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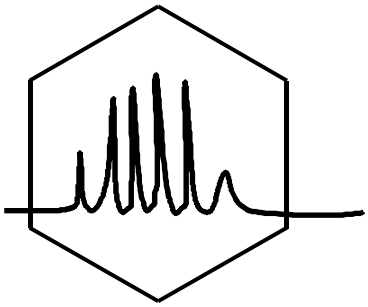
KATOWICE, POLAND



THE XXXVIth SYMPOSIUM

CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS

JUNE 5th -7th, 2013

KATOWICE – SZCZYRK

POLAND

PROGRAM

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SESSION I WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS: Monika Waksmundzka-Hajnos and Roman Kaliszan

9.25 – 9.30 am OPENING ADDRESS

9.30 – 10.00 am

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

B. Chankvetadze

10.00 – 10.30 am

2. Capillary liquid chromatography

J. Silberring

10.30 -11.00 am

3. Recent results on food chemistry using nano-liquid chromatography

S. Fanali

11.00 – 11.30 am

4. Bioactive compounds obtained by structural manipulation of natural products

M. A. Castro

11.30 am COFFEE BREAK

1.00 pm LUNCH

SESSION II WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS: Danica Agbaba and Bezhan Chankvetadze

2.00 – 2.30 pm

5. Quantitative structure-retention relationships (QSRR) in proteomics and metabolomics

R. Kaliszan

2.30 – 3.00 pm

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

I. Stanimirova

3.00 – 3.30 pm

7. The best thermal conditions for SFC separation

K. Kaczmarski

3.30 – 4.00 pm

8. Chemometric analysis of chromatographic retention data

Ł. Komsta

POSTER SESSION I WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS: Agnes Móricz and Maja Natić

4.00 – 6.00 pm (COFFEE BREAK)

6.00 pm BONFIRE

SESSION III THURSDAY, JUNE 6th, 2013

CHAIRPERSONS: Irena Vovk and Živoslav Tešić

9.30 – 10.00 am

9. Selected problems in chromatographic separation of ionizable drugs

M. Waksmundzka-Hajnos

10.00 – 10.30 am

10. Optimized RP-HPLC method to determine mCPBG from biological matrices

K. Tekes

10.30 – 11.00 am

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

H. Kalasz

11.00 – 11.30 am

12. New applications of sulfones in synthesis and organocatalysis

D. Diez

11.30-11.45 am

13. Innovative solutions of Shimadzu in chromatography or organic compounds

P. Stalica

11.45 am COFFEE BREAK

1.00 pm LUNCH

SESSION IV THURSDAY, JUNE 6th, 2013

CHAIRPERSONS: Huba Kalasz and Hubert Pealinck

2.00 - 2.30 pm

14. High performance liquid chromatography as an indispensable tool in plant chemosystematics and chemical ecology

Ch. Zidorn

2.30 – 3.00 pm

15. Chromatography of flavanols, procyanidins and methylxanthines in plant extracts and chocolate

I. Vovk

3.00 – 3.30 pm

16. Effect-directed isolation of plant antibacterials

A. Móricz

3.30 - 4.00 pm

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

K. Demeyer

POSTER SESSION II THURSDAY, JUNE 6th, 2013

CHAIRPERSONS: Eveline De Mey and Łukasz Cieśla

4.00 – 6.00 pm (COFFEE BREAK)

6.00 pm DINNER

SESSION V FRIDAY, JUNE 7th, 2013

CHAIRPERSONS: Teresa Kowalska and Krzysztof Kaczmarski

10.00 – 10.30 am

18. On the mechanism of $DPPH^{\cdot}$ reaction with free radical scavengers in solvents and on the surface of TLC plates

Ł. Cieśla

10.30 – 11.00 am

19. Pharmaceutical nanoparticles and methods of their characterization

J. Jampilek

11.00 – 11.30 am

20. New fillers for dental composites – examination by means of IGC

A. Voelkel

11.30 – 11.50 am

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

W. Prus

11.50 am CLOSING REMARKS

12.00 am LUNCH

POSTER SESSION I

1.

Probing an artificial polypeptide receptor library using a series of novel histamine H3 receptor ligands

A. Bąk, M. Daszykowski, V. Kozik, K. Jarzembek, Z. Kaminski, K. Kiec-Kononowicz, K. Kuder, J. Fraczyk, B. Kolesinska, P. Ciosek, J. Polanski

2.

UPLC analysis for diosgenin detection infenugreek extracts obtained with different methods

J. Ciura, M. Kozioł, M. Leśko, W. Zapała, M. Tyrka, K. Kaczmarski

3.

Piperidine and piperine: extraction and content assessment in black pepper

E. De Mey, M. Marek-Swędzioł, I. Fraeye, M. Sajewicz, H. Paelinck, T. Kowalska

4.

Analysis of perindopril and quinapril, the ACE inhibitors by GC-FID

R. J. Ekiert, J. Krzek, M. Stolarczyk

5.

HPLC/DAD, HPLC/ELSD, and LC-MS investigation of spontaneous oscillatory reactions of *L*-phenylalanine and *L*-hydroxyproline in 70% aqueous acetonitrile solutions

A. Godziek, A. Maciejowska, M. Sajewicz, T. Kowalska

6.

Gas chromatography in determination of fuel char reactivity and gasification process efficiency - experimental study on steam gasification of various fuels in fixed-bed reactor

N. Howaniec, A. Smoliński

7.

Specific mobility of selected phytochemicals in thin-layer chromatographic systems and its possible relevance to pharmacokinetics

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

8.

Chromatographic methods of investigating spontaneous oscillatory reactions of *L*-phenyloalanine and *L*-proline in aqueous solutions

A. Maciejowska, A. Godziek, M. Sajewicz, T. Kowalska

9.

Synthesis new terephtalamides and structure characterization

M. Matussek, V. Kozik, K. Jarzembek, K. Pytlakowska, S. Michalik, A. Bąk, M. Rojkiewicz

10.

Polyphenolic profiles of Serbian polyfloral honeys and discrimination of the geographical origin

M. Natić, U. Gašić, D. Dabić, S. Kečkeš, Ž. Tešić

11.

A comparison of the plant extraction methods upon an example of common thyme (*Thymus vulgaris*)

M. Orłowska, I. Stanimirova, D. Staszek, K. Rykulska, S. Słomczyńska, M. Sajewicz, M. Waksmundzka-Hajnos, T. Kowalska

12.

Chromatographic fingerprints and chemometrics as a tool for prediction of the total antioxidant capacity of rooibos infusions

J. Orzel, M. Daszykowski, B. Walczak, D. de Beer, E. Joubert, A. E. Schulze, T. Beelders, A. J. de Villiers

13.

Chemical characterization of sour cherry wine produced in Serbia

M. Pantelić , D. Dabić, U. Gašić, M. Natić, Ž. Tešić, R. Baošić

14.

Development and validation of a TLC-densitometric method for the quantitative determination of amygdalin

A. Radoičić, R. Petronijević, H. Majstorović, M. Stanojević, Ž. Tešić,
D. Milojković-Opsenica

15.

Application of gas chromatography in the study of the polycyclic aromatic hydrocarbons concentrations in gas sampled from the burning mine waste dump located in Ruda Śląska, Upper Silesia, Poland

A. Smoliński , P. Kuna Gwoździewicz, N. Howaniec

16.

Chromatographic vs. calculated lipophilicities of selected cosmetic raw materials

A. W. Sobańska, E. Brzezińska

17.

Analysis of new stationary phase for amines detection by UHPLC utilizing multiple detection method

S. Swinarew, S. Golba, J. Gabor, M. Łężniak, T. Flak, B. Swinarew

18.

Analysis of volatile fraction from selected thyme (*Thymus* L) species by means of GC-MS and HS-GC-MS

D. Staszek, M. Orłowska, J. Rzepa, G. Szymczak, T. Kowalska, M. Waksmundzka-Hajnos

19.

Application of chromatographic data to build an analytical model of ligand-G protein-coupled receptors interaction

G. Żydek, E. Brzezińska

POSTER SESSION II

1.

Chemometric comparison of the retention of 35 model compounds in HPLC gradient conditions with four columns and two gradient modifiers

E. Bartuzi, Ł. Komsta

2.

Structure activity relationship studies of thiosemicarbazone derivatives

J. Bogocz, J. Polański

3.

Application of TLC and HPLC to quantification of protoporphyrin IX, Zn-protoporphyrin IX, and hemin in Parma ham

H. De Maere, M. Jaros, M. Dziewięcka, I. Fraeye, M. Sajewicz, H. Paelinck, T. Kowalska

4.

Principital component Analysis and Hierarchical Clustering Analysis as novel approach for studying bioactivity of α-adrenergic and imidazoline receptors ligands

S. Filipić, K. Nikolic, A. Smoliński, D. Agbaba

5.

Gas chromatography combined with mass spectrometry as a tool for food quality control

J. Gabor, M. Lężniak, S. Golba, T. Flak, A. Swinarew

6.

GPC as a tool for anionic polymerization of propylene oxide control

S. Golba, J. Gabor, M. Łężniak, T. Flak, S. Swinarew,

7.

Determination flavonols and phenolic acids in *Andrographis paniculata* and dietary supplements

J. Kadłubowska, A. Filipiak-Szok, M. Kurzawa, E. Szłyk

8.

Investigation of cyclohexene oxidation mechanizm over nanogold catalyst using NMR and GC-MS methods

M. Kapkowski, P. Bartczak, J. Polański

9.

Determination of purine alkaloids in some Asiatic plants

D. Kasiorkiewicz, A. Filipiak-Szok, M. Kurzawa, E. Szłyk

10.

Application of analysis of variance to different forms of HPLC-UV/VIS data

M. Kazura, P. Zerzucha, B. Walczak, D. de Beer, E. Joubert, A. E. Schulze, T. Beelders, A. J. de Villiers

11.

Multivariate curie resolution In thin layer chromatography

M. Kobyłka, Ł. Komsta

12.

Investigation of the Sonogashira coupling reactions in a heterogeneous system using chromatographs techniques

M. Korzec, P. Bartczak, J. Polański

13

Comparative analysis of diesel oil samples of different origin based on chromatographic fingerprints

B. Krakowska, M. Daszykowski, I. Grabowski, G. Zaleszczyk, M. Sznajder

14.

Estimation of linear isotherm model parameters in Supercritical Fluid Chromatography (SFC)

M. Leśko, D. P. Poe , K. Kaczmarski

15.

Analysis of selected non-steroidal anti-inflammatory drugs for animals using the chromatographic techniques

M. Lężniak, J. Gabor, S. Golba, T. Flak, A. Swinarew

16.

Stability of lipophilic vitamins in fodder premixes

J. Maćkowiak, A. Voelkel, Z. Okulus

17.

TLC determination of tiapride hydrochloride and its impurities in pharmaceuticals

K. Ranković, S. Filipić, K. Nikolić, D. Agbaba

18.

Determination of water-soluble vitamins in European and Asiatic spices

M. Smolińska, A. Filipiak-Szok, M. Kurzawa, E. Szłyk

19.

Chemometric approach to lipophilicity of selected cosmetic raw materials

A. W. Sobańska, E. Brzezińska

20.

Single chromatographic run approach to lipophilicity of selected cosmetic raw materials

A. W. Sobańska, E. Brzezińska

SESSION I

WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS:

Monika Waksmundzka-Hajnos

and Roman Kaliszan

1.

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

Bezhan Chankvetadze

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

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

References:

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2. K.S.S. Dossou, P.A. Edorh, P. Chiap, B. Chankvetadze, A.-C. Servais, M. Fillet, J. Crommen, J. Sep. Sci., 34 (2011) 1772-1780.
3. G. Jibuti, A. Mskhiladze, N. Takaishvili, L. Chankvetadze, M. Karchkhadze, T. Farkas, B. Chankvetadze, J. Sep. Sci., 35 (2012) 2529-2537.
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6. A. Mskhiladze, M. Karchkhadze, A. Dadianidze, S. Fanali, T. Farkas, B. Chankvetadze, Chromatographia, in press.
7. M. Demetrashvili, L. Chankvetadze, T. Farkas, B. Chankvetadze, Chromatographia, in preparation.
8. K. Lomsadze, M. Merlani, V. Barbakadze, T. Farkas, B. Chankvetadze, Chromatographia, 75 (2012) 839–845.

2.

Capillary liquid chromatography

Marek Smoluch, Przemyslaw Mielczarek, Jerzy Silberring

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

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

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2) Noga M., Sucharski F., Suder P., Silberring J. (2007) A practical guide to nano-LC MS troubleshooting. J. Separation Sci. 30, 2179-89.
3) Li Y.-M., Brostedt P., Hjertén S., Nyberg F. and Silberring J. (1995) Capillary LC-FAB MS using high resolution, continuous chromatographic beds; application to studies on neuropeptide peptidases. J. Chromatogr. B. 664, 426-430.

3.

Recent Results on Food Chemistry Using nano-Liquid Chromatography

Chiara Fanali[1], Anna Rocco[2], Salvatore Fanali[2]

[1]Center for Integrated Research - CIR, University Campus Bio-Medico di Roma, via Álvaro del Portillo 21, 00128 Rome, Italy
[2]Institute of Chemical Methodologies, Italian National Council of Research, Area della Ricerca di Roma I, Via Salaria Km 29,300 – 00015 Monterotondo (Rome) Italy

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

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

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

4.

BIOACTIVE COMPOUNDS OBTAINED BY STRUCTURAL MANIPULATION OF NATURAL PRODUCTS

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

Departamento de Química Farmacéutica. CIETUS. IBSAL. Facultad de Farmacia. Campus Miguel de Unamuno. 37007 Salamanca. Spain.

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

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

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

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

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

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

References:

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3. Miguel del Corral, J.M.; Gordaliza, M.; Castro, M.A.; Nahiques, M.M.; Chamorro, P.; Molinari, A.; García-Grávalos, M.D.; Broughton, H.B.; San Feliciano, A. *J. Med. Chem*. **2001**, *44*, 1257-1267.
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5. Castro, M. A.; Miguel del Corral, J. M.; Rodríguez, M.L.; San Feliciano, A. *Tetrahedron Lett*. **2012**, *53*, 519-521.

SESSION II

WEDNESDAY, JUNE 5th, 2013

CHAIRPERSONS:

Danica Agbaba

and Bezhan Chankvetadze

5.

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

Roman Kaliszan

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Gen. J. Hallera 107, 80-416 Gdańsk, Poland;
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At the molecular level, processes at the basis of drug action are physicochemical interactions, which do not involve formation of the new or breaking of the existing covalent bonds within the interacting molecules. Analogous intermolecular interactions are assumed to determine chromatographic retention. Therefore, chromatographic retention data of structurally defined analytes - usually after chemometric processing - can be used to model their activity in biological systems. The basic research strategy consists in analysis of Quantitative Structure-Retention Relationships (QSRR).
Example QSRR will be demonstrated as predicting in a reliable manner the physicochemical properties of xenobiotics, which are considered to be decisive for their pharmacokinetics and pharmacodynamics. Emphasis will be put on the combination of QSRR with mass spectrometry to help to identify bioanalytes in proteomics and metabolomics. QSRR models will be discussed for the prediction of retention of peptides and hence, for verification of their correct identification, based on our proposed semiempirical structural descriptor based on determination of retention of only 7 out of 20 existing natural amino acids. Another QSRR model will be presented, which has been proposed to help identify bioanalytes resulting from doping in sport. The QSRR models derived have generally been based on structural descriptors of hypothetical compounds, generated solely by the calculation chemistry methods.

6.

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

I. Stanimirova[1], D. de Beer[2], E. Joubert[2,3], A. E. Schulze[3], T. Beelders[3], A. de Villiers[3]

[1]*Department of Theoretical Chemistry, Institute of Chemistry, University of Silesia, Katowice, Poland*

[2]*Post-Harvest and Wine Technology Division, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa*

[3]*Departments of Food Science, and Chemistry and Polymer Science, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa*

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

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

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

References

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[2] I. Stanimirova, M. Kazura, D. de Beer, E. Joubert, A. E. Schulze, T. Beelders, A. de Villiers, B. Walczak, under revision in Talanta.

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7.

The best thermal conditions for SFC separation

Donald P. Poe[a], Jordan Zauner[a], Ryan Lusk[a], Steven Koski[a], Abhijit Tarafder[b], Georges Guiochon[b,c] and Krzysztof Kaczmarski[d]

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b) Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA

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ABSTRACT

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

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

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

8.

Chemometric analysis of chromatographic retention data

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

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

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

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

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

SESSION III

THURSDAY, JUNE 6th, 2013

CHAIRPERSONS:

Irena Vovk and Živoslav Tešić

9.

Selected Problems in Chromatographic Separation of Ionizable Drugs

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

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

10.

Optimized RP-HPLC method to determine mCPBG from biological matrices

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

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

11.

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

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

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

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

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

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

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

12.

NEW APPLICATIONS OF SULFONES IN SYNTHESIS AND ORGANOCATALYSIS.

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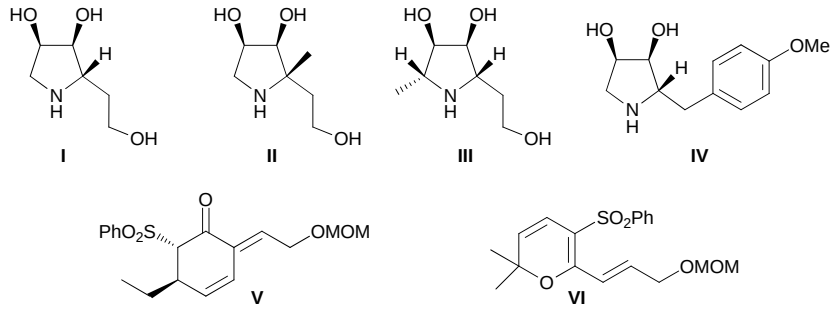
The sulfone group is one of the more versatile functionalities in organic chemistry, for its physical and chemical properties. It has been used for the stabilization of anions in α position and as leaving group in the synthesis of many of the most demanding and sophisticated complex molecules.[1] In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



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

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13.

INNOVATIVE SOLUTIONS OF SHIMADZU IN CHROMATOGRAPHY AND MASS SPECTROMETRY

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

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

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

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

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

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

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

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

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

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SESSION IV

THURSDAY, JUNE 6th, 2013

CHAIRPERSONS:

Huba Kalasz and Hubert Pealinck

14.

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

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

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

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

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

15.

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

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

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

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16.

Effect-directed isolation of plant antibacterials

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

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

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

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17.

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

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

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

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

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

SESSION V

FRIDAY, JUNE 7th, 2013

CHAIRPERSONS:

Teresa Kowalska

and Krzysztof Kaczmarski

18.

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

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

19.

Pharmaceutical Nanoparticles and Methods of Their Characterization

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

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

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

This study was supported by the Czech Science Foundation – GAČR P304/11/2246.

20.

NEW FILLERS FOR DENTAL COMPOSITES – EXAMINATION BY MEANS OF IGC

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

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

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

21.

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

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

POSTER SESSION I

WEDNESDAY, JUNE 5 th, 2013

CHAIRPERSONS:

Agnes Móricz and Maja Natić

1. Probing an artificial polypeptide receptor library using a series of novel histamine H3 receptor ligands

Andrzej Bak[1] , Michal Daszykowski[1], Violetta Kozik[1], Krystyna Jarzembek[1], Zbigniew Kaminski[2], Katarzyna Kiec-Kononowicz[3], Kamil Kuder[3], Justyna Fraczyk[2], Beata Kolesinska[2], Patrycja Ciosek[4] and Jaroslaw Polanski[1]

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Artificial receptors that are capable of selectively binding small chemical molecules under controlled conditions are not only interesting for modeling molecular recognition effects. This method can be also used for the development of flexible sensors and diagnostic platforms or as molecular probes enable to interfe with an actual biological event. Combinatorial libraries can provide us with an important tool not only for the design and discovery of novel chemical molecules but also for the exploration of interactions that are too complex for qualitative and quantitative rationalization. A systematic exploration of the provided data is of the great importance to design and synthesize a library of receptors which can emulate the interactions between drugs and living organisms is made possible. Therefore, the question of how to efficiently design a library to probe a certain receptor type and/or biological effect is as important as the problem of the analysis of the data that is acquired. Unfortunately, the nature of the interactions that govern the binding process of any ligand to its biological target, which is understood in terms of inter/intramolecular forces, is a complicated phenomenon.

Hence, the application of the potential that is represented by libraries of artificial targets binding a given guest molecule in physiological conditions as a model system for the emulation of ligand-receptor interactions has recently been reported. The ensemble of artificial 'biosensors' formed by the self-organization of *N*-lipidated peptides immobilized on cellulose arranged as a molecular probe matrix proved to be an efficient tool to recognize the shape, size and polarity of ligands.

The aim of this study was to probe a library of artificial receptors using a series of novel histamine H_3 receptor inhibitors. An artificial polypeptide receptor (APR) library was created by using the self-organization of *N*-lipidated peptides attached to cellulose via m-aminophenylamino-1,3,5-triazine. The response of the library was probed using a series of novel H_3 receptor ligands. Since no guidelines on how to design an APRs selective vs. certain receptor types exist, a diverse set of amino acids (Ala, Trp, Pro, Glu, His, Lys and Ser) were used and coupled with one of three gating fatty acids (palimitic, ricinoleic or capric). A competitive adsorption-desorption of an appropriate reporter dye was used for the indirect visualization of the interactions of guests with particular receptors. The resulted library response to individual inhibitors was then arranged in a matrix, preprocessed and analyzed using the principal component analysis (PCA) and partial least squares (PLS) method. The most important conclusion obtained from the PCA analysis is that the library differentiates the probed compounds according to the lipophilicity of the gating unit. The PC3 with a dominant absolute contribution of the receptors containing Glu allowed for the best separation of the ligands with respect to their activity. This conclusion is in agreement with the fact that Glu 206 is a genuine ligand counterpart in the natural histamine receptor.

2. UPLC analysis for diosgenin detection infenugreek extracts obtained with different methods

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Fenugreek (*Trigonella foenum-graecum*) is one of the several plant sources that produce diosgenin. Diosgenin, a steroidal sapogenin belonging to the group of triterpenes, is a very important compound in the pharmaceutical industry. It is used primarily as a precursor for the synthesis of steroidal drugs, oral contraceptives and sex hormones. Diosgenin plays an important role in the control of cholesterol metabolism and has anticancer activity, antagonistic effect on rheumatoid arthritis, cardiovascular -, and antimalarial action. The aim of this work was comparison of different extraction methods of diosgenin and identification this compound with the use of UPLC method and two way peaks detection by DAD and CAD detectors.

We tested efficiency of five different methods of diosgenin extraction. Method (1) proposed by Savikin-Fodulovic used hydrolysis of plant material with sulfuric acid in isopropanol and then extraction with hexane. Next, in the three liquid phase (TLPS) procedure (2), diosgenin was extracted with ethanol, ammonium sulphate and petroleum ether. In a protocol described by Li (3), the lyophilized material was subjected to ultrasonication and then hydrolyzed under reflux with sulfuric acid and extracted with petroleum ether. Another method (4)used hydrolysis under reflux with hydrochloric acid and then extraction with chloroform. The last (5) method is simple extraction with only two solvents: hexane and methanol.

To investigate of the extraction efficiency, the chromatographic separation of the plant extracts was performed in Waters Acquity UPLC^{TM} system (Waters Corp., Milford, MA, USA) with DAD and CAD detector. An Acquity UPLC^{TM} BEH RP18 column (50 mm×2.1 mm I.D., 1.7 μm) also from Waters was used. The column and sample temperature were maintained at 30°C. The mobile phase consists acetonitrile and water (9:1 V/V). The injection volume was 50 μL. The detection wavelength (DAD detector) was 203, 236 and 248 nm. The flow rate was set at 0.4 mL/min.

3. Piperidine and piperine: extraction and content assessment in black pepper

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Piperidine is a cyclic secondary amine, which can be considered as a parent molecular structure for many alkaloids. The mature black pepper fruit contains a specific substance – piperine – which chemically is an amide and a source of pungent taste, highly valued with this culinary spice. Piperine is a piperidine derivative as a condensation product with an aromatic acid derived from 3,4-dimethoxycinnamic acid. Nowadays, it is widely used in various herbal cough syrups, and it is also employed in the anti-inflammatory, anti-malarial, and anti-leukemia treatment [1]. Piperine can contribute to an increased bioavailability of many substances according to a number of mechanisms. It inhibits several enzymes responsible for metabolizing nutritional substances and it stimulates amino acid transporters in the intestinal lining. Moreover, recent studies demonstrate that piperine can reduce the fat level in the bloodstream by inhibiting the fat cell differentiation [2].

In our studies, the classical solvent extraction and the Accelerated Solvent Extraction (ASE) of alkaloids from the ground black pepper fruit was performed. Based on the literature [1], we selected the following system for running the thin-layer chromatographic analysis of piperine: stationary phase, silica gel; mobile phase, acetone + *n*-hexane, 3:2 (*v*/*v*). In our efforts to determine piperine in the commercially traded spices, we decided for TLC/densitometry as a well suited and very promising analytical technique. As main advantages of this technique, we consider its simplicity, rapidity, and cost-friendliness, and the quantitative results obtained with its aid are often comparable with those originating from HPLC. The results obtained in this study confirm an excellent performance of TLC/densitometry in the analysis of piperine contained in botanical material.

We also quantified piperine and piperidine by means of high-performance liquid chromatography (HPLC). The analysis was carried out with use of Pursuit 5 C18 chromatographic column and pure methanol as mobile phase. For piperidine, the ELSD detector was employed, and piperine was quantified with DAD detector at a selectively operating wavelength λ = 343 nm. The obtained quantitative results were tabulated and a comparison was made with respect of the employed measuring technique.

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4. Analysis of perindopril and quinapril, the ACE inhibitors by GC-FID

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Perindopril and quinapril are angiotensin-converting enzyme (ACE) inhibitors, commonly used in therapy of hypertension and other circulatory system diseases. The compounds have related chemical structures and similar physicochemical properties. The aim of presented study was to analyze these compounds together, using capillary gas chromatography with flame-ionization detector (GC-FID). Despite FID is the most common detector in gas chromatography, there were no elaborations in this area yet. Simultaneous analysis of both compounds in one run was successfully executed. Each compound served as internal standard for another. Direct analysis without derivatization step was performed. Analysis was possible despite compounds high boiling points, because of their sufficient volatilization in applied conditions. The retention times (t_R) equaled about 3,8 min. and 7,9 min. for perindopril and quinapril respectively. Gas chromatography was performed with a Trace GC (Thermo Finnigan), Compounds were separated on a 15 m × 0.25 mm i.d. WCOT column (Hewlett–Packard) coated with polydimethylsiloxane. Helium served as carrier gas. The temperature program was set as followed: 200˚C to 300˚C with temperature rise 10˚C/min. The total run time was 10 minutes. The inlet temperature was set at 300˚C, whereas injection volume was 4 μL and split 30. The detector, with base temperature of 260˚C was supplied. In the next step, in order to prove method suitability, the analysis in pharmaceutical products was done. No interferences were found, though other peaks were eluted.

5.

HPLC/DAD, HPLC/ELSD, and LC-MS investigation of spontaneous oscillatory reactions of L-phenylalanine and L-hydroxyproline in 70% aqueous acetonitrile solutions

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In previous studies, it has been shown that profen drugs, amino acids and hydroxy acids in aqueous or non-aqueous solutions can undergo spontaneous oscillatory condensation. For example in the studies with L-lactic acid dissolved in pure acetonitrile and 70% ethanol, it was found out that this hydroxy acid can undergo oscillatory oligopeptidization, and also a theoretical model was proposed to describe this phenomenon.

To check that another pair of amino acids (L-hydroxyproline–L-phenylalanine) can undergo spontaneous oscillatory oligomeryzation, we used the achiral high performance liquid chromatography with spectrophotometric detection (HPLC/DAD), the light scattering detection (HPLC/ELSD) and the mass spectroscopic detection (LC-MS). The choice of these amino acids was due to their important functions in human body. L-Hydroxyproline is major component of the protein known as collagen and it is responsible for its stability, and hence, for the tissue architecture and strength. L-Phenylalanine is a precursor for tyrosine, dopamine, noradrenaline, adrenaline, and the skin pigment melanin, and therefore it is an amino acid necessary for proper functioning of human body.

The obtained results demonstrate the oscillatory instability of the pair of amino acids (L-Phe–L-Hyp). In this spontaneous oscillatory reaction homo- and heterooligopeptides are obtained and most probably the additional reaction products (like esters) are also generated.

6.

Gas chromatography in determination of fuel char reactivity and gasification process efficiency - experimental study on steam gasification of various fuels in fixed-bed reactor

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Increasing energy demand, unstable market of fossil fuels and environmental concerns reflected in relevant regulations enforce development of technologies of sustainable energy production. Much attention is given to the development of integrated gasification combined cycle based on gasification process, with a use of which power and hydrogen, as an environment friendly energy carrier, are produced. Utilization of renewable energy resources, including biomass, is also supported. Since waste biomass is estimated to satisfy only approximately 7.5% of the world energy demand, cultivation of energy crops is considered on wastelands and post-industrial areas. Improvement in energy systems efficiency, mitigation of CO_2 emission and more effective utilization of biomass are key aspects of the development of Polish energy sector, based in approximately 90% on coal. Gasification technologies make feasible more efficient utilization of fuels when compared to combustion systems, with a use of variety of gaseous, liquid and solid fuels and production of synthesis gas of wide application potential in heat and power generation, chemical synthesis and liquid fuel production. These technologies have been successfully implemented in over 140 plants of the total syngas capacity of 71,000 MW_{th}. Nevertheless, there are still some technical and technological aspects requiring further optimization, especially when waste and biomass utilization is considered in gasification systems, including optimization of feeding system, fuel pretreatment (drying, grinding, torrefaction, pelletization), selection of operating parameters and type of reactors suitable for a particular fuel and required product gas composition as well as development of effective measures of prevention of low melting point ash and tars accumulation.

Gas chromatography (GC) is one of the key techniques applied in the above mentioned research works making feasible assessment of fuel char reactivity in the gasification process and process efficiency in terms of gas composition, gas heating value, carbon conversion and energy efficiency. Here, the results of application of the GC technique in the study on steam gasification of various fuels in a laboratory scale fixed bed reactor installation are presented.

7.

Specific mobility of selected phytochemicals in thin-layer chromatographic systems and its possible relevance to pharmacokinetics

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On video images of the chromatograms present in numerous publications on the TLC analysis of medicinal plant extracts, striking skewness of the separated chromatographic bands with many phytochemicals can be seen, although no attention has ever been paid to this odd mass transfer effect. Lateral relocation (i.e., sidewise deviation of the analytes' migration route from linearity) has been reported in our earlier studies on the thin layer chromatographic enantioseparation of chiral low molecular weight carboxylic acids belonging to the groups of profen drugs, amino acids, and hydroxy acids. Then we found out that lateral relocation was observed with these analytes only, which structurally resembled molecular rotors. In this study, we investigate the role played by the thin-layer chromatographic stationary phases in lateral relocation of botanically relevant molecular rotors. Thus, three carboxylic acids were selected as the test analytes, all of them resembling molecular rotors and abundantly present in the medicinal plant extracts. We selected two of the most popular thin-layer chromatographic stationary phases: silica gel (characterizing with microcrystalline chirality) and alumina (achiral). These results show that the mobility of tested compounds on the chiral stationary phase surface characterizes with lateral relocation. A conclusion was drawn that the chiral stationary phase makes a complementary contribution to lateral relocation (along with the propeller-like molecular structure of the analytes). In our view, specially devised thin-layer chromatographic systems can prove a convenient nano-platform for future investigation of the drug transport patterns, advantageous in the pharmacokinetic studies.

8.

Chromatographic methods of investigating spontaneous oscillatory reactions of L-phenylalanine and L-proline in aqueous solutions

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In our earlier studies, it has been shown that the low molecular weight chiral compounds from the groups of profen drugs, amino acids, and hydroxy acids in the solution can undergo spontaneous oscillatory condensation. These reactions are characteristic of single compounds or the mixtures of compounds in the aqueous or non aqueous solvents. In the studies with a mixture of L-proline and L-hydroxyproline dissolved in 70% methanol, it was found out that these compounds not only undergo oscillatory peptidization, but a theoretical model was also developed which describes this phenomenon.

In this study, another pair of amino acids (L-proline–L-phenylalanine) was investigated. To this effect, we used the achiral high performance liquid chromatography with spectrophotometric detection (HPLC/DAD), the light scattering detection (HPLC/ELSD), and the mass spectroscopy detection (LC-MS). The choice of these amino acids was due to their important functions in human body. L-Proline is major components of the protein known as collagen and it is responsible for its stability, and hence, for the tissue architecture and strength. L-Phenylalanine is an exogenous amino acid, one of twenty common amino acids which build proteins. It is a precursor for tyrosine, dopamine, noradrenaline, adrenaline, and the skin pigment melanin, and therefore it is an amino acid necessary for proper functioning of human body.

The obtained results demonstrate the oscillatory instability of the investigated amino acids, which consists in the spontaneous oscillatory oligopeptidization, i.e., in the sequential formation and decomposition of homo- and heterooligopeptides as the products of the spontaneous peptidization process.

9.

TEREPHTHALAMIDES OBTAINED FROM METHYL ESTERS OF CHOSEN AMINOACIDS

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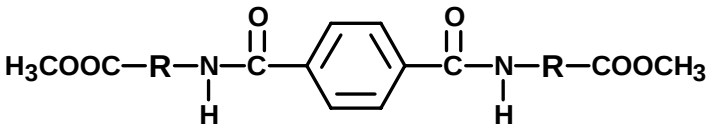
This study was aimed at synthesizing terephtalic acid diamides from methyl esters of chosen aminoacids**,** examining physicochemical properties of the new compounds and determining their crystallographic structure.



Przykłady:

R =

(Gly-OMe) —CH_2— , (βAla-OMe) —CH_2—CH_2— , (Ala-OMe) —CH(—CH_3)— , (Asp-OMe) —CH(—CH_2—$COOCH_3$)—

(Val-OMe) —CH(—$HC(CH_3)_2$)— , (Phe-OMe) —CH(—CH_2—C_6H_5)— , (Ile-OMe) —CH(—$HC(CH_3)$—CH_2—CH_3)— , (Met-OMe) —CH(—CH_2—CH_2—S—CH_3)—

Chemical structure of intermediate products and diamides was confirmed by 1H NMR and 13C NMR. Additionally, FTIR and ESI-MS spectra were recorded. Solubility and melting point temperature were determined for new diamides. Crystallization was attempted in order to obtain monocrystals.

As the result of our investigations seventeen (17) aminoacid methyl ester hydrochlorides were obtained, as well as seventeen (17) terephtalic acid diamides, so far unreported in the literature.
The structure of all the obtained compounds was corroborated by spectroscopic methods.

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10.

Polyphenolic profiles of Serbian polyfloral honeys and discrimination of the geographical origin

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Honey is a natural sweet product produced by honeybees. It is essentially an aqueous solution of saccharides, primarily glucose and fructose, and other substances, such as organic acids, amino acids, proteins, polyphenolic compounds, minerals and other chemicals. In this paper, a total of 58 polyfloral honey samples from different regions in Serbia were studied to determine their polyphenolic profile, total phenolic content and antioxidant capacity, as well as the relationships between them. Total phenolic content (TPC) was determined by the modified Folin-Ciocalteu method, and radical scavenging activity (RSA) by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. A good correlation (R = 0.865) was observed between TPC and RSA. Total phenolic content ranged from 0.03 to 1.39 mg/g and radical scavenging activity ranged from 1.31 to 25.61%. UHPLC–Orbitrap–MS made possible identification of a total of 38 different compounds, and quantification was done using 14 available standards. Data on polyphenols allowed the discrimination and classification of honeys in accordance to their geographical origin using pattern recognition technique, Linear Discriminant Analysis (LDA). LDA showed significant separation between honey extracts originating from different regions in Serbia: Zlatibor, South, East, Vojvodina, and Central.

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11.

A comparison of the plant extraction methods upon an example of common thyme (*Thymus vulgaris*)

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Pharmacognosy is an area of science involved in the investigation of botanical materials and of chemical composition thereof. It is based mostly on plant anatomy and morphology, but also on chemical analysis of plant constituents and their biological properties [1]. Until quite recently, basic knowledge about herbal preparations used for the curative purposes originated from the experience and tradition of folk medicine. With time and owing to the growing sophistication of analytical and pharmacodynamic methods of evaluating curative preparations, increased information has been cumulated on the properties and medical efficacy of plants and the plant-derived medicaments [2].

One of more important plants which are grown on a commercial scale in Poland is common thyme (*Thymus vulgaris*). Phenolic acids contained in this plant show an indisputable pharmacological activity, e.g., as antibacterial and anti-inflammatory agents [1,3].

At the preliminary stage of this study, phenolic acids were extracted from the plant tissue by means of two techniques. One technique was classical extraction in Soxhlet apparatus with use of pure methanol as an extraction agent, and the second one was the Accelerated Solvent Extraction (ASE), with use of the aqueous methanol mixtures in different volume proportions. Central composite design was adopted to optimize the solvent concentration and temperature of the ASE. At the second stage, all the obtained extracts were analyzed with use of 1D isoctratic thin-layer chromatography, in order to compare the respective extraction yields.

The obtained extracts were point-wise spotted on to the chromatographic plates in the 10-μL aliquots with use of an automatic applicator. Then the chromatograms were developed to the distance of 15 cm in the horizontal sandwich-type chromatographic chambers. Silica gel was used as stationary phase and ethyl acetate + acetic acid + formic acid + water in volume proportion 100:11:11:13 as mobile phase. For visualization of the chromatograms, two visualizing agents were used, i.e., the 1% methanol solution of (2-aminoethyl) diphenyl borate and the 10% methanol solution of sulphuric acid. In that way, two fingerprint-type chromatograms were obtained for each plant extract, which were then twice photographed (before and after visualization) upon illumination with UV light at two different wavelengths (λ=254 and 366 nm). For the assessment of the chromatograms, scanning densitometry was also used. The densitograms were recorded before and after visualization at the two different wavelengths (λ=340 and 380 nm). All this information in a digitalized form was then utilized for chemometric comparison of the extraction performance with the two employed extraction methods.

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12.

Chromatographic fingerprints and chemometrics as a tool for prediction of the total antioxidant capacity of rooibos infusions

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Over the last years antioxidant compounds have gained popularity due to their ability to scavenge free radicals – reactive species causing damage in living organisms. Food (e.g., fruits, vegetables, and herbs) is a natural source of such compounds. Food consists of complex mixtures of many antioxidant compounds, complicating their analysis. The total antioxidant capacity (TAC) of samples can be determined using chemical methods that exploit the reaction between artificially generated free radicals and all the antioxidants contained in the tested sample. The reaction is monitored with a suitable instrumental technique (e.g., spectrophotometry or fluorometry). Examples of these methods are FRAP, ORAC or DPPH assays [1]. Despite the simplicity of chemical methods (just to monitor the reaction) they are time and reagent consuming and expensive. Determining the TAC of samples using chromatographic techniques is challenging, as well as time and reagent consuming. On the other hand, chromatographic fingerprints could be used to characterize the antioxidant compounds in foods.

Our goal is to propose an approach to predict TAC of complex natural samples (rooibos infusions) directly from their chromatographic fingerprints (HPLC-DAD chromatograms) without the need to quantify potential antioxidants. The proposed model describes the TAC parameter determined with standard chemical method (DPPH assay) on the basis of a set of fingerprints. The partial least squares regression, PLS [2] is used as a multivariate calibration method. The proposed approach offers satisfactory prediction properties, eliminating the need for additional TAC tests, and considerable reduction of time and chemical reagents.

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13.

Chemical characterization of sour cherry wine produced in Serbia

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Wines contain a number of polyphenolics which contribute to beneficial effects on human health and because of that have attracted much attention in recent years. In this study, wine of sour cherry cultivar Oblačinska and five red wines (Vranac Potkranjski, Cabernet Sauvignon (Radovanović), Cabernet Sauvignon (Rogljevo), Cabernet Sauvignon (Bailo) and Frankovka Banoštor) were purchased directly from different wineries in Serbia during 2011. The aim of this study was the determination and comparison of the total phenolic (TPC), total anthocyanin content (TAC), and radical scavenging activity (RSA) in cherry wine and five red wines. Polyphenolic compounds possess antimicrobial, anti-inflammatory, antimutagenic, antitumor and antioxidative activity. Liquid chromatography coupled with a hybrid mass spectrometer (UHPLC- MS/MS Orbitrap) was used for a study of the phenolic components of wines. RSA was determined with DPPH reagent using slightly modified standard method. The content of total phenolics and anthocyanins was compared with RSA. TPC ranged from 1.19 to 2.50 g GAE/l wine. The highest content of total anthocyanins was found in Frankovka Banoštor wine (0.219 g cyn-3-glu/l wine). Correlation analysis was used to explore relationships among RSA, TPC and TAC. RSA is a relatively high correlated with TPC (r = 0.990).

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

14.

Development and validation of a TLC-densitometric method for the quantitative determination of amygdalin

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Amygdalin became popular like a potential anti-cancer drug. Although there is no scientific evidence to support claims that it may treat cancer, recently, interest in amygdalin is gradually increasing and its use as secondary cancer therapy has been encouraged. Amygdalin can also be used for the treatment of asthma, bronchitis, diabetes, for preventing and treating migraine, hypertension, chronic inflammation etc. In view of its pharmacological effects, an efficient and simple method for amygdalin analysis from natural sources is highly desirable.

Soxhlet extraction is the most commonly used technique for amygdalin extraction while for its determination different HPLC methods were used. In this study, we have explored accelerated solvent extraction (ASE) as an alternative to Soxhlet extraction. Instead of HPLC the TLC is chosen as cheap, simple and sensitive liquid chromatographic method.

The reversed-phase TLC system was consisted of RP-18 silica as the stationary phase and ACN-water, 50:50 (v/v) as mobile phase. Densitometric evaluation was performed at 210 nm.

Validation of the proposed method was carried out with respect to the following parameters: linearity and range, precision, limit of detection and limit of quantitation. The linear regression data for the calibration plots showed good linear relationship with $R^2 = 0.998$ in the concentration range 2.5-50.0 μg per spot. The average recovery was found to be 97.34%. The limits of detection and quantitation were 1.28 μg and 4.28 μg per spot, respectively.

Statistical analysis proves that the developed TLC method is repeatable, selective, and accurate and can be applied for the identification and quantitative determination of amygdalin.

15.

Application of gas chromatography in the study of the polycyclic aromatic hydrocarbons concentrations in gas sampled from the burning mine waste dump located in Ruda Śląska, Upper Silesia, Poland

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Polycyclic aromatic hydrocarbons (PAHs) is a group of environmental contaminants, which constitute an extremely large and diverse class of organic molecules [1], includes over a hundred of various compounds, formed typically during incomplete combustion of organic matter at high temperatures produced primarily from anthropogenic sources [2]. Mine waste dumps are highly heterogeneous, i.e. they are composed of various materials, often flammable, posing a potential risk of autoxidation and self-ignition. Burning mine waste dumps cause a threat to the environment increasing with time, due to the potential fire propagation, and pollution of the atmosphere with dust and noxious gases [3]. The autoxidation and self-heating processes result in intensive release of various gases, including PAHs. Therefore, mine waste dumps may be considered to be a serious source of organic contaminants, including PAHs, demonstrating persistent, bio-accumulative properties [4]. The main objective of the study presented was to determine the PAHs concentrations in gases released from the burning mine waste dump in Ruda Śląska (Upper Silesia, Poland). All samples were extracted using Accelerated Solvent Extraction ASE 200 equipment (DIONEX) and hexane as a solvent. Liquid chromatography method (HPLC 1200 Series Agilent Technologies) with a column ZORBAX Eclipse PAH (4.6 mm × 150 mm, 3.5 μm) was applied in the analyses.

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16.

Chromatographic vs. calculated lipophilicities of selected cosmetic raw materials

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The chromatographic behavior of 22 selected cosmetic raw materials (sunscreens, preservatives and vitamins) was investigated using thin-layer chromatography on RP-18 stationary phase using water-organic modifier (acetone, methanol, dioxane, acetonitrile, dimethylformamide or tetrahydrofurane) binary mixtures as mobile phases. Good linear correlations (R^2>0.98) were found between R_m values and volume concentrations of the organic modifiers. Obtained by extrapolation to zero concentrations of six organic modifiers R_m^0 values were correlated with lipophilicities calculated *via* different methods (ALOGPs, AClogP, ABlogP, milogP, ALOGP, MLOGP, KOWWIN, XLOGP2, XLOGP3, ACDLab). Comparison of correlations between R_m^0 and calculated logP values revealed that methanol, acetone and dioxane are better modifiers in chromatographic lipophilicity measurements than dimethylformamide, acetonitrile or tetrahydrofurane. This conclusion was further supported by analysis of relationships between R_m^0 and experimental $logP_{o/w}$ found for 9 out of 22 investigated compounds.

Acknowledgements
This work was supported by an internal grant from the Medical University of Lodz, Poland (no. 503(3-016-03)503-01.
Thanks are due to Merck and BASF for free samples of sunscreens used through this investigation.

17.

ANALYSIS OF NEW STATIONARY PHASE FOR AMINES DETECTION BY UHPLC UTILIZING MULTIPLE DETECTION METHODS

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Keywords: liquid chromatography, gas chromatography, molecularly imprinted polymers, quality control.

Molecularly imprinted polymers (MIPs) are synthetic polymeric materials with specific recognition sites complementary in shape, size and functional groups to the template molecule, involving an interaction mechanism based on molecular recognition. These recognition sites mimic the binding sites of biological entities such as antibodies and enzymes. Their stability, ease of preparation and low cost for most of the target analytes make them attractive for numerous applications. The use of MIPS as stationary phases for HPLC is one of the best studied application of imprinted polymers, largely because it provides a convenient method for quantitative assessment of the quality of imprints produced by a particular strategy. A wide range of chemical compounds have been imprinted successfully, ranging from small molecules, such as drugs, to large proteins and cells. The best results have been obtained for molecules with molecular weights in the range of 200–1200 Da. The resulting polymers are robust, inexpensive and, in many cases, possess affinity and specificity that is suitable for industrial applications.

In this announcement we present an investigation of new polymeric stationery phase for amines detection using the Nexera UHPLC equipped with a Pinnacle DB PAH 1.9 μm, 50 x 2.1 mm column and the multiple DAD and FLD detectors enabling detection of trace-level components. The described method allows for selective absorption of amines molecules.

18.

Analysis of volatile fraction from selected thyme (*Thymus* L) species by means of GC-MS and HS-GC-MS

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The influence of aromas on physical and psychical well-being of humans has long been recognized in psychology and philosophy. Therefore in aromatherapy, the curative potential of essential oils (plant hormones) is utilized. Essential oils are among the main constituents of plants which are used in plant pharmacy and homeopathy, although too rarely in classical pharmaceuticals. Along with a recognized curative potential, essential oils can also exert toxicity. The high quality pure essential oils are available on the Polish market in the drugstores, selling spots specialized in trading medicinal plants, and in the centers for aromatherapy [1,2].

Essential oils are liquids characterizing with relatively high boiling point (ca. 105^0C) and considerable volatility. From the chemical point of view, essential oils are mixtures of hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers, which are mainly mono- and sesquiterpenes, and also the phenylpropane derivatives. Essential oils exert diverse biological activity which depends on pharmacological activity of their predominant constituents (this activity can be, e.g., antibacterial, decongestant, sedative, anti-inflammatory, etc.). Essential oils can by derived from plants, e.g., by means of hydrodistillation or pressing [3,4].

Common thyme (*Thymus vulgaris* L.) is the plant originating from Mediterranean zone. It is a semi-shrub with characteristic, pleasant aroma of thymol, which contains ca. 3.5% (and sometimes even up to 5.4%) essential oils, and up to 10% tannins, polyphenolic acids, and flavonoids. It exerts a recognized decongestant, antispasmodic, antifungal, and antibacterial activity [5,6].

Plant material utilized in this study originated from Botanical Garden of Maria Curie-Skłodowska University in Lublin. Three different thyme species were investigated, i.e., *Thymus pulegioides*, *Thymus vulgaris*, and *Thymus kosteleckyanus*. We compared the volatile fraction contained in each investigated species by using two different methods of extraction and analysis the volatile compounds. In first approach, essential oils were separated through hydrodistillation in the Deryng apparatus, and then analyzed by means of GC-MS. In second approach, the analysis was carried out by means of headspace-GC-MS.

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19.

Application of chromatographic data to build an analytical model of ligand-G protein-coupled receptors interaction

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The purpose of this study was to examine the structure-activity relationships of agents acting on G protein-coupled receptors. Selected set of GPCR agonists and antagonists was identified from the literature. The chromatographic data and calculated molecular descriptors obtained for representative set of compounds were used in SAR analysis.

Normal phase thin layer chromatography system was used for determination of the chromatographic data. The analysis was carried out in two variants of the mobile phase: acetonitrile-methanol-ammonium acetate buffer (pH 7.4; 0.02 M) (40:40:20, v/v/v) and acetonitrile-methanol-methylene chloride- ammonium acetate buffer (60:10:10:20, v/v/v/v). Glass TLC silica gel 60 F_{254} plates (20×20 cm, Merck, Darmstadt, Germany) were used as the stationary phase. The stationary phase was modified by impregnation with 0.03 M L-aspartic acid in automatic TLC spray chamber (ChromaJet DS20, Desaga, Germany). Such modified stationary phase was used as analytical model of ligand-receptor interaction.The plates were scanned densitometrically at 254 nm by means of a Desaga CD 60 densitometer with Windows-compatible ProQuant software (Desaga, Germany).

Principal component analysis and stepwise discriminant analysis were employed to obtain the relationship between the descriptors and receptor binding affinities, expressed as pK_i values. The set of ligands was classified into two groups according to their degree of biological activity.

This work was supported by the Medical University in Lodz, Poland, Research Programs No. 503/3-016-03/503-01.

20.

Separation aminoacid methyl ester hydrochlorides sample

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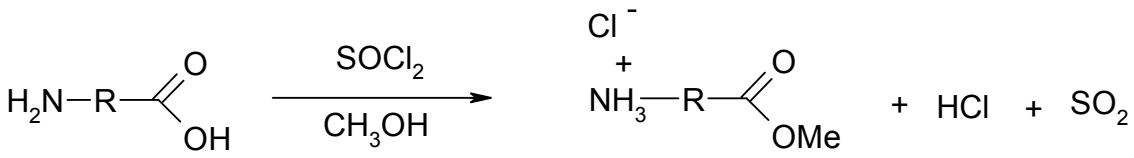
This study was aimed at synthesizing hydrochloride methyl esters of chosen aminoacids**,** examining physicochemical properties of the compounds and separation sample enantiomers.



Chemical structure of intermediate products was confirmed by 1H NMR and 13C NMR. Additionally, FTIR and ESI-MS spectra were recorded. Solubility and melting point temperature were determined for compouds. Crystallization was attempted in order to obtain crystals.

As the result of our investigations a few aminoacid methyl ester hydrochlorides were obtained. The structure of all the obtained compounds was corroborated by spectroscopic methods.

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POSTER SESSION II

THURSDAY, JUNE 6 th, 2013

CHAIRPERSONS:

Eveline De Mey and Łukasz Cieśla

1.

Chemometric comparison of the retention of 35 model compounds in HPLC gradient conditions with four columns and two gradient modifiers

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This study is a HPLC continuation of our earlier TLC studies. We have performed chemometric interpretation of 35 simple compounds in different TLC conditions and investigated the best ways to determine lipophilicity: on the RP18 plates[1], silica plates[2] and cyanopropyl plates[3]. The compounds were also subjected to an additional study about a closer look of extrapolation ways and averaging methods[4]. The current study is done with the same compounds on an unified HPLC system. We have used four columns LiChroCart 125 mm (C18, C18 endcapped, CN and DIOL) with two modifiers: acetonitrile and methanol. Each system was run in an unified gradient along 20 minutes with linear increasing modifier concentration in water from 10 to 90 %. Flow was set to 1 ml/min.

It can be shown, that the longest retention times were obtained with C18 encapped and methanol, whereas the shortest ones were noticed with DIOL column and acetonitrile. In general, the retention times are multicollinear (intercorrelated); two of compounds (antraquinone and 8-hydroxyquinoline) were outliers in DIOL systems (a very long retention time). PCA analysis on the retention time matrix explained 85.9% of total variance in PC1, 10.9% in PC2. However, PC2 information was related only to difference of the two outlying substances. When the two outliers are removed, PC1 explained 97.3% of variance, and PC2 1.3%. First PC represented an average retention, second PC represented differences in retention. It can be concluded, that differences on retention can be modelled as one main trend: some compounds have CN and DIOL retention times simultaneously increased, with parallel decrease of the retention in C18 endcapped column with methanol as the modifier.

Robust correlations between Log P and retention times were in range from 0.51 to 0.86, the best correlation was obtained on C18 non-endcapped column with methanol as a modifier.

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2.

STRUCTURE ACTIVITY RELATIONSHIP OF THIOSEMICARBAZONE DERIVATIVES

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Abstract: Structure-Activity Relationship is an approach designed to find relationship between chemical structure and biological activity of studied compounds. It is the concept of linking chemical structure to a chemical property or biological activity.

Thiosemicarbazones (TSC) are a class of compounds exhibiting a broad range of biological activity such as: anticancer, antibacterial, antiviral and antiparasitic. An increasing number of publications devoted to TSCs indicated the growing interest in this group of compounds. In this study we performed a query of chemical databases in a search for (Q)SAR that could explain the molecular basis of the TSC activity. The maximum common substructure and ADMET properties was used as molecular descriptor in our analyses.

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3.

Application of TLC and HPLC to quantification of protoporphyrin IX, Zn-protoporphyrin IX, and hemin in Parma ham

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Porphyrins are naturally occurring organic heterocycles, which play an important role in metabolism of living organisms. All compounds which belong to this group can structurally be derived from the simplest porphyrin, known as porphin. The molecule of each porphyrin is built of four pyrrole rings coupled together with methane bridges (=CH-)[5]. Due to that, all these molecules are flat, stable, strongly coloured, and prompt to form complexes with metal ions. Porphyrins absorb light and have characteristic absorption spectra both in the visible and ultraviolet wavelength range[6].

This study is devoted to quantification of three porphyrins, i.e., protoporphyrin IX, Zn-protoporphyrin IX, and hemin in Parma ham by means of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Protoporphyrin IX plays a specific role among all tetrapyrroles, as it is a kind of a template for a wide variety of naturally occurring compounds, e.g., for the investigated Zn-protoporphyrin IX and hemin. All these compounds affect the colour properties of meat, and moreover, Zn-protoporphyrin IX contributes to the formation of a characteristic stable red colour of Parma ham[7].

Sample of Parma ham was pre-treated for the TLC and HPLC analyses, following the available data from the literature, with minor modifications of our own only[3]. The calibration curves for protoporphyrin IX, Zn-protoporphyrin IX, and hemin were elaborated both with aid of TLC and HPLC.

Thin-layer chromatographic analysis was carried out under the following working conditions: The ready-made chromatographic glass plates were pre-coated with RP-18 stationary phase and methanol was used as mobile phase. Development of the chromatograms was carried out to the distance of 15 cm in the normal chromatographic chambers after Stahl. After the development and drying, the chromatograms were first visually inspected in UV light and then densitometrically scanned.

High-performance liquid chromatographic analysis was performed with use of the C18 type stationary phase, in the isocratic mode with use of the following mobile phase: A + B, 9: 1 (*v*/*v*). A: MeOH + DCM, 9:1 (*v*/*v*); B: H_2O + CH_3COOH, 97:3 (*v*/*v*). The assumed mobile phase flow rate was 0.8 mL min^{-1}.

Upon the results obtained, the adequate conclusions were drawn.

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4.

Principal Component Analysis and Hierarchical Clustering Analysis as novel approach for study bioactivity of α-adrenergic and imidazoline receptors ligands

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Abstract

Pharmacological profile of 29 drugs, α-adrenergic/imidazoline receptors ligands and related compounds, were studied by Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA). Chromatographic retention parameters (logKw at pH 4.4, *S* at pH 4.4, logKw at pH 7.4, *S* at pH 7.4, logKw at pH 9.1, and *S* at pH 9.1), capillary electrophoresis migration parameters (μ_{eff} at pH 4.4, μ_{eff} at pH 7.4, and μ_{eff} at pH 9.1), and computed molecular descriptors [1-6] of the 29 ligands were used as variables in the chemometric clustering approach. The PCA and HCA of the 29 drugs in the space of the 34 descriptors provided clustering of the compounds in terms of studied descriptors. The final analysis of the HCA dendrogram, sorted according to the Ward linkage method with the data color map, provided additional sub-clustering of the formed HCA sub-clusters and allowed specific grouping of the examined drugs with the highest agreement with their activities on α-adrenergic and imidazoline receptors and with their pharmacological and side effects.
The presented chemometric approach could be used as simple and reliable tool for initial investigation of pharmacological activities of novel α-adrenergic and imidazoline receptors ligands.

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5.

GAS CHROMATOGRAPHY COMBINED WITH MASS SPECTROMETRY AS A TOOL FOR FOOD QUALITY CONTROL

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Keywords: gas chromatography, quality control, mass spectrometry.

Quality control for different kind of beer presented on the market were performed using a gas chromatograph Shimadzu GCMSQP2010 Plus, with a capillary column ZB5 MSi 30 m length and a diameter 0.25 mm, with film thickness 0.25 μ and installed precolumn 5 m length. Injector temperature was set to 250 °C, column temperature was changed in the range from 100 °C (1 min isothermal) to 250 °C at the rate of 25 °C/min, transfer line temperature was equal to 250 °C. Identification of compounds was based on comparative analysis of the spectra obtained from a library of mass spectra JWS (John Wiley and Sons), and then by comparing the mass spectra and retention times of test compounds and standards.

The analysis was performed using an internal standard method with caffeine as a reference compound. Samples were degassed and mixed in a 1:1 ratio with the standard solution.

Quality control was performed due to determine the amount of propylene glycol in beer. The amount of propylene glycol was in the range from 4 to 67 mg/dm3

6.

GPC AS A TOOL FOR ANIONIC POLYMERIZATION OF PROPYLENE OXIDE CONTROL

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Keywords: size exclusion chromatography (SEC), mass spectrometry, MALDI TOF.

In novel chemical laboratory there is a lot off technique to analyze material during the synthesis process. Each of technique can measured different properties. Sometimes is necessary to confirm results or combine them. But only all techniques together are able to well describe analyzed material.

This work is a attempt to describe polymers and control the polymerization process in real-time using three analytical methods mainly GPC because of its simplicity and TTL properties . Every of analyzed materials are typical for industry. For all polymers were used matrix-assisted laser desorption/ionization time of flight (MALDI TOF) supported with gas chromatography combined with mass spectrometry (GC-MS) as a reference technique.

7.

Determination flavonols and phenolic acids in *Andrographis paniculata* and dietary supplements

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Andrographis paniculata (AP) annual shrub (family *Acanthaceae,* literally "king of bitters" or known as an Ayurveda herb *Kalamegha*) grows abundantly in India, Sri Lanka, Taiwan, China and Thailand. The aerial parts of the plant (leaves, roots and stems) are used for extraction of the active phytochemicals. Andrographis paniculata Nees is used for several applications in traditional Chinese medicine to treat many diseases and infections. In Europe is a component of dietary supplements.

In the present work, a method involving water-bath extraction (with ethanol or water), HPLC – RP- C18 column chromatography with photodiode array detection was developed for determining the level of quercetin derivatives (e.g., quercitrin, hyperoside, rutin, and keampherol) and derivatives of benzoic and cinamic acids (gallic, caffeic, chlorogenic, ferulic, p-hydroxybenzoic acid) in *Andrographis paniculata* and dietary supplements containing AP. The impact of hydrolysis digestion was also tested.

The HPLC -PDA system was applied. Phenolic acids were detected at two wavelengths: 254and 325 nm, using a 45-min program, while flavonols were identified at 360 nm, in 50-min gradient program. Analyses were carried out on Discovery RP-C_{18} column (5 µm particle size, 150×4,6 mm, SUPELCO), maintained at 30°C. Mobile phase for phenolic acids was A: 2% acetic acid and phase B: methanol, whereas for flavonols was A: isopropanol-water (95:5 v/v) and B: isopropanol-water-THF (50:40:10 v/v). The rate-flow was 1ml/min in both programs. The total amount of studied flavonols and phenolic acids was compared with total content of polyphenols using Folin-Ciocalteu's method.

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8.

Investigation of cyclohexene oxidation mechanism over nanogold catalyst using NMR and GC-MS methods.

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Abstract

Gold nanoparticles catalyze a wide range of the oxidation reactions.[1] Simple hydrocarbons, due to their structural minimalism are excellent model objects, which provides to obtain useful oxidation model, given the importance of such conversion currently we are still looking the new catalysts which will be more efficient in this type of reactions. One of the first alkene oxidised over Au/C or Bi-Au/C was cyclohexene reacted with tert-butyl hydroperoxide (TBHP) in 1,2,3,5-tetramethylbenzene.[2]

The aim of this study was to optimize the conditions of the selective oxidation of cyclohexene using nanogold catalysts of varying Au percent (0.1%, 1.0%, 5.0%, 10.0%) on a matrix of SiO_2, using 30% aqueous hydrogen peroxide solution (H_2O_2). The optimization of the oxidation process involved: the percentage of gold, temperature and reaction times.

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9.

Determination of purine alkaloids in some Asiatic plants

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Alkaloids usually have strong, sometimes toxic physiological effects on the human body. Many toxic alkaloids respectively served in low doses are an effective drug for various diseases and conditions (such as morphine, codeine or quinine). Many alkaloids are also components of stimulants (caffeine, theobromine, nicotine). Purine alkaloids, such as caffeine, theobromine and theophylline were detected and analyzed in some Asiatic plants (*Cola accuminata, Emblica officinalis, Andrographis paniculata, Puearia lobata* and *Garcinia cambogia*). Alkaloids were also identified in tea and coffee samples.

In the present work, HPLC-PDA method is developed for determining the level of purine alkaloids. The HPLC system equipped with auto sampler and photodiode array, SHIMADZU (Kyoto, Japan) was applied. Analyses were carried out on Discovery RP-C_{18} column (5 μm particle size, 150×4,6 mm, SUPELCO), maintained at 25°C. The impact of extraction method (SPE, ultrasonic, water-bath), solvent (methanol, ethanol, water, chloroform, and sodium carbonate), pH, procedure and various extraction conditions (time and temperature) were tested. The optimal conditions were ultrasonic extraction at pH=9.0 (sodium carbonate and methanol, 2:3, v/v), then extracts were shared in two fraction. One was at SPE, second was shaking with chloroform. These two fractions were evaporated, the residue dissolved in methanol and analyzed by HPLC-PDA method. The recovery for caffeine standard was 101.23%, while for theophylline 91.05%. The developed method was validated for specificity, repeatability, recovery and accuracy.

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10.

Application of Analysis of Variance to different forms of HPLC-UV/VIS data

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Nowadays, hyphenated chromatographic techniques are standard analytical tools to solve complex analytical problems. The power of combining separation techniques with multivariate detection methods (usually spectroscopic techniques) has been demonstrated over the years for both quantitative and qualitative analysis of unknown compounds in complex natural extracts. Chromatography produces pure or nearly pure fractions of chemical components of the studied mixture and multivariate detection methods produces selective information for identification using standards or library spectra [1]. Hyphenated techniques are often the methods of choice in order to obtain fingerprints of complex mixtures, such as blood or urine samples, herbal extracts, etc. where the goal is to find differences among samples and to identify components responsible for these differences. These techniques are known to provide a huge amount of data, since each sample is characterized by a two-way data table [2]. Having at our disposal the HPLC-UV/VIS data (signals registered for rooibos tea samples), we can build different forms of data representation for the set of m samples. These can be: a collection of two dimensional individual signals, mean chromatograms, and a peak table. All of these forms require intensive signal preprocessing, prior to data analysis. In our study we propose a new form of data representation well suited for chromatographic data originating from multi-channel detection, namely inbound-pairwise representation. The biggest advantage is that any kind of chemometric analysis can be performed directly for hyphenated chromatographic data, because the proposed approach (inbound-pairwise representation), eliminates the need for time consuming warping of the studied signals and allows elimination of all problems associated with the peak correspondence. In our study Analysis of Variance was performed for all those different forms of a studied data set.

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11.

Multivariate curie resolution In thin layer chromatography

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It was recently proven by us, that the most popular spot purity approach in TLC, based on correlation of spectra measured in three places of the spot, cannot detect even large amount of contamination in certain cases[8]. These results should be taken seriously, as common spectral similarity between drugs and impurities can additionally increase the risk of presence of unnoticed inhomogeneities.

The chemometric techniques are very helpful in testing for homogeneity. The main difference between HPLC and TLC is the nonlinearity of detector response and this was the subject of our second recent study[9], where we tested chemometric approaches of peak purity testing. It was concluded, that the simplest recommended method could be visual inspection of Principal Component Analysis (PCA) scores, as this method is most sensitive to inhomogeneity in the case of nonlinearity, spectral similarity and high overlap.

If the inhomogeneity is detected inside the spot, an average analyst wants then to obtain spectral profiles, estimated by the application of curve resolution methods. These methods try to find bilinear decomposition of spot matrix with one important constraint: spectral and concentration profiles must be nonnegative. This is the last part of our study and the subject of current poster. It discusses usefulness of self modelling multivariate curve resolution (nonnegative matrix factorization) as a chemometric tool for analysis of inhomogeneous spots captured by densitometer in multivariate way. Two examples are analyzed: a spot of decomposed aspirin with comparative spot of pure salicylic acid, and spots of overlapped ciprofibrate and clofibric acid.

In general, this approach works well in the case of TLC and the algorithm finds reliable spectral and concentation profiles, even with high overlap and spectral similarity. Nonlinearity does not affect this algorithm in visible manner.

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12.

The Investigation of the Sonogashira coupling in a heterogeneous system using chromatographic techniques.

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Sonogashira Coupling is used to form the C-C bonds between atoms of various hybridizations. Currently, most of the reactions are carried out in homogeneous systems using palladium catalysts such as: $Pd(PPh_3)_2Cl_2$, supported by a various compounds of copper(I), Ag_2O, silver or other. The Mechanism of Sonogashira coupling is not clearly understood.

In our work we used a novel heterogeneous catalysts in Sonogashira coupling. We revealed that, the whole system is influenced by temperature, time and the amount of phosphine. Various chromatographic methods were used for the purpose of this study. This includes: TLC, HPLC and HPLC-ESI-MS. These techniques provide qualitative and quantitative specification of the reaction products. Based on our studies and results we hypothesized that in the Sonogashira coupling a couple of products appeared, which depends on conditions such as: temperature, time and the amount of phosphine.

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13.

Comparative analysis of diesel oil samples of different origin based on chromatographic fingerprints

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Diesel oil is a very popular fuel used for transport purposes. It is a mixture of hydrocarbons refined from petroleum and contains additives modifying physico-chemical properties. To ensure certain quality of diesel fuel a constant monitoring of its chemical composition is required. Gas chromatography (GC) is one of the most popular techniques used for this purpose. It has a potential to separate and quantify chemical components. Gas chromatography aims to describe a sample by its chromatographic profile regarded as a fingerprint. However, the comparative analysis of chromatographic fingerprints requires application of advanced chemometric techniques.

In this work chromatographic fingerprints of diesel oils differed with respect to geographical location were characterized using gas chromatography with the flame ionization detection, GC-FID. Prior to analysis raw chromatograms were baseline corrected with the penalized asymmetric least squares approach (P-ALS) [1]. Then, correlation optimized warping (COW) [2] was used to correct misalignment of chromatographic peaks. Preprocessed fingerprints were a subject to further comparative analysis.

In order to visualize the differences between diesel oil samples principal component analysis (PCA) [3] was used. This exploration method allows distinguishing two groups of samples. It is possible to conclude the observed groups differ in content of fatty acid methyl esters, FAME. It is important to stress that preprocessing of chemometric fingerprints is an important step in comparative analysis and can greatly influence its results and conclusions.

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14.

Estimation of linear isotherm model parameters in Supercritical Fluid Chromatography (SFC)

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ABSTRACT

Supercritical fluid chromatography is one of the most attractive separation methods among chromatographic techniques. The use of mainly the carbon dioxide as mobile phase which is environmentally friendly, nontoxic, non-flammable, inexpensive makes SFC forward – looking separation technique. This is also consistent with so called "green chemistry" which is currently preferred in industrial applications. Moreover, industrial laboratories require faster and more efficient separation techniques such as SFC which allow achieve shorter retention times than HPLC ever shorter than in Ultra - High Performance Liquid Chromatography. Because of this advantages SFC is still develop.

Mathematical modelling of the chromatography process requires knowledge of the isotherm model. Therefore a necessary step in numerical calculations is estimation of isotherm model parameters. In this study the inverse method has been successfully used to estimation of linear isotherm model parameters in SFC. Estimation was done directly on the base of retention times of experimental profiles obtained in SFC by inverse method which minimize difference between calculated and experimental retention times. Experimental data were obtained for alkylbenzenes. The mobile phase was carbon dioxide-methanol, 95/5% v/v. The 250 mm x 4.6 mm i.d. column packed with 5 – micron Luna C18 particles was used. Separation was done for outlet pressure from 100 bar to 150 bar in different sets of experimental conditions: (1) column is operated under convective air; (2) column is operated in still air conditions. To estimate five parameters of linear isotherm model five experimental data were chosen which were obtained for column operated in still air conditions where radial gradients of temperature can be neglected. The estimation method was validated by comparing the appropriate experimental data with calculated on the basis of a mathematical model and estimated parameters of linear isotherm model. It was achieved good agreement.

15.

ANALYSIS OF SELECTED NON-STEROIDAL ANTI- INFLAMMATORY DRUGS FOR ANIMALS USING THE CHROMATOGRAPHIC TECHNIQUES.

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Non-steroidal anti-inflammatory drugs for small animals are used to control the pain and inflammation associated with osteoarthritis in pets. From the wide range, were selected four examples of anti- inflammatory drugs and were compared with standards (sample 1-4). In the present study the HPLC technique was used as the tool for analysis of active substance and cover of the analgesics pill as well as the quality of active substance control. The analgesics were respectably separated by the use of HPLC column Pinnacle DB PAH Dimensions: 50mm x 2.1mm ID Particle Size: 1.9µm Pore Size: 140Å, temp.: 30°C sample eluent: ACN/MeOH, conc from.: 20µg/mL each component inj. vol.: 5µL, mobile Phase A: water B: MeOH/ACN. Flow: 0,2-0,9mL/min. The chosen UHPLC system was equipped with micro-mixer, DAD and FLD detectors. For better identification and system efficiency determination the active compounds from each medicament were determined by the use of GC/MS system. Several chemicals were chosen for analysis, in the group were selected respectably: sample 1 - carprofen, sample 2 - tolfenamic acid, sample 3 - meloxicam, sample 4 – robenacoxib.

16.

STABILITY OF LIPOPHILIC VITAMINS IN FODDER PREMIXES

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Spectrophotometric and chromatographic method were applied in the examination of the stability of selected lipophilic vitamins, i.e. vitamin A and vitamin E. These were used in the form of their acetates what ensures their higher stability in the final product. However, the storage of the products, e.g. fodder premixes, is accompanied by the significant decrease of the content of both vitamins what results in the decrease of products quality. The series of fodder premixes of different composition were examined both standard procedures. Fourier transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC) and MS-ESI techniques were also used in the examination of the products after prolonged storage time. The results of the experiments allowed to present the preliminary conclusions concerning the magnitude and the factors influencing the vitamins degradation.

17.

TLC determination of tiapride hydrochloride and its impurities in pharmaceuticals

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Tiapride (N-[2-(diethylamino)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide) hydrochloride) is a substituted benzamide with the D_2/D_3 dopamine receptor antagonist activity and it belongs to the atypical antipsychotics with general properties similar to those of sulpiride.

Hydrolysis under the forced acidic and basic conditions leads to the hydrolysis of the amide bond to give 2-metoxy-5-methylsulphonylbenzoic acid (impurity I) and to the cleavage of the methoxy group (which is a vinylogous ester and therefore activated to hydrolytic cleavage by the virtue of being *ortho* to the ester functionality and *para* to the sulphone), to yield N-(-2-diethylaminoethyl)-2-hydroxy-5-methylsulfonylbenzamide (impurity II). Also, tiapride can be oxidized under relatively drastic conditions to give N-oxide, i.e., N-(2-diethylaminoethyl)-2-methoxy-5-methylsulphonylbenzamide-N'-oxide (impurity III).

A simple, rapid, accurate and precise HPTLC method is presented for simultaneous determination of tiapride hydrochloride and its three degradation impurities (I, II, and III), which have to be monitored in pharmaceutical dosage forms according to the manufacturer requirements of 0.2 %. Analysis of tiapride hydrochloride and impurities I, II, and III, was performed on silica gel 60 F_{254} HPTLC plates using methylene chloride-methanol-concentrated ammonia, 9:1.6:0.1 (v/v) as mobile phase. Detection was performed at 240 nm. All validation requirements, specificity, linearity ($r \geq 0.997$), recovery (95.08 %-100.39 %), limit of quantification (0.1-0.2 %), and robustness were examined and fulfilled, and the proposed method can be successfully applied for the quality control analysis of commercially available tablets and injections.

18.

Determination of water-soluble vitamins in European and Asiatic spices

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Vitamins are a broad group of organic compounds that are minor, but essential, constituents of food required for normal growth, self-maintenance, and functioning of human and animal bodies. These compounds can be classified in two main groups – water-soluble and fat-soluble vitamins. Because of the critical role of vitamins in nutrition and their relative instability, qualitative and quantitative analyses are important issues and a challenging task for food manufacturers.

In the present work, a method involving water-bath extraction, SPE (Bakerbond C_{18} (500-mg) cartridges) and HPLC – RP- C18 column chromatography with photodiode array detection is developed for determining the level of vitamins (e.g. ascorbic acid, folic acid, thiamine (vitamin B_1), D-biotin (vitamin B_7), niacinamide (vitamin B_3), calcium D-Pantothenate (vitamin B_5), pyridoxine hydrochloride (vitamin B_6), folic acid, and riboflavin (vitamin B_2) in European (pepper, basil, curcuma, thyme, cinnamon and ginger) and Asiatic spices (*Ocimum sanctum*, *Curcuma longa*, Ceylon cinnamon, Ginger root). The impact of procedure and various extraction conditions were tested.

The HPLC-PDA system was applied. Analyses were carried out on Discovery RP-C_{18} column (5 µm particle size, 150×4,6 mm, SUPELCO), maintained at 35°C. Mobile phase was A: 0.1 M KH_2PO_4 (pH 7.0) and B: methanol, in ratio 90:10 at isocratic condition The rate-flow was 0.7 ml/min. Identification of compounds was achieved by comparing their retention times and UV spectra with those of standards stored in a data bank. The accuracy of the method was tested by measuring average recovery; values ranged between 97.65 and 99.40%.

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19.

Chemometric approach to lipophilicity of selected cosmetic raw materials

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Retention parameters R_f and R_m were obtained for 16 cosmetic raw materials by thin-layer chromatography on RP-18 stationary phase using water-organic modifier (acetone, methanol, dioxane, acetonitrile, dimethylformamide or tetrahydrofurane) binary mixtures as mobile phases. First principal components (PC1) were calculated for R_f and R_m values obtained for each organic modifier used throughout this study and correlated with lipophilicities calculated *via* different methods (ALOGPs, AClogP, ABlogP, milogP, ALOGP, MLOGP, KOWWIN, XLOGP2, XLOGP3, ACDLab) and with experimental $logP_{o/w}$ found for 9 out of 16 investigated compounds. Analysis of correlations of PC1 calculated for R_f and R_m values revealed significant differences between the organic modifiers investigated with respect to chromatographic estimation of lipophilicity of studied compounds.

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20.

Single chromatographic run approach to lipophilicity of selected cosmetic raw materials

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Retardation factors R_f were obtained for 22 cosmetic raw materials by thin-layer chromatography on RP-18 stationary phase using water-organic modifier (acetone, methanol, dioxane, acetonitrile, dimethylformamide or tetrahydrofurane) binary mixtures as mobile phases. R_m values were calculated for different concentrations of organic modifiers (60, 70, 80 and 90 % v/v) in mobile phase and correlated with lipophilicities obtained *via* different theoretical methods (ALOGPs, AClogP, ABlogP, milogP, ALOGP, MLOGP, KOWWIN, XLOGP2, XLOGP3, ACDLab) and with experimental $logP_{o/w}$ found for 9 out of 22 investigated compounds. Analysis of correlations of R_m values with calculated and experimental logP values suggested that R_m values obtained for a single chromatographic run may be an interesting alternative to R_m^0 (retention factor extrapolated to zero concentration of an organic modifier) for compounds of moderate lipophilicity (0<logP<6).

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Thanks are due to Merck and BASF for free samples of sunscreens used through this investigation.