+)-naproxen

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Discovery of chemical processes running in abiotic systems according to non-linear dynamics and then tracing the kinetics thereof is not a simple experimental task. If the non-linear processes run in the colorless organic solutions (thus escaping the straightforward visual inspection), then the most reliable measuring techniques probably are the chromatographic ones.

In our earlier studies [1-3], we described the results of investigating the remarkable phenomenon of spontaneous *in vitro* oscillatory chiral conversion of the selected optically pure profen drugs (e.g., *S*(+)-ibuprofen, *S*(+)-naproxen, *S*(+)-ketoprofen, *S*(+)-flurbiprofen, and *R*(-)-flurbiprofen), when dissolved in the abiotic aqueous and non-aqueous media and then aged for certain periods of time at ambient temperature, in the stoppered glass vials. These investigations were carried out with aid of the chiral thin-layer chromatography and polarimetry.

Later, we managed to experimentally prove that the spontaneous *in vitro* oscillatory chiral conversion of the low-molecular-weight carboxylic acids is accompanied by the spontaneous oscillatory polycondensation of these compounds and our polycondensation results originated from the non-chiral high-performance liquid chromatography [4,5].

In this study, we present the results of a simple yet more advanced experiment carried out with *S*(+)-naproxen dissolved in 70% aqueous ethanol, then placed on Petri dishes in an optical zooming scanner, and scanned in UV light at 254 nm, in the selected time intervals for ca. four hours. The employed technique was aimed to highlight the dynamic aspect of the discussed supramolecular self-organization of the investigated profen drug solution in a given period of time and to demonstrate the fluctuating density inhomogeneity thereof. The sequence of the snapshots obtained with use of the zooming scanner was discussed in the context of the obtained high-performance liquid chromatographic data.

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14.

On the influence of impregnation with *L*- and *rac*-arginine on retention of naproxen in the thin-layer chromatographic systems

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Silica gel impregnated with *L*-arginine was for the first time used to enantioseparate the racemic ibuprofen mixture in 1996 [1]. In order to better understand the mechanisms governing profen enantioseparation and to further explore the potential of *L*-arginine for this particular purpose, extensive investigations have been carried out with other profens (e.g., with naproxen), and comparisons were made between the performance of the impregnated and the non-impregnated silica gel layers [2-5]. Upon the results (i.e., densitograms and videoscans) obtained, it was assumed that the crystalline chirality of the silica gel layers enables partial enantioseparation of profen antimers in the direction perpendicular to that of the mobile phase flow, whereas the molecular chirality of *L*-arginine is responsible for enantioseparation parallel to this direction [6].

In this study, we present a further and important step in the aforementioned research, which depends on a comparison of the influence exerted by impregnation of the silica gel and the silica gel – kieselguhr layers with *L*-arginine and *rac*-arginine on the retention of the selected test enantiomers. To this effect, we used 70% aqueous solutions of *S*-(+)-naproxen and *rac*-naproxen as the test solutions and six different stationary phases, listed below:

- silica gel impregnated with *L*-arginine,
- silica gel impregnated with *rac*-arginine,
- non-impregnated silica gel,
- silica gel – kieselguhr mixture impregnated with *L*-arginine,
- silica gel – kieselguhr mixture impregnated with *rac*-arginine,
- non-impregnated silica gel – kieselguhr mixture.

A comparison of the chromatograms obtained on silica gel impregnated with *L*-arginine and *DL*-arginine confirmed our earlier findings on the influence of the molecular chirality of the impregnating agent on the retention and enantioseparation of naproxen. A comparison of the chromatograms obtained on the silica gel and the silica gel – kieselguhr mixture emphasized the influence of the adsorbent's chromatographic activity on the retention of the test samples.

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15.

Photostability Study of Alopres® tablets

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Amlodipine besylate is a long-acting calcium channel blocker (dihydropyridine class), that is in use for the treatment of angina [1] and hypertension [2]. Today can be found many products with amlodipin or its combination with other substances on the market. In the study were investigated the Alopres® tablets (Zdravlje-Actavis, Serbia), which contain only amlodipine besylate as an active substance. Since the amlodipine besylate is photosensitive compound [3,4] the main aim of this study was to examine the photostability of the Alopres® tablets (Zdravlje-Actavis, Serbia). Also, the photostability of the Alopres® tablets was compared with related commercially available products. All the examined tablets were stored under accelerated photo conditions, in accordance with ICH regulative [5]. The content of the amlodipine besylate in the irradiated samples was determined by use of validated RP-HPLC method.

Key words: Alopres, amlodipine, tablet, photostability.

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16.

Solid phase extraction for the determination of biogenic amines in dry fermented sausages

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During the fermentation of dry sausages the amount of biogenic amines (BAs) increases due to the microbial decarboxylation of amino acids. The monitoring of the BAs is therefore recommended in the course of biogenic amine intoxication. The BAs were separated and detected by means of C18-HPLC-UV after derivatization with dabsyl-chloride. Prior to analysis, the target compounds were extracted from the meat samples with 0.4 M $HClO_4$. In complex protein-fat matrices, an extra clean-up step can improve the baseline separation of the target analytes.

In this study the feasibility of a C18-SPE as purification step was explored. An experiment was set up to determine the best possible conditions for the SPE procedure. Therefore the different eluted fractions were collected separately and each one was analyzed for the target compounds. Initially, SPE purification was directly applied after the perchloric acid extraction. After activation of the cartridge with subsequently water and acetonitrile, the sample was loaded and washed with water. Elution of the BAs was forced with acetonitrile. Under these conditions, the BAs were distributed over the different fractions, i.e. loading, washing and elution, due to the great polarity differences of the underivatized BAs. Therefore, the extract was, prior to SPE, derivatized with dabsyl chloride. In that way, the BAs become more apolar and were better retained in the course of washing. But even then, still a small percentage of BAs, mainly phenethylamine, eluted during loading and washing. To ensure the retention of all BAs during washing, 0.4 M $HClO_4$ was used instead of water.

The final SPE procedure existed of following steps: activation with 0.4 M $HClO_4$ and acetonitrile, loading of the primarily dabsylated extract, two washing steps with perchloric acid and two elution steps with acetonitrile. Introduction of this modified SPE procedure improved the sensitivity of the method by the possibility to concentrate the sample in an absence of interfering compounds.

17.

Determination of the soil-water, octanol-water, and air-water partition coefficients for the twelve benzodiazepines by the means of the reversed-phase thin-layer chromatography

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The omnipresent and raising concern about environmental fate of pharmaceuticals has been the main driving force in developing and establishing novel methods for rapid and inexpensive estimation of many of the ecologically important parameters such as the soil-water or air-water partitioning constants. In the present study reversed-phase thin-layer chromatography using octadecyl-modified silica as a stationary phase and dioxane-water or acetonitrile-water mixtures as mobile phases was employed. Mathematical models relating retention parameters of several standard compounds with experimentally obtained partition coefficients were established, with satisfactory statistical properties. Calibration models were further used to predict – determine the soil-water (log K_{OC}), octanol-water (log K_{OW}), and air-water (log K_{AW}) partition constants of twelve benzodiazepine compounds mostly used on Serbian market. The obtained values were further compared with the calculated ones by the several computational methods mostly employed trough the EPI suite software, which is freely available from the US Environmental Protection Agency (EPA). Good accordance was established among several parameters, which indicate that reversed-phase thin-layer chromatography could be used as a relatively cheap and fast method in assessment of the aforementioned parameters.

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18.

Degradation of C.I. Reactive Black 5 using water falling film dielectric barrier discharge. An investigation of carboxylic intermediates by IC

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Textile manufacturing is one of the largest industrial producers of wastewater, which have high concentrations of organic and inorganic compounds and strong color, caused by residual dyes that were not fixed to the fibers in the dyeing process. Azo dyes were chosen due to the fact that they are the major colorants in the textile industry; they provide colors with outstanding colorfastness and wide spectrum. At the same time, azo dyes are the most toxic, mutagenic and carcinogenic commercial dyes [1].

In the present paper, the degradation of commercial reactive azo dye C.I. Reactive Black 5 was studied using Advanced Oxidation Processes (AOPs) in a non-thermal plasma reactor, based on coaxial water falling film Dielectric Barrier Discharge (DBD) [2]. Degradation products, which resulted from the oxidative degradation process, were determined by the use of ion chromatography. The effectiveness of subsequent recirculation of dye solution through the DBD i.e. dye conversion into carboxylic intermediate was monitored. Initial dye concentrations in the solution were 40, 80, 200, 500 and 1000 mg/L. Anions of interest were separated on Dionex AS11 column with conductivity detector, with potassium hydroxide as mobile phase. Due to low retention of acetate and formiate and high retention of oxalate and fumarate on aforementioned column gradient elution was applied.

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19.

Revisiting thin layer chromatography as a lipophilicity determination tool. Part II. Is silica gel a reliable adsorbent for lipophilicity investigation ?

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As many approaches regarding lipophilicity determination with TLC (different modifiers and treatment of retention data) are mixed together and almost randomly chosen in every-day published studies, the subject needs some contribution of standardization. The problem is very comprehensively discussed in the case of HPLC, comparative studies by TLC were almost undone.

As the recent articles often present the use of silica gel layers (normal phase) in lipophilicity estimation, the purpose of the second part of our study[1] was to compare several approaches of TLC lipophilicity determination on this adsorbent: a single TLC run, extrapolation of a retention, principal component analysis of a retention matrix, PARAFAC on a three-way array and a PLS regression.

All techniques were applied to 35 model solutes with simple molecules, using silica gel 60 F254 thin layer plates and nine concentrations of six modifiers: acetone, dioxane, ethyl acetate, methylethylketone, propan-2-ol and tetrahydrofuran.

Comparative analysis formed several general recommendations, similar to previous part on RP18 plates:

1. Propan-2-ol and tetrahydrofuran were the best modifiers, while acetone gave the worst correlation of retention with lipophilicity.
2. Surprisingly good correlations were obtained for single TLC runs and this method is underestimated in the literature.
3. Advanced chemometric processing proposed recently, such as PCA, PARAFAC and PLS did not show a visible advantage comparing to classical methods.

Although the retention mechanism on silica is not connected with partitioning between two phases, there is a significant correlation between experimental lipophilicity and retention in silica gel. This adsorbent can be used to estimate lipophilicity, but the correlation is a little bit worse than on reversed-phase systems.

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1 The first part was presented in Szczyrk in 2010 and then published in J. Pharm. Biomed. Anal. 2010, 53, 911-918

20.

Densitometric RP TLC-DPPH method for quantitative evaluation of free radical scavenging activity of N,N`-bis(acetylacetone)ethylenediimine and corresponding copper(II) complex

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Schiff bases and their complexes with metals have provoked wide interest in their diverse spectra of biological and pharmaceutical activities, such as anticancer, antitumor and antioxidative due to structural similarities with natural biological systems. Besides, the Schiff base complexes can be greatly modified by introducing different substituents. They provide models for different chemical processes and investigations. Free radicals are species that contain unpaired electrons. Therefore, they may induce some oxidative damage to biomolecules thus accelerating ageing, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation. There is a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants. One such method that is currently popular is based upon the use of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).

The aim of this work was development of method which gives the possibility of determination of antioxidative activity of compounds independent of applied solvent. N,N`-bis(acetylacetone) ethylenediimine and corresponding copper(II) complex were used for this investigation. Antioxidant activity was determined in vitro and has been evaluated using a RP TLC method that involves reaction between DPPH (2,2-diphenyl-1-picrylhydrazyl) and Schiff base and its metal complex. Investigated compounds were applied by autosampler followed by application of methanolic solution of DPPH on the same spots. The antioxidant activities, expressed in Trolox Equivalent Antioxidant Capacity (TEAC), were determined on the basis of a calibration plot, peak height being a function of Trolox concentration. Their antioxidant capacities were quantitatively evaluated using densitometry with detection at 515 nm. The strength of antioxidant activity of Schiff base metal complex was shown to be significantly stronger than antioxidant activity of its ligand.

21.

Application of gas chromatography in experiments on steam gasification and co-gasification of coal and biomass

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Sustainable energy management requires utilization of renewable energy resources to the possibly widest extent, especially in the light of continuous increase in energy demand and recognition of environmental problems related to fossil fuel processing. In Poland, with the energy sector traditionally based on coal, this issue has also gained a considerable recognition reflected in targets related to diversification of energy resources set in the energy policy until 2030. An increasing trend in the renewable resources share in the final energy use (to about 15% in 2020) as well as support given to the development of distributed energy systems and highly efficient technologies, like gasification is expected.

In the paper the results of experimental comparative study on steam gasification of lignite, hard coal and energy crop – derived biomass (Salix Viminalis) in a laboratory-scale fixed bed reactor at the temperature of 700^{o}C were presented. The amount and composition of product gas were measured and analyzed online via flow meter and gas chromatograph Agilent 3000A, respectively. The main component of synthesis gas produced in the process of steam gasification was hydrogen. The highest hydrogen content in product gas was observed for lignite samples (66-67%vol.). The respective values for hard coal and biomass samples were comparable (59-64%vol. and 59-62%vol., respectively). The experiments on fuel reactivity in the process of steam gasification proved the biomass to be highly reactive in the process of steam gasification. However, the calorific values of product gas in biomass gasification tests were relatively lower than the respective values for lignite and hard coal. The product gas yields in coal gasification tests were over twice the volumes of the biomass-based gas. These results constituted the basis for further experiments focused on co-gasification of coal and Salix Viminalis blends of biomass mass fraction of 20, 40, 60 and 80wt%. Hydrogen content in product gas increased with an increase in process temperature for all biomass/coal ratios. A synergy effect at all tested temperatures was observed in co-gasification tests for blends with 20 and 40wt% content of biomass, consisting in an increase in the volume of product gas, when compared to the tests of coal and biomass gasification. In tests of co-gasification of blends of higher biomass mass fraction (i.e. 60 and 80wt%) the opposite trend was observed.

22.

Application of gas chromatography in experiments of underground lignite gasification to hydrogen-rich gas

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The efforts of the world research society focus on the development of new, environmentally friendly, economically attractive and commonly accessible energy carriers. Hydrogen is considered as the clean alternative fuel, the production of which may be based on the process of fossil fuels' gasification. In-situ coal processing might be an attractive option of hydrogen-rich gas production. In the process, the gasifying medium such as air, oxygen and/or steam is injected into a coal seam through a surface well or from the post mining space on the level of coal deposits.

In the paper the results of the experimental study on simulated in-situ lignite gasification to hydrogen-rich gas with oxygen and steam are presented. The experiments were conducted in an ex-situ reactor, in which real underground conditions can be simulated both in respect to the coal seam and the surrounding rocks strata. The reactor was designed to carry out gasification experiments on extra large coal samples, so that to preserve the natural structure of the coal under study. The composition of the outlet gas mixture was analyzed online via gas chromatograph Agilent 3000A, whereas the amount of the gas produced in the gasification process was measured with a flow meter. The experiment was initially divided into three stages: the ignition stage, the oxygen stage and the steam stage. Gas produced in the steam gasification stage was characterized by the calorific value of 7.8 MJ/m^3 and average hydrogen content of 46.3% vol. A rapid decrease in the temperature levels and in the amount of produced gas proved that the tested lignite of 53% vol. moisture content was not suitable for steam gasification. A great amount of thermal energy was consumed for water evaporation which led to a considerable heat loss. An addition of stoichiometric amount of water in the system by adding steam caused the seam to extinguish. Thus only oxygen could be used as the gasifying medium in the gasification of the tested lignite. The average calorific value of gas produced during stable operation in oxygen gasification stage equaled 5.2 MJ/m^3 with the average gas production rate of 16.0 m^3/h and the average hydrogen content in the produced gas of 26.4% vol.

23.

The HPLC analysis of the selected extract fractions derived from a variety of the sage (Salvia) species

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The sage (*Salvia*) genus embraces over 900 different sage species, which are widespread all over the globe [1]. Many of these species are used in natural medicines of different cultures, basically due to a wide spectrum of their biological activity (like, e.g., antioxidative, anti-tumor, and antibacterial properties). With time, classification of the different sage species has become increasingly more difficult, as in terms of morphology, many of them look quite similar. Moreover, many different sage species appear in form of the subspecies, holding their individual botanical names [2].

Recently, a lot of attention has been paid to biologically active and water-soluble compounds contained in sage. These are basically polyphenol flavonoids, phenolic acids, and simple coumarins. The majority of phenolic acids contained in the different sage species are caffeic acid derivatives. Caffeic acid depsides (i.e., rosmarinic and chlorogenic acid) are considered as the main biologically active compounds contained in sage [1]. According to the literature, rosmarinic acid is the main compound responsible for antioxidative properties of the sage genus [3]. The analysis of chemical composition of the sage extracts most probably can help to better understand the biological potential of this genus and the systematic relations among the individual sage species.

At the first stage of this study, we focused our attention on spectrophotometric determination of the sums of phenolic acids and flavonoids in the sage extracts with use of the pharmacopeial procedures [4]. To this effect, two separate extractions were carried out, with use of the Arnov's reagent (phenolic acids) and aluminium chloride (flavonoids). These preliminary measurements allowed to select nine out of twenty six sage species with the highest contents of phenolic compounds (which were *S. amplexicaulis, S. azurea, S. cadmica, S. glutinosa, S. pratensis, S. pratensis ssp. Haematodes, S. sclarea, S. staminea* oraz *S. triloba*). At the second stage of this study, the nine selected sage species underwent an exhaustive liquid – solid extraction in the Soxhlet apparatus with the liquid – liquid purification step. Finally, the qualitative and quantitative assessment was performed with use of high-performance liquid chromatography (HPLC) of individual fractions containing flavonoid glycosides and aglycones, free phenolic acids and flavonoids, and also phenolic acids and flavonoids liberated through the acidic and basic hydrolysis. This study makes part of a wider research project targeting chemical composition of a variety of the sage species.

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24.

Thin layer chromatography with micellar mobile phase of aromatic biogenic amines in magnetic field

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Constant need for solving various new analytical problems results in research on new methods and techniques. In some cases, it is enough to modify an old technique to overcome the difficulties and receive a satisfying solution.

Using micellar mobile phase in liquid chromatography is one of those cases. By adding a surfactant in concentration higher than CMC, chemical properties of mobile and stationary phases (and in consequence the whole chromatographic system) change drastically, and open a totally new area in chromatographic analysis. Although the exact mechanism of retention in micellar liquid chromatography is still unrecognised, it helps to solve many problems where other methods failed to come researchers' expectations.

Biogenic amines are a group of biologically active compounds responsible for very important processes in living organisms such as protein synthesis, DNA replication, metabolism and the growth of cells. They are also transmitters. Their conventional chromatographic separation, quantitative and qualitative analysis is very sophisticated. That is why, applying the external magnetic field, as an additional factor modifying chromatographic system, has been taken under consideration in this project.

The use of thin layer chromatography in our experiment allowed to compose and examine far great number of chromatographic systems than it could be possible with any other chromatographic method at the same time. It made possible to lower organic solvent usage reducing environmental and financial costs of the whole project. It also simplified applying the external magnetic field to the experiment thanks to small dimensions of chromatographic chambers and plates.

In our survey, we decided to focus on three main subjects and ways of data analysis, concerning:

- optimisation and efficiency of separation of investigated amines in micellar chromatography – through the analysis of chromatographic data obtained for different kinds and concentrations of surfactants, organic modifiers, for various pH values and stationary phases ;
- influence of magnetic field on chromatographic systems with micellar mobile phase – by comparing results of chromatograms developments carried out in magnetic field and outside it.
- gathering additional information on biological behaviour of aromatic biogenic amines and their role in living organisms under varying chemical (pH changes) and physical (presence of external magnetic field) conditions – by the analysis of the whole data obtained during the experiment.

It is obvious that it is impossible to find all the answers in one project, but we hope that we will get closer to final solutions of problems presented above, and our work will be a small contribution useful in future discoveries.

25.

Comparison of GC/MS, GC/ECD and HPLC/DAD techniques in determination of PBDEs in water samples

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The reason for undertaking this research was the Water Framework Directive, which defines framework of cooperation in the field of water policy. The directive gives parameters of water chemical state assessment made by indicating substances with proven or highly probable, especially harmful effect on ecosystems and water of so called priority substances.

The aim of this work was to devise chromatographic methods of determination Penta-BDE, Octa-BDE and Deca-BDE in water and waste water samples. For determination of analyzed substances, researchers used a gas chromatograph compound with mass spectrometer (GC/MS), high pressure liquid chromatograph with diode array detector (HPLC-DAD) and gas chromatograph with electron capture detector (GC-ECD). The following two methods of samples extraction were compared: the liquid-liguid extraction and solid phase extraction using different extraction solvents and absorption phases.

The best devised method with lowest limit of quantification was GC-ECD, which allows determination of Penta-BDE in water samples according to ordinance of the Ministry of Environment 20 August 2008 regarding the way of classification of surface water deposits state (DZ. U. Nr 162, poz. 1008).

26.

DEHYDRATION AND ANALYSIS OF 18-CROWN-6 USED FOR ANIONIC POLYMERIZATION OF PROPYLENE OXIDE

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Keywords: gas chromatography, size exclusion chromatography, anionic polymerization, crown ether.

18-Crown-6 made by four different companies with the declared purity on the level about 99.8 percent (Tab.1, Fig. 1) was used as an activator in the anionic polymerization of polypropylene oxide by oligo(potassium glicydol) as the macroinitiator. Two series of polymerization were conducted, i.e. before and after crown ether dehydration. The influence of 18-crown-6 purity was observed on the rate of polymerization and on the molecular mass of polymers obtained.

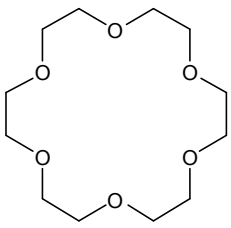

Fig. 1. 18-crown-6

1,4,7,10,13,16-hexaoxacyclooctadecane
Chemical Formula: $C_{12}H_{24}O_6$
Exact Mass: 264,16
m/z: 264,16 (100,0%), 265,16 (13,5%), 266,16 (2,0%)
Elemental Analysis: C 54.53; H 9.15; O 36.32

Tab. 1.

L.p.	Declared purity	Measured purity before dehydration	IR test
1	GC≥98,5%	GC/MS≥99,08%	passed
2	GC≥98%	GC/MS≥97,46%	passed
3	GC≈99%	GC/MS≥99,06%	untested
4	GC≥98%	GC/MS≥97,40%	passed

It was found that the SEC technique allowed to control the crown ether and poly(propylene oxide) purity. The GC-MS technique was successfully applied for qualitative and quantitative identification of impurities.

27.

New CZE-DAD and LC-DAD methods for honeybee venom analysis and standardization of the product

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Honeybee venom possesses diverse biological and pharmacological properties. It has been demonstrated its effectiveness in treating pathological conditions such as arthritis, rheumatism, pain, cancerous tumors and skin diseases. However, bee venom is a toxin and in order to develop pharmaceutical formulations for the safe administration, comprehensive information about its toxicology, side effects and chemical composition is needed. Moreover as it is natural product of high complexity uniform guidelines for standardization of this product are required.

The aim of this study was to develop and compare the new LC and CZE methods for honeybee venom characterization. The developed methods have been applied for analysis of bee venom samples of different strains of the bees, country of origin, year and season of the venom collection. At least nine honeybee venom constituents were separated and the content of four of them (apamine, mast cell degranulating peptide, phospholipase A_2 and melittin) have been determined. Applying LC and CZE the differences in chemical composition of honeybee venom were evaluated. Probably this was the first study in which the internal standard in chromatographic and electrophoretic assays of honeybee venom has been used.

The following steps and parameters were took into account for the validation of the method: selectivity, precision (injection repeatability, analysis repeatability), accuracy (recovery), linearity and operating range, limit of detection (LOD) and limit of quantitation (LOQ). All steps of validation proved that the developed analytical procedures were suitable for their intended purpose (standardization). It was stated that CZE and LC data did not differ significantly. Developed methods due to their simplicity can be easily automated and incorporated into routine operations both in the bee venom identification, quality control and assay tests. Moreover CZE was found to be a cheaper method than LC because of lower consuming of reagents used.

28.

Using gas chromatography to control the functioning of polytrioxane production installation

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The paper presents possible uses of gas chromatography method in the analytic control of the functioning of polytrioxane production installation. The polytrioxane belongs to the group of polyacetals which due to their proprieties are widely used by different branches of indrusty.

The basic material to produce polytrioxane is trioxane. In the Polish technology the method of trioxane production is based on trimeryzation of aqueous formaldehyde solution in the presence of sulphuric acid as a catalyst. For this purpose formaldehyde sofution of ca. 60% is used. The chemical reaction takes place at the boiling point of the reaction mixture. The process of obtaining trioxane is not entirely selective. During the synthesis, different by-products of reaction are obtained in the reactor such as: methanol, formic acid, methyl formate and methylal. As the concentration of trioxane for polymerization should be at least 99,50%, it has to be further concentrated and purified throught distillation, extraction and crystallization.

For the production of polytrioxane to be accurate, it is necessary to carry out regular analytic control of individual production stages and final product quality. Gas chromathography method, which offers a possibility of analyzing samples of complex organic mixtures, can be used in the ongoing control of trioxane synthesis.

29.

Application of GC analysis in the control of trioxane synthesis using ion-exchange resins as catalysts

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The application of the GC method turns out to be useful in selecting a catalyst for the trioxane synthesis process. The method makes it possible to quickly identify and determine the concentration of reaction products and consequently the relevance of a catalyst.

Trioxane is a cyclic trimer of formaldehyde, a major monomer used in the polymerization to obtain polytrioxane. Polytrioxane is thermoplastic with very good mechanical properties, widely used in automotive, electronic and mechanical industries.

The production of trioxane consists in the trimerization of aqueous formaldehyde solution in the presence of acidic catalysts. The trioxane synthesis reaction is conducted at the boiling point of 103 – 110 ^{0}C.

The chemical equation is following:

$$HCHO \leftrightarrow (HCOH)_3$$

Sulfuric acid is commonly used as a catalyst in this reaction. The use of sulfuric acid as a catalyst causes the following problems:

- low yield of the reaction,
- low selectivity of the process (large quantity of by–products such as: methanol, formic acid, methyl formate, methylal),
- corrosion of installations,
- precipitation of paraformaldehyde.

Experiments were conducted to replace sulfuric acid with a different catalyst, witch would ensure a higher yield and selectivity of the reaction. Three acidic ion-exchange resins produced by Purolite were chosen for the experiment. The method of gas chromatography was used to determine the quality and quantity of the reaction products resulting from trioxane synthesis process. The GC method was used in order to determine the yield and selectivity of the reaction.

30.

Determination of volatile components and phenolic acids in *Satureja montana* by GC/MS

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Our work presents the results of chromatographic analysis of the volatile fraction and the phenolic acids fraction contained in different samples of the culinary plant winter savory (*Satureja montana*). This herb is highly appreciated and widely utilized as a food additive and a medicinal plant (which improves functioning of human digestive system).

The first part of this study focused on the volatiles (i.e., on essential oils) contained in *Satureja montana*, which have been isolated by means of hydrodistillation. Two types of the hydrodistillation apparatus were used:

- the Clevenger apparatus (recommended by European Pharmacopoeia), and
- the Deryng apparatus (recommended by Polish Pharmacopoeia).

The aim of our research was to find out which system provides a better option for derivation of essential oils. The obtained results were additionally compared with those obtained, when using the headspace-GC/MS system as a reference technique in the analysis of the volatile fraction. The headspace analysis was carried out at 100°C (to imitate the temperature regime utilized in hydrodistillation). Chemical composition of essential oils was in each case determined by means of the GC/MS technique. The major components of the volatile fraction contained in winter savory proved to be thymol, carvacrol, α-terpinene, myrcene, α-thujene, and linalool.

The second part of our study was focused on the analysis of phenolic acids. To isolate these compounds from plant material, we used the techniques of solid phase extraction (SPE) and accelerated solvent extraction (ASE) of the residual plant material from hydrodistillation. Within the framework of the SPE technique, we carried out the acidic and basic hydrolysis of the samples first (to split the glycosidic and ester bonds, and to liberated free phenolic acids). Then the extracts were percolated through the octadecyl and quaternary amine SPE columns. The eluted samples were evaporated to dryness with an air stream and derivatized with the BSTFA+TMCS (99:1) mixture in pyridine. The parallel ASE extraction of phenolic acids was carried out from the plant material by means of petroleum ether. The phenolic acids containing extracts derived from *Satureja montana* by means of the SPE and ASE technique were analyzed with use of the GC/MS analytical system.

31.

Stability study of acetylsalicylic acid in solutions by the use of UHPLC/ESI-Q-TOF method

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Acetylsalicylic acid also known as a aspirin is a very popular analgesic and antipyretic agent with a very labile properties to reveal its degradation to salicylic acid. However the stability of aspirin is well known, the literature data concerning its stability in organic solutions is very poor.

Ultra high performance liquid chromatography (UHPLC) coupled with accurate quadrupole-time-of-flight (Q-TOF) mass spectrometry was used to the stability study of acetylsalicylic acid in different solutions: methanol, ethanol, propanol, butanol, acetonitrile, tetrahydrofuran, dioxane and water. The separation was performed on Zorbax Extend-C18 (2.1x50mm, dp=1.8 μm) HT column and mixture of acetonitrile (A) and 0.1% solution of formic acid in water (B) was used as a mobile phase.

The gradient elution was carried out at constant follow 0.4 ml/min from 15%A (85%B) to 80%A (20%B) 0 - 13 min and than 15%A (isoctaric) 13-14 min. Assay was monitored by the use of DAD (190-400 nm) and Q-TOF (negative ionization) detection. Mass spectrometry was performed in auto MS/MS mode in mass range: 50-1100 *m/z* and with acquisition rate: 1.41 spectra/s. In this mode during one run full MS/MS spectra and also MS (TOF) spectra were recorded. Extracted ion chromatograms (EIC) from MS spectra for acetylsalicylic acid (179.03490 m/z) and salicylic acid (137.02440 m/z) were used for quantitative analysis of aspirin and its main degradation product in chosen solutions. Calibration range was 0.4 – 14 μg/ml for acetylsalicylic acid and 0.02 – 8 μg/ml for salicylic acid. Correlation coefficients were >0.999 in both cases.

Stability of acetylsalicylic acid in eight solutions at concentration 10 μg/ml was tested in 24h period. Every 2 hour full MS and MS/MS spectra were collected and qualitative and quantitative analysis was performed. In all tested solutions only one degradation product – salicylic acid was found, however significant differences in the rate time of degradation were observed. The most stable solutions were: acetonitrile and dioxane above 80% of initial concentration of aspirin was found in this case (after 24h). The most fast degradation of analyzed compound was observed in butanol (after 10 hour aspirin was totally degradated) and in methanol and ethanol solutions (below 30% of initial concentration of aspirin was found after 24h). Interesting results were observed in the case of water solution (concerning 1% of methanol from stock solution), only about 30% of acetylsalicylic acid was resolved to salicylic acid after 24h.