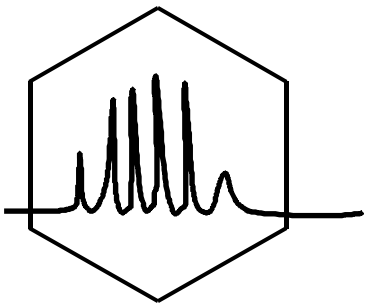
INSTITUTE OF CHEMISTRY, UNIVERSITY OF SILESIA,
KATOWICE, POLAND



THE XXXVIIIth SYMPOSIUM

CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS

MAY 27th-29th, 2015

KATOWICE – SZCZYRK

POLAND

PROGRAM

SCIENTIFIC COMMITTEE OF THE SYMPOSIUM

Teresa Kowalska (Katowice, Poland)

Jarosław Polański (Katowice, Poland)

Danica Agbaba (Belgrade, Serbia)

Andrzej Bąk (Katowice, Poland)

Michał Daszykowski (Katowice, Poland)

Josef Jampilek (Brno, Czech Republic)

Huba Kalasz (Budapest, Hungary)

Imre Klebovich (Budapest, Hungary)

Łukasz Komsta (Lublin, Poland)

Debby Mangelings (Brussels, Belgium)

Agnes Moricz (Budapest, Hungary)

Maja Natić (Belgrade, Serbia)

Hubert Paelinck (Ghent, Belgium)

Kornelia Tekes (Budapest, Hungary)

Monika Waksmundzka-Hajnos (Lublin, Poland)

Yvan Vander Heyden (Brussels, Belgium)

Jim Van Durme (Ghent, Belgium)

Irena Vovk (Ljubjana, Slovenia)

Christian Zidorn (Innsbruck, Austria)

ORGANIZATION COMMITTEE OF THE SYMPOSIUM

Teresa Kowalska (Katowice, Poland)

Jarosław Polański (Katowice, Poland)

Krystyna Jarzembek (Katowice, Poland)

Violetta Kozik (Katowice, Poland)

Marcin Rojkiewicz (Katowice, Poland)

Andrzej Bąk (Katowice, Poland)

SESSION I WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Danica Agbaba and Monika Waksmundzka-Hajnos

9.25 – 9.30 am **OPENING ADDRESS** (T. Kowalska; in memory of Dr. habil. Friedrich Geiss)

9.30 – 10.00 am

1. Characterization and classification of stationary phases in SFC (and HPLC)

Ch. Galea, D. Mangelings, Y. Vander Heyden

10.00 – 10.20 am

2. Method development for impurity profiling in supercritical fluid chromatography: The selection of a dissimilar set of stationary phases

Ch. Galea, D. Mangelings, Y. Vander Heyden

10.20 -10.40 am

3. Retention behavior of model compounds in thin layer chromatography with novel green mobile phases

Ł. Komsta, R. Skibiński, K. Modelska, A. Mularczyk, E. Piękoś

10.40 – 11.10 am

4. Chromatographic columns for HPLC: An overview of today's columns technology and their applications for analysis of some pharmaceutical products in drug quality control laboratories

M. Khalifa, O. El- Sayed Omar

11.10 – 11.40 am

5. TNF-α analysis and characteristic by the use of combined chromatography and mass spectrometry generated under influence of various genospecies of *Borrelia burgdorferi* sensu lato

A. Swinarew

11.40 – 12.00 am

6. Multi-dimentional chromatographic systems coupled with mass spectrometry

P. Stalica (Shim-Pol A. Borzymowski)

1.00 pm LUNCH

SESSION II WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Huba Kalasz and Ivan Vander Heyden

2.00 – 2.30 pm

7. Chemical changes in resins and relations with the mechanical properties of abrasive articles

A. Voelkel, B. Strzemiecka, J. Zięba-Palus, T. Lachowicz

2.30 – 3.00 pm

8. Impact of gas sampling in polymeric bags on the quality of quantitative VOC measurements: a critical evaluation

J. Van Durme

3.00 – 3.30 pm

9. Non-chromatographic applications of porous monolithic materials

M. Pietrzyńska, A. Voelkel

POSTER SESSION I WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Josef Jampilek and Jim Van Durme

4.00 – 5.30 pm

4.00 – 5.30 pm COFFEE BREAK

6.00 pm BONFIRE

SESSION III THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Kornelia Tekes and Hubert Paelinck

9.30 – 10.00 am

10. Thin-layer chromatography with biological detection as a method for screening drugs candidates in natural mixtures

M. Waksmundzka-Hajnos

10.00 – 10.30 am

11. Analysis of food supplements by planar chromatography

I. Vovk, V. Glavnik, A. Albreht, B. Simonovska

10.30 – 11.00 am

12. Detection and characterization of antibacterial components of *Onopordum acanthium*

Á. M. Móricz, Á. Alberti, A. Böszörményi, Sz. Béni, P. G. Ott

11.00 – 11.30 am

13. Natural products from seagrasses

Ch. Zidorn

11.30 -11.50 am

14. Hyphenated techniques in phenolic profiling of wild fruits grown in Serbia

D. Dabić, Ž. Tešić, V. Glavnik, I. Vovk, M. Fotirić, M. Natić

11.50 – 12.10 pm

15. Innovative sample introduction, extraction and separation technology for analytical systems such as HPLC, MS, GC and NMR

E. Cybulska (Donau Lab Sp.z o.o.)

1.00 pm LUNCH

SESSION IV THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Irena Vovk and Łukasz Komsta

2.00 - 2.30 pm

16. Update of a chiral separation strategy in capillary electrochromatography using chlorinated and non-chlorinated polysaccharide-based selectors

D. Mangelings, A. Hendrickx, D. Albals, B. Chankvetadze, Y. Vander Heyden

2.30 – 3.00 pm

17. Molecular lipophilicity profile in drug discovery: A comparative study for a set of the terephthalamides derivatives

A. Bąk , V. Kozik

3.00 – 3.20 pm

18. Testing of complementarity of PDA and MS detectors using chromatographic fingerprinting of genuine and counterfeit Viagra®

D. Custers, B. Krakowska, P. Courselle, M. Daszykowski, S. Apers, E. Deconinck

3.20 - 3.40 pm

19. Characterizations of *Ipomoea reptans* extracts through HPLC and HPTLC fingerprint development, phytochemical profiling and in vitro cytotoxicity and antioxidant activity determination

M. Hefny Gad, N. Elsawi, E. Elmewafy, S. Younes, Y. Vander Heyden, K. Demeyer, D. Mangelings

POSTER SESSION II THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Andrzej Bąk and Andrzej Swinarew

4.30 – 6.30 pm

4.30 – 6.30 pm COFFEE BREAK

POSTGRADUATE STUDENT SECTION THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Maja Natić and Adam Voelkel

4.00 – 4.20 pm

20. How to read and to write scientific publications: A personal view on an important piece of our individual scientific lives

H. Kalász

4.20 – 4.35 pm

21. Inverse liquid chromatography as a tool for biomaterials surface characterization

K. Adamska, K. Kadlec, A. Voelkel

4.35 – 4.50 pm

22. Determination of pesticides in herbs by gas chromatography

P. Marczewska, D. Szeremeta, M. Mucha, J. Rzepa, M. Sajewicz

4.50 – 5.05 pm

23. Investigation of the peptide nanofibers and nanospheres formation by chromatographic and microscopic techniques

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

5.05 – 5.20 pm

24. Investigation of peptide structures resulting from spontaneous oscillatory reactions of biogenic amino acids

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

5.20 – 5.35 pm

25. The GC-MS investigation of hydrogen–deuterium exchange amon selected 1,10-phenanthroline-4,7-dion

Marcin Szala, Karolina Czyż, Jacek E. Nycz

6.30 pm DINNER

SESSION V FRIDAY, MAY 29th, 2015

CHAIRPERSONS: Debby Mangelings and Agnes Moricz

10.00 – 10.30 am

26. Distribution of selegiline in the rats and rabbits

K. Tekes, H. Kalász

10.30 – 11.00 am

27. Determination of drugs loaded to silica nanoparticles in brain tissue

M. Oravec, J. Jampílek

11.00 – 11.30 am

28. Determination of interactions of drug and alcohol, caffeine, smoking and their clinical relevance with different bioanalytical technics

I. Klebovich

11.30 am CLOSING REMARKS

12.00 am LUNCH

POSTER SESSION I

1.

Synthesis of 1,10-phenantroline derivatives; the GC-MS investigation

K. Czyż, M. Szala, J.E. Nycz

2.

Analysis of vitamin C (ascorbic acid) in raw, pasteurized and ultra high temperature cow's milk using HPLC–PDA–ESI (+)-MS as a highly sensitive and highly confirmatory analytical tool

W. A. S. Gafour, A. F. Zayn, O. Al Sayed Omar

3.

The preliminary studies of the selected aqueous mosses using HPLC and TLC fingerprint methods

A. Hawrył, M. Hawrył, A. Turula, R. Świeboda, G. Jóźwiak

4.

The fingerprint analysis of selected *Cirsium* species using the HPLC and TLC methods

A. Hawrył, M. Hawrył, A. Ziobro, R. Świeboda, M. Waksmundzka-Hajnos

5.

TLC isolation of biologically active compounds from extracts obtained from different *Potentilla* species

G. Jóźwiak, M. Waksmundzka-Hajnos, W. Zieliński

6.

Searching for urine biomarkers of prostate cancer using LC-MS and GC-MS metabolomics approach

M. Kordalewska, W. Struck-Lewicka, R. Bujak, A. Yumba Mpanga, M. Markuszewski, J. Jacyna, M. Matuszewski, R. Kaliszan, M. J. Markuszewski

7.

Identification of tributyltin in environmental water samples supported by means of discriminant models

B. Krakowska, M. Daszykowski, K. Fabiańczyk, M. Korzeń

8.

The encounter of macrofunghi research and direct bioautography

D. Kruzselyi, J. Vetter, P. G. Ott, A. M. Moricz

9.

Optimization of the extraction of total heme from meat for determination by HPLC

H. De Maere, E. De Mey, M. Baca, M. Sajewicz, T. Kowalska, I. Fraeye, H. Paelinck

10.

Detection of ovarian cancer based on metabolomic data obtained from liquid chromatography coupled with mass spectrometry

P. Matuszewski, M. Daszykowski

11.

Between batch differentiation of stamping inks from the same manufacturer by means of thin layer chromatography

A. Menżyk, M. Sajewicz, G. Zadora

12.

Optimization of chromatographic condition for separation of ziprasidone and its impurities by TLC

D. Obradović, S. Filipić, K. Nikolić, M. Čarapić, D. Agbaba

13.

Bioautography as a method of determination of antibacterial properties with selected thyme species

M. Orłowska, W. Jesionek, B. Majer-Dziedzic, I.M. Choma, G. Szymczak, M. Waksmundzka – Hajnos, T. Kowalska, M. Sajewicz

14.

Qualitative evaluation of excise duty marker Solvent Yellow 124 in diesel oil samples

J. Orzel, M. Daszykowski

15.

Determination of acrylamide in coffee samples using the fluorescence spectroscopy extended with chemometric modeling approaches as a simple and cost-effective alternative to separation-based methods

A. Psiuk, J. Orzeł, M. Daszykowski

16.

Identification and separation of TNF-α after infection of three genospecies of *Borrelia burgdorferi* sensu lato by the use of chromatographic techniques. Preliminary results

B. Rozwadowska, J. Gabor, M. Łężniak, T. Flak, H. Okła, D. Wcisło-Dziadecka, U. Mazurek, K. Jasik, A.S. Swinarew

17.

Determination of resveratrol in fruit juices and herbal infusions

M. Skorek, K. Pytlakowska, K. Kocot, T. Kowalska, M. Sajewicz

18.

Determination of hexachlorocyclohexane (HCH) isomers and their biodegradation products in environment samples by GC

D. Szeremeta, P. Marczewska, M. Knaś, J. Rzepa, M. Sajewicz

19.

Application of smoke condensates in smoking process

A. Włosowicz; O. Goemaere, A. De Winne , J. Van Durme

20.

LC-MS and GC-MS based untargeted metabolomics to study urologenital tract cancer heterogeneity

A.Yumba Mpanga, W. Struck-Lewicka, R. Bujak, M. Markuszewski, M. Roslan, M. J. Markuszewski, R. Kaliszan

21.

Analysis of insect signalling using ion-trap mass spectrometry a case of *dendrolimus pini*

D. Staszek, K. J. Rudzinski, M. Asztemborska, M. Cieślak, J. Raczko,
R. Szmigielski

POSTER SESSION II

1.

Synthesis and physicochemical properties of terephthalamides

R. Biegun, K. Bochenek, A. Jędrzejowska, V. Kozik

2.

Top 100 drug bestsellers are getting older

J. Bogocz, A. Tkocz, J. Polanski

3.

Synthesis of a new nanographene model containing seven-membered ring

P. Dybał, I. Rodríguez Márquez, A. González Campaña, J. Manuel Cuerva Carvajal, V. Kozik

4.

Mercapto-modified graphene oxide for determination of divalent metal ions and arsenic species

P. Janik, B. Zawisza, E. Talik, E. Margli, I. Queralt, R. Sitko

5.

Synthesis, properties and applications of thioterephthalamides

A. Jędrzejowska, M. Matussek, V. Kozik, A. Bąk, Ł. Pieszczek, P. Kuś

6.

Investigation of alcohols and polyols oxidation products over Au_{NPs} and Pd_{NPs} catalysts using ^{1}H, ^{13}C NMR and 2D techniques

M. Kapkowski, M. Słota, J. Polański

7.

Application of microextraction procedures of determination of trace elements by spectroscopy techniques

K. Kocot, R. Sitko

8.

Synthesis of building blocks in the quasi-heterogeneous reactions with nano-Pd/Cu catalyst - monitoring the progress of reaction by TLC

M. Korzec, R. Rzycka, S. Senkała, J. Polański

9.

Investigation of antioxidant activity of pomegranate juices by means of electron paramagnetic resonance (EPR) spectroscopy and UV-VIS spectrophotometry

V. Kozik, A. Bąk, K. Jarzembek, M. Rotkiewicz, A. Jędrzejowska, P. Dybał, K. Pytlakowska, J. Polak, M. Bartoszek, S. Oślizlok, M. Przybyszewska, A. Stasiuka, N. Niestrój, A. Kurpanik, K. Nowosińska

10.

Bio-corrosion in biopurification of air fromVOC's mixture

V. Kozik, A. Bąk, D. Kasperczyk, S. Kuś, K. Barbusiński

11.

Synthesis of phthalimides and isophthalamides obtained from methyl esters of selected amino acids

A. Kruk, P.Sobota, K. Jarzembek, P. Kuś

12.

Chromatographic and spectroscopic methods for the identification of two new psychoactive substances contained I “designer drugs”

M. Majchrzak, M. Rojkiewicz, A. Mazurkiewicz, R. Celiński, M. Sajewicz

13.

Differences in polyphenolic and elemental composition of red and white Serbian wines

M. Natić, M. Pantelić, J. Mutić, Ž. Tešić

14.

Elemental composition and antioxidant activity of selected juices

K. Pala, K. Pytlakowska, M. Skorek, V. Kozik, R. Sitko

15.

Analytical techniques used in the synthesis of novel thiosemicarbazones based on 5-bromosalicylaldehyde

M. Rejmund , J. Polański

16.

One-pot synthesis of 1,4-disubstituted-1,2,3-triazoles using nano-Pd/Cu catalyst

R. Rzycka, S. Senkała, M. Korzec, J. Mularski, J. Polański

17.

Studies on the Cadiot - Chodkiewicz coupling reaction in heterogeneous system using chromatographic techniques

S. Senkała, R. Rzycka, M. Korzec, J. Polański

18.

Determination of lipid concentration in liposomal drug formulation

J. Procek, M. Langer, M. Przybyło

19.

An analysis of fragmental drug-likeness topology

A. Tkocz, J. Bogocz, J. Polanski

20.

Application of high-performance counter-current chromatography for the isolation of coumarins from the non-polar extract of Mutellina purpurea L

M. Walasek, K. Skalicka-Woźniak, T. Mroczek

21.

A new similarity measure for comparative analysis of two-way chromatographic fingerprints

K. Drab, M. Daszykowski

22.

Chromatographic separation of products of 4-bromotoluene nitration

J. Bebłot, J. Polański

SESSION I

WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Danica Agbaba and Monika Waksmundzka-Hajnos

1.

Characterization and Classification of stationary phases in SFC (and HPLC)

Charlene Galea, Debby Mangelings, Yvan Vander Heyden

Department of Analytical Chemistry and Pharmaceutical Technology, Centre for Pharmaceutical Research, Vrije Universiteit Brussel - VUB, Laarbeeklaan 103, 1090 Brussels, Belgium

Packed column supercritical fluid chromatography (pSFC) is an attractive technique in drug discovery related analysis because it offers several advantages over the more commonly used high-performance liquid chromatography (HPLC) technique. Column characterization aims to obtain a quantitative understanding of the properties of a column that influence the selectivity of a separation. Determining column properties allows the rapid selection of dissimilar columns for method development for a particular application. Columns have been extensively characterized in HPLC using several approaches. However, limited column characterization has been done in SFC.

Common methods used to characterize stationary phases in HPLC include thermodynamic methods, spectroscopic techniques, and chromatographic test methods. The linear solvation energy relationship (LSER) model (Abraham's model) and the carotenoid method are two methods which are used to characterize columns in both HPLC and SFC. LSER is a quantitative structure retention relationship (QSRR) model in which solute parameters, such as polarizability, dipolarity, steric and hydrogen-bonding properties are linked to the solute's retention through linear regression in order to get a better understanding of the applied stationary-phase properties. A closer look at the LSER coefficients will help understanding the retention differences observed between SFC and HPLC conditions, for columns with similar chemistries. The carotenoid test consists of the analysis of carotenoid pigments and evaluates polar surface activity, absolute hydrophobicity and the steric separation factor of octadecylsilica (ODS) stationary phases.

The aim of this presentation is to give an overview of the approaches used to characterize stationary phases in SFC (and HPLC), and to highlight topics that may still need to be researched further.

2.

Method development for impurity profiling in supercritical fluid chromatography: The selection of a dissimilar set of stationary phases

Charlene Galea, Debby Mangelings, Yvan Vander Heyden

Department of Analytical Chemistry and Pharmaceutical Technology, Centre for Pharmaceutical Research, Vrije Universiteit Brussel - VUB, Laarbeeklaan 103, 1090 Brussels, Belgium

Supercritical fluid chromatography (SFC) is gaining considerable interest as a separation technique in the pharmaceutical industry. The use of SFC as a technique for drug impurity profiling is examined here. To define potential starting conditions in method development for drug impurity profiling, a set of dissimilar stationary phases should be screened in parallel.

This study evaluates the possibility to select a set of dissimilar columns in SFC using the retention factors (k-values) for a set of 64 compounds measured on 27 columns. Experiments were carried out at a back pressure of 150 bar and 25◦C with a mobile phase consisting of CO_2 and methanol containing 0.1% isopropylamine (5–40% over 10 min) at a flow rate of 3 mL/min.

The k-values of the drugs were then used to calculate correlation coefficients between two columns on the one hand and to perform principal component analysis on all column data on the other. The Kennard and Stone algorithm, dendrograms and correlation-coefficient colour maps were used to select a set of dissimilar stationary phases. Derringer's desirability functions, a multi-criteria decision making technique was used to rank stationary phases used according to overall column performance. The stationary phase characterization results from this study were compared to those from previous studies found in the literature. The dissimilarity of the selected subset of stationary phases was finally validated using mixtures of compounds with similar properties and structures, as one can expect in a drug impurity profile.

3.

Retention behavior of model compounds in thin layer chromatography with novel green mobile phases

Łukasz Komsta, Robert Skibiński, Katarzyna Modelska, Aneta Mularczyk, Ewa Piękoś

Department of Medicinal Chemistry, Faculty of Pharmacy
Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

Nowadays, the green mobile phases have an increasing interest in separation science and this strategy fits the current ecological trends. The aim of the green chromatography is the use of non-toxic and environmental friendly substances in the mobile phase preparation.

As the literature on such trials in thin layer chromatography is very limited, the aim of our study was to check the ability to use simple and non-toxic organic substances in aqueous solutions as mobile phases on different adsorbents.

In this study, we used 35 simple model organic compounds (including drugs), which were also used in our previous research (see for example [1]) on separation behavior in TLC. They belong to different chemical groups and possess various chemical properties.

These compounds were separated on RP18, RP8, DIOL, CN, NH2 and cellulose plates with the use of: urea, N-methylurea, N,N-dimethylurea, O-methylisourea, 2-hydroxyethylurea, N,N'-bis(hydroxymethyl)urea and guanidine aqueous solutions as mobile phases, in concentration varying from 0.5 mol/l to 4 mol/l. Plates were developed in 9 cm distance in horizontal non-equilibrated DS chambers.

All the results were subjected to multivariate chemometric analysis methods which allowed explanatory data analysis and decomposed the retention data to several independent trends.

The best results were achieved on DIOL, RP8 and RP18 plates. CN plates gave well-established spots, but they suffer from long development time and curvy migration front. There is no significant difference in spots shape between used modifiers.

[1] Komsta, Ł. Skibiński, R. Gowin, E. Ma□czka, P. Exploring hidden trends in classic and Micellar thin-layer chromatographic retention of model compounds by chemometric methods. J. Liq. Chrom. 36:17, 2348-2362.

4.

CHROMATOGRAPHIC COLUMNS FOR HPLC: An Overview Of Today's Columns Technology And Their Applications For Analysis Of Some Pharmaceutical Products In Drug Quality Control Laboratories

Moustafa Khalifa[a] *and Omar El- Sayed Omar*

Drug and Food Quality Control Laboratories , Ministry of Health , Kuwait .

E-mail corresponding author : drma.moustfa@gmail , tel. +96566643183 .

It is true that the possibilities of HPLC continually being extended through the development in HPLC- column technology, advances in instrumentation design and performance. The HPLC column is the heart of the HPLC – instrument and essential to its success. Today's HPLC column technology offering high efficiency, high resolution, short analysis time, use of minute volumes and a wide pH range of mobile phase. Drug analysis in drug quality control laboratories of Kuwait have been benefiting from these advanced features of today's HPLC column technology considering the main advantages over conventional HPLC columns. This will be done by reporting some of our recent results obtained by using these columns (Symmetry , Symmetry Shield and XTerra columns) for analysis of some pharmaceutical preparation according to the requirements of drug manufacturers specification or drugs pharmacopeias. Determination of molecular size distribution as a quality control test for Human Albumin in pharmaceutical preparations was done using Size Exclusion (SE) columns. HPLC – column for Mass Spectrometry was employed as analytical column (C18, 150 mm X 2.1mm and 5um particle size, Symmetry 300 Waters, Milford, MA, USA) for screening studies which was conducted to investigate the presence of three synthetic PDE-5-inhibitors, Sildenafil (S), Tadalafil (T) and Vardenafil (V) illegally adulterated in natural herbal products. These herbal products have been a subject for registration by Kuwait Drug and Food Quality Control Administration (KUFDA) as natural herbal products for improving sexual performance for man in the period from 2003 to 2012. Analytes detection was done simultaneously by PDA and MS. Nowadays in our laboratories, instead of Atomic Absorption Spectroscopy (AAS), HPLC with conductivity detector and cation or /anion columns were employed for analysis of cations such as , Na^{+}, K^{+} , Mg^{++} , Ca^{++} in Balance Salt Solution (BSS) and anions such as Cl^{-} in Movicol sachets (for the relief of constipation). Based on Ion Exclusion Chromatographic mechanism (polymethacrylate – based weak acidic cation exchange resin HPLC column) with detection UV, a simple,selective and sensitive method for the determination of carboxylic acids in Renal Dialysis solutions was used in our laboratories. Finally and on the base of our results, it can be said that using today's column technology by the analysts in Drug Quality Control Laboratories, the lab productivity and accuracy will be increased.

5.

TNF-α analysis and characteristic by the use of combined chromatography and mass spectrometry generated under influence of various genospecies of *Borrelia burgdorferi* sensu lato

A.S. Swinarew[1*],B. Rozwadowska[2],J. Gabor[1], M. Łężniak[1], T. Flak[1], H. Okła[1,2], K. Jasik[2],

[1]*Institute of Materials Science, University of Silesia, 40-007 Katowice, Poland*

[2]*Department of Skin Structural Studies, Medical University of Silesia, 41-200 Sosnowiec, Poland*

andrzej.swinarew@us.edu.pl

A rapid separation and characterization method for online detection of TNF-α (fig.1) generated under exposition of various genospecies of *B. burgdorferi* s.l. was developed based on UHPLC, GPC and MALDI-ToF analyses (fig. 2).

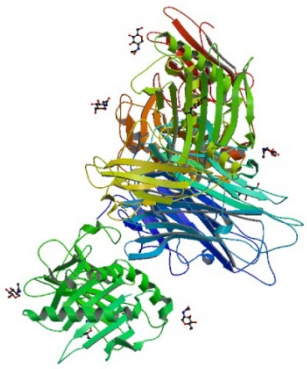


Fig. 1. Crystal structure of TNF-α

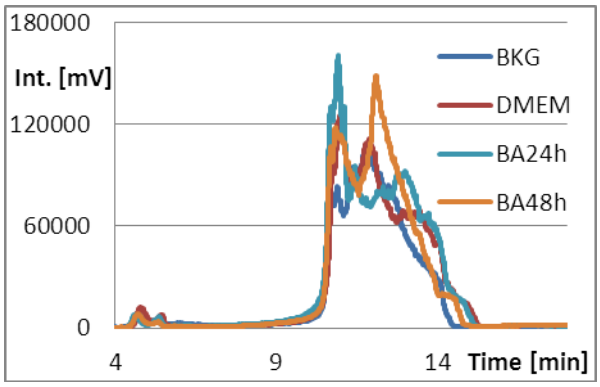


Fig. 2. GPC separation of TNF-α obtained after human fibroblasts (NHDF) were infected with genospecies of *B. burgdorferi* s.l.

TNF-α (tumor necrosis factor alpha) is a cytokine with multiple biological activities. It stimulates the immune cells to secrete cytokines, the epithelial cells to expansion adhesion molecules important for binding leukocytes, has an pyrogenic effect.

The HPLC equipped with micro mixer, DAD and FLD detectors was used as the tool for QA/QC analysis of TNF-α.HPLC Pinnacle DB PAH column dimensions: 50 mm x 2.1 mm, particle size:1.9 µm pore size:140 Å, temp.:30 °C sample eluent: physiologic salineconcentration from:800 ppm each component inj. vol.:1 µL, mobile phase A: water B: acetonitrile. flow rate: 0,2-0,9 mL/min was used. For identification of TNF-α excitation and emission wavelength, separated standard samples were investigated by the use of spectrofluorometer. Additional, for better separation and structure investigation UHPLC-GPC equipped with fraction collector and MALDI ToF^2 MS^2 techniques were used.

6.

Multi-dimentional chromatographic systems coupled with mass spectrometry

R. Zera

Shim-Pol A. Borzymowski

Początki obecnej firmy „SHIM-POL A.M. Borzymowski" E.Borzymowska-Reszka A.Reszka Spółka Jawna sięgają roku 1986. W tym roku mgr inż. Marek Borzymowski stworzył wyłączne przedstawicielstwo koncernu SHIMADZU w Polsce pod nazwą SHIMADZU Polska Analityka. W kolejnych latach, w celu zapewnienia swoim klientom kompleksowej oferty, nasza firma rozpoczęła współpracę ze światowymi liderami w zakresie produkcji sprzętu laboratoryjnego i innymi. Należy podkreślić, że SHIM-POL A.M. Borzymowski reprezentuje na zasadzie wyłączności koncerny międzynarodowe Shimadzu, Phenomenex®, Chromacol, ANTEC, PEAK SCIENTIFIC oraz jest dystrybutorem niewyłącznym kilku innych firm międzynarodowych oferujących akcesoria do aparatury analitycznej.

W 2001 roku firma zmieniła nazwę na SHIM-POL A.M. Borzymowski, w 2005 roku została utworzona spółka cywilna SHIM-POL A.M. Borzymowski s.c. Od roku 2007 nasza firma funkcjonuje pod obecną nazwą „SHIM-POL A.M. Borzymowski" E. Borzymowska-Reszka, A. Reszka Spółka Jawna.

Od początku istnienia, nasza firma zainstalowała w całym kraju już ponad 1700 aparatów w laboratoriach prywatnych, uniwersyteckich i instytucjach państwowych prowadzących badania z zakresu ochrony środowiska, farmacji, badania żywności, petrochemii oraz innych. Atrakcyjność aparatury SHIMADZU polega na jej różnorodności, wysokiej jakości, konkurencyjnych cenach oraz na profesjonalnej obsłudze serwisowej. Oferujemy naszym klientom nie tylko wsparcie w zakresie obsługi oferowanych przez nas urządzeń, lecz także zapewniamy wsparcie techniczne i aplikacyjne.

Oferujmy następujące rozwiązania:

chromatografy UHPC, HPLC i GC; systemy MS: GC-MS(MS), LC-MS(MS), LCMS-IT-TOF, MALDI-TOF-TOF, spektrofotometry UV-VIS, FTIR, RF i AAS, analizatory TOC; spektroskopy do analizy powierzchni ESCA-XPS, SIMS, ISS i Auger oraz analizatory fluorescencji rentgenowskiej (EDX). Oferujemy również akcesoria: PHENOMENEX, ANTEC LEYDEN, RHEODYNE, CHROMACOL, PEAK SCIENTIFIC – generatory, PIKE - akcesoria IR, HORIZON TECHNOLOGY - automaty do ekstrakcji, zatężania i osuszania próbek, w tym automatyczny koncentrator XcelVap, SUPERCRITICAL FLUID TECHNOLOGIES - aparaty do prowadzenia ekstrakcji cieczą w stanie nadkrytycznym oraz reaktory wysokociśnieniowe.

SESSION II

WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Huba Kalasz and

Ivan Vander Heyden

7.

Chemical changes in resins and relations with the mechanical properties of abrasive articles

Adam Voelkel[1], Beata Strzemiecka[1], J. Zięba-Palus[2], T. Lachowicz[3]

[1] Poznan University of Technology, Institute of Chemical Technology and Engineering, Berdychowo 4, 60-965 Poznań, Poland

[2] Institute of Forensic Research, Westerplatte 9, 30-033 Kraków, Poland

[3]Jagiellonian University, Faculty of Chemistry, Ingardena 3, 30-060, Krakow, Poland

Adam.Voelkel@put.poznan.pl , Beata.Strzemiecka@put.poznan.pl

Aging of adhesives is important practical problem that influences on the functional properties of many products such as glues, furniture, bonded abrasive articles. In this paper aging of phenol-formaldehyde resins were studied. Tested resins are used as a binder in abrasive articles. Chemical changes occurring during storage of phenolic resins were assessed by different instrumental methods: Fourier Transform Infrared Spectroscopy (FT-NIR), Inverse Gas Chromatography (IGC), X-ray Photoelectron Spectroscopy (XPS). Thermo-mechanical changes of the model final products were determined by Dynamic Mechanical Thermal Analysis (DMTA). Presented results showed subtle chemical changes during storage. However, thermo-mechanical properties of model final products changed more significantly.

8.

Impact of gas sampling in polymeric bags on the quality of quantitative VOC measurements: a critical evaluation

Jim Van Durme*

*Research Group Molecular Odor Chemistry, KU Leuven Technology Campus Ghent, Gebroeders De Smetstraat 1, B-9000 Ghent, Belgium (*corresponding author: jim.vandurme@kuleuven.be)*

ABSTRACT

In the field of environmental analysis, researchers rely on the accurate qualitative and quantitative assessment of odorous compounds in the gas phase. In recent years, attention has been mainly given to advanced hyphenated analytical techniques and the development of new detectors (e.g. e-noses, SIFT-MS, etc.). However, the sample collection step is equally important and strongly influences reproducibility and accuracy. Whole air sampling using polymeric bags is still one of the most frequently used sample collection methods in the field. The degree of scalping, which is defined as sorption of the volatiles on the inner surface of polymeric sampling bag, is often underestimated, in particular in the field of environmental sampling. Own experiments revealed that after introducing a wide range of volatiles in a two-phase system containing Nalophan, recoveries decreased down to 57% in a period of 22 hours.

In this lecture chromatographic expertise is been used to develop a Phase Ratio Variation (PRV) method as a fast and efficient manner for predicting the degree of scalping for individual compounds, and thus enabling to compensate for sorption phenomena. This method requires limited measurements, without the need for time-consuming calibrations. Moreover, a correlation was found between partitioning coefficients and the liquid molar volume for a number of aliphatic, aromatic and oxygenated compounds.

9.

Non-chromatographic applications of porous monolithic materials

M. Pietrzyńska, A. Voelkel

Poznań University of Technology, Institute of Chemical Technology and Engineering,

ul. Berdychowo 4, 60-965 Poznań, e-mail: monika.pietrzynska@put.poznan.pl

Monolithic columns are commonly used in high performance liquid chromatography (HPLC). The development of monolithic stationary phases based on either silica or polymer skeleton is a relatively recent achievement in the preparation of chromatographic columns [1]. Monolithic stationary phases prepared from hydrophobic, hydrophilic or charged monomers can be employed for a wide range of applications.

Monolithic materials found also increasing role as sorbents for a sample preparation [2]. In most cases they are located in capillary column [3] or poly(ether ether ketone) (PEEK) tube [4]. The first report of a polymer monolith used for SPE was presented by Xie et al. in 1998 [4]. Less known use of monoliths was described by Svec in the review [1]. Miyazaki et al. prepared monolithic silica extraction tip. In this way the solid-phase extraction (SPE) tool was combined with pipette-tip shape [5]. Monolithic porous polymer for on-chip solid-phase extraction was invented by Yu et al. [6] Microextraction in a packed syringe (MEPS) was introduced by Abdel-Rehim [7]. In this device, a solid support is inserted directly into a syringe as a plug (with a filter at either end of the plug holding the solid phase), and fitted manually into the syringe. Stir cake sorptive extraction using monoliths as extractive medium was developed by Xiaojia Huang [8]. Shintani et al. dealt with monolithic silica column for in-tube solid-phase microextraction coupled to high-performance liquid chromatography [9].

Incorporation of a monolithic material in the needle for the extraction purposes is a quite new proposal [10]. In-needle extraction technique was up-today limited to bulk sorbents, which can be displaced in the needle or even removed from the needle. The application of monolithic filling of the in-needle device should prevent changes occurring in the sorbent layer and increase the efficiency of this sample preparation tool.

Monolithic scaffolds modified with nanostructures are not only used for chromatographic separations but are finding use in a broad range of applications [11]. Recently, monolithic scaffolds incorporating these nanoparticles were applied in cell cultivation and tissue engineering [12]. Porous monolithic inorganic/polymeric hybrid materials in a disk format were prepared using ring-opening metathesis polymerization (ROMP).

[1] F. Svec, T. B. Tennikova, Z. Deyl, Eds., Monolithic Materials: Preparation, Properties,and Applications. Elsevier, Amsterdam, 2003
[2] O. G. Potter , E. F. Hilder J. Sep. Sci. 31 (2008) 1881-1908
[3] J. Urban, F. Svec, J. M.J. Fréchet, J. Chromatogr. A, 1217 (2010) 8212-8221
[4] S. Xie, F. Svec, J. M. J. Fréchet, Chem. Mater. 10 (1998) 4072-4078
[5] Y. P. Zhang, X.W. Ye, M. K. Tian, L. B. Qu, S. H. Choi, A, I. Gopalan, K. P. Lee, J. Chromatogr. A, 1188 (2008) 43-49
[6] M. Motokawa , H. Kobayashi , N. Ishizuka , H. Minakuchi, K. Nakanishic, H. Jinnai, K. Hosoya, T. Ikegami, N. Tanaka, J. Chromatogr. A, 961 (2002) 53-63
[7] M. Abdel-Rehim, J. Chromatogr. A 1217 (2010) 2569-2580
[8] X. Huang, L. Chen, F. Lin, D.Yuan, J. Sep. Sci. 34 (2011) 2145-2151
[9] O. G. Potter , E. F. Hilder J. Sep. Sci. 31 (2008) 1881-1908
[10] M. Pietrzyńska, A. Voelkel, K. Bielicka-Daszkiewicz, Analytica Chimica Acta 76(2013) 50-56
[11] J. Krenkova, F. Foret, F. Svec, J. Sep. Sci., 35 (2012) 1266–1283
[12] Weichelt, F., Frerich, B., Lenz, S., Tiede, S., Buchmeiser, M. R., Macromol. Rapid Commun. 31 (2010) 1540– 1545.

SESSION III

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Kornelia Tekes and Hubert Paelinck

10.

Thin-Layer Chromatography with biological detection as a method for screening drugs candidates in natural mixtures

Monika Waksmundzka-Hajnos

Department of Inorganic Chemistry, Medical University of Lublin, Poland

Thin-layer chromatography coupled with biodetection is very useful for screening of wide range of plant extracts for fishing out bioactive compounds. It should be emphasized that TLC is most often the only method for detection of pharmacologically active compounds. TLC gives various possibilities to separate several samples in parallel in the same conditions and time to obtain a lot of results simultaneously. The experiments can be delivered with the crude plant extracts. It gives time and labor saving. Investigator has an open access to the adsorbent bed with separated mixtures and can perform observations in various conditions and obtain scans at various wavelength. Then one can perform biological detection. In such situation one wants to see only those compounds which possess desired activity, and does not care about the rest. The comparison of physicochemical vs. biological detection gives possibility to recognize which compounds or compound groups exhibit specific activity. It is the first step to obtain information about drug candidates before search of compound identity, isolation of it and investigation of activity which should follow-up preliminary experiments.

In such a way various active substances can be detected such as: new antibiotics and chemotherapeutics, new drugs for the therapy of: Alzheimer's disease, hypertension, diabetes, depression, obesity and free radical scavengers, which prevent deleterious effects of oxidative stress.

Detection of antibiotics can be performed by bioauthography (contact, agar-overlay or direct one) with bacteria growing on the chromatographic plate (or its imprint). After visualization using bacteria-coloring agent antibiotics appear as white zones against a color background.

Detection of enzyme inhibitors such as acetylcholinoesterase inhibitors, α- and β-glucosidase inhibitors, lipase inhibitors, xanthine oxidase inhibitors relays on the making contact of the plate with the selected enzyme and after that incubation under proper conditions of temperature and humidity followed by derivatization. Inhibitors should appear as white inhibition zones on the color background.

For detection of free radical scavengers various tests can be performed by use of stable radicals such as DPPH', ABTS or by β-carotene - linoleic acid assay.

The results of TLC-biodetection for various groups of natural compounds are also discussed.

11.

Analysis of food supplements by planar chromatography

Irena Vovk, Vesna Glavnik, Alen Albreht, Breda Simonovska

National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia; irena.vovk@ki.si

The endless possible combinations of the bioactive ingredients and excipients in different formulations such as hard/soft capsules, tablets, and liquids make the analysis of food supplements very difficult. Bioactive ingredients can be bioactive natural compounds, vitamins, minerals, plant extracts or even several plant extracts in one dietary supplement product, chemically modified compounds (e.g., encapsulated), etc.. Additional problems are the lack of available reference standards, standard reference materials (SRM), and marker compounds of particular plant materials, plant extracts or plant extract fractions, as well as a variety of chemical structures including isomeric compounds.

We will show the state-of-the-art and the potential of the planar chromatography in the analysis of food supplements with carotenoids, flavanols, stilbenes and methylxanthines as bioactive ingredients. The examples will include fast chemical fingerprinting, identification and characterization of biomarkers and analysis of adulterants with a focus on the instrumental detection possibilities (densitometry, image analysis, mass spectrometry) in solving the analytical challenges related to different combinations of bioactive ingredients in multi-ingredient food supplements.

12.

Detection and characterization of antibacterial components of *Onopordum acanthium*

Ágnes M. Móricz[1], Ágnes Alberti[2], Andrea Böszörményi[2], Szabolcs Béni[2], Péter G. Ott[1]

[1] *Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman O. Str. 15, H-1022 Budapest, Hungary*
[2] *Department of Pharmacognosy, Faculty of Pharmacy, Semmelweis University, Üllői Str. 26, H-1085 Budapest, Hungary*
e-mail: moricz.agnes@agrar.mta .hu

To fight against various diseases there is an increasing demand for effective compounds applicable in human and animal medicine as well as in plant protection. It is especially true for the antimicrobials, because the origin of a broad range of diseases is infection, and the incidence of (multi)drug resistance in pathogens is increasing against the widely used antimicrobials.

The aim of this study was to isolate and characterize the antibacterial components sourced from scotch thistle (*Onopordum acanthium*) plant extracts. High-throughput thin-layer chromatography - direct bioautography was utilized as bio-monitoring system for the detection, purification and isolation of the active compounds. The test organisms were Gram negative pepper pathogen *Xanthomonas vesicatoria*, the luminescence gene-tagged *Arabidopsis* pathogen *Pseudomonas syringae* pv. *maculicola*, the naturally luminescent marine *Vibrio fischeri* bacteria and the Gram positive soil bacterium *Bacillus subtilis*.

One major antibacterial component was found in each samples (root and leaf), which showed activity against all tested bacteria. The active compounds were enriched and isolated by means of gravimetric column and flash chromatography as well as preparative TLC, OPLC and HPLC. OPLC and HPLC are preferable, providing better separation and the possibility of the on-line fraction collection.

The isolated components with confirmed antibacterial effect were characterized and/or identified by LC-ESI-MS/MS (with positive and negative ionization modes) and NMR.

A lignin derivative and long chain aldehydes were found as active major components. Sesquiterpene alcohol and sesquiterpene lactones were also established as active components, compounds that are characteristic secondary metabolites of the *Asteraceae* family.

This work was supported by OTKA grant PD83487, and Á.M. Móricz was supported by Bolyai grant.

13.

Natural Products from Seagrasses

Christian Zidorn

Institut für Pharmazie/Pharmakognosie, Leopold-Franzens-Universität Innsbruck, Centrum für Chemie und Biomedizin, Innrain 80/82, A-6020 Innsbruck, Austria.

Current address: Istituto di Chimica Biomolecolare – Consiglio Nazionale delle Ricerche, Via Campi Flegrei, 34, 80078 Pozzuoli (NA), Italy.

Seagrasses are the only higher plants which are occurring in marine environments. Seagrasses are flowering plants (Division Angiospermae) belonging to four closely related plant families (Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, and Zosteraceae), which grow in marine, fully saline environments. There are 12 genera of seagrasses with about 60 species known. The secondary metabolite profile of seagrasses is of particular interest, a) because of their unique ecology as the only marine higher plants and b) from an evolutionary perspective, because seagrasses are descendants from higher land plants which have reverted to marine life. Thus, seagrasses are the only marine organisms featuring the spectrum of secondary metabolites also commonly found in higher land plants but on the other hand seagrasses contain very different secondary metabolites from sympatric other groups of plants such as brown, red, and green algae.

The secondary metabolite profile of seagrasses and the methods used to analyse it will be discussed. These chemosystematic data will be compared with data from the closest relatives of seagrasses, i.e. other families of the order Alismatales s.l., which predominantly encompass freshwater species such as the taxa of the Potamogetonaceae.

Seagrasses contain many groups of secondary metabolites commonly found in higher plants such as caffeic acid derivatives and flavonoids. Additionally, some rare groups of natural products including diarylheptanoids, unusual diterpenes, sulfated flavonoid glycosides, and sulfated phenolic acids have been found. Though seagrasses are of particular ecological relevance for the marine environments they are growing in, little is known about the ecologial role of secondary metabolites from seagrasses, both in response to abiotic factors as well as in the response to other marine organisms feeding on seagrasses.

Besides the description of seagrass secondary metabolites and the discussion of analytical studies for their detection and quantification, the presentation will strive to identify gaps in the current knowledge of the phytochemistry of seagrasses and their ecological role, and to identify some priorities for future research.

14.

Hyphenated techniques in phenolic profiling of wild fruits grown in Serbia

Dragana Dabić[a], ŽivoslavTešić[b], V. Glavnik[c], Irena Vovk[c], Milica Fotirić[d], Maja Natić[b]

[a]*Innovation Centre, Faculty of Chemistry Ltd., University of Belgrade, Studentskitrg 12-16, 11158 Belgrade, Serbia*

[b]*Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia*

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

[d]*Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Serbia*

Wild fruits are unfairly forgotten and pushed aside in comparison to other cultivated species. Our study on *Morus alba* species showed that mulberries are rich in health beneficial secondary metabolites [1]. The characteristic phenolic acids and flavanoids were identified using Ultra High-Performance Liquid Chromatography coupled with Linear Trap Quadrupole and OrbiTrap mass analyzer (UHPLC-LTQ OrbiTrap MS). Quantification of polyphenolics was realized using UHPLC coupled with a diode array detector and triple-quadrupole mass spectrometer (UHPLC DAD-MS/MS). Differences in the contents of anthocyanins, phenolic acids and non-anthocyanins among all the samples were evident. Except for derivatives of chlorogenic acids, this was the first report on the identification of individual hydroxycinnamic acid esters in *Morus alba* L. fruits. Our latest research in the field of phenolic profiling of indigenous fruits from Serbia was conducted on Elderberry (*Sambucus nigra*), Rose hip (*Rosa* sp.), and Cornelian cherry (*Cornus mas*). UHPLC DAD-MS/MS was used in order to quantify characteristic phenolics. In order to trace other polyphenols accurate mass search was performed using LTQ OrbiTrap MS. As a third hyphenated technique thin-layer chromatography coupled with mass spectrometry gave rise to distinctive profiles of the extracts. Monomeric and polymeric flavan-3-ols were found to be the major polyphenols extracted from the Rose hip samples, while anthocyanins were characteristic for Elderberry and Cornelian cherry.

[1] Maja M. Natić, Dragana Č. Dabić, Adele Papetti, Milica M. Fotirić Akšić, Vladislav Ognjanov, Mirjana Ljubojević, Živoslav Lj. Tešić, Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruit grown in Vojvodina, North Serbia, Food Chemistry 171 (2015) 128-136.

15.

Innovative sample introduction, extraction and separation technology for analytical systems such as HPLC, MS, GC and NMR

E. Cybulska

www.donaulab.pl
Donau Lab Sp. z o. o. 05-816 Reguły k / Warszawy
ul. St. Bodycha 73 office-pl@donaulab.com

Sample integrity, safety and traceability of samples play a central role in Spark's instrument innovation. Their aim is to provide front-end sample handling and separation systems that effectively eliminate every instrumental source of analytical errors caused by sample degradation, sample contamination, false sample identification, sample cross contamination, etc. Spark Holland front-end systems can be trusted as offering the best possible sample care technology, enabling seamless integration of automated front-end sample preparation into analytical systems.
Spark high performance SPE cartridges are at the heart of our online SPE technology, the intelligent solution to any SPE challenge. Dimensions are optimized to combine high extraction capacity with small elution volumes. Naturally, the cartridge will resist typical HPLC system pressures.
The Symbiosis technology for online SPE is based on pressure resistant high performance SPE cartridges that can be automatically exchanged. SPE solvent delivery is provided by proprietary High Pressure Syringe pumps HPD for accurate delivery, independent of cartridge backpressure. The operating principle of the Symbiosis online SPE systems is based on Automatic Cartridge Exchanger ACE which has two clamps for SPE cartridges enabling parallel operation of extraction and LC analysis.
All Symbiosis™ online SPE Systems processes multiple batches/assays fast and reliably, with the highest analytical performance, fully unattended. They also have all the tools to perform method development using a systematic approach which generates a working online SPE method in just a few days

Why to chose unique solutions for integrated online SPE-LC-MS or online SPE-HPLC?

- Full automation
- SPE and LC in parallel for high throughput
- Increased sensitivity
- No sample pre-treatment
- Reduced matrix effects
- Sample concentration
- Small and large molecules
- Ideal for biological and water samples
- Works with any LC or MS system
- Can also be used as Offline SPE solution

Water Purification Systems

For sensitive organic analysis like HPLC, LC-MS and also GC, TOC we recommend the ADRONA Water Purification Systems specially designed to produce highest purity water grade HPLC ultrapure water conductivity: < 0,055 µS/cm Low TOC concentration < 2 ppb Bacteria: < 1 cfu/ml Endotoxins: < 0,15 EU/ml particles > 0,22 µm: < 1 per ml
We can deliver complete solutions for laboratory water purification, wide range of models which fits exactly to your needs, working with low running costs.

SESSION IV

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Irena Vovk and Łukasz Komsta

16.

Update of a Chiral Separation Strategy in Capillary Electrochromatography using Chlorinated & Non-Chlorinated Polysaccharide-based Selectors

Debby Mangelings[1], Dima Albals[1], Ans Hendrickx[1], Bezhan Chankvetadze[2], Yvan Vander Heyden[1]

[1]*Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit Brussel –VUB, Laarbeeklaan 103, B -1090 Brussels, Belgium*

[2]*Institute of Physical and Analytical Chemistry, Molecular Recognition and Separation Science Laboratory, School of Exact and Natural Sciences, Tbilisi State University, Tbilisi, Georgia*

Capillary electrochromatography (CEC) is a hybrid separation technique that combines the separation principles of two techniques capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Advantages of CEC are highly efficient separations due to the plug-like electro osmotic flow (EOF) as driving force in the capillary column, a higher sample loading capacity due to the presence of a stationary phase, and overall low mobile-phase and sample consumption.

Chiral drug molecules often display different pharmacological properties. Because most pharmaceutical compounds possess chiral properties, the development of chiral separation methods is an important research topic the pharmaceutical industry to isolate the therapeutically active enantiomer, the eutomer, from the other, the distomer. Consequently, the possibility to commercialize safe drugs with less or no side effects will be achieved.

The development of chiral separation methods is often a trial-and-error procedure. For that reason, a generic chiral separation strategy was proposed earlier using CEC as separation technique. For the separation of acidic compounds, a low pH mobile phase was needed while for the separation of basic compounds, a high pH mobile phase was used. The final CEC strategy was therefore composed of two sub-strategies, i.e. one for acidic and one for non-acidic compounds.

In this study, an update of the existing CEC strategy was conducted by evaluating the potential of newer types of chiral stationary phases (CSP) that use chlorinated polysaccharide derivatives as chiral selector. In a first part, the earlier defined screening conditions were tested on the newer types of CSP. Then the most enantioselective and most complementary CSP were chosen to update the screening steps for acidic and non-acidic compounds. In a second phase, the applicability of the existing optimisation steps was verified and adapted where necessary. The final result was an updated CEC strategy with a higher success rate, which uses both chlorinated and non-chlorinated polysaccharide-based CSP.

17.

Molecular Lipophilicity Profile in Drug Discovery: A Comparative Study for a Set of the Terephthalamides Derivatives

Andrzej Bak , Violetta Kozik

Institute of Chemistry, University of Silesia, 40-006 Katowice, Poland
e-mail: Andrzej.Bak@us.edu.pl

Lipophilicity is generally regarded as a first-rate physicochemical parameter increasingly relevant in specification both the pharmacokinetic (ADMET) and pharmacodynamic aspects of drug-receptor/enzyme interactions which often correlates well with the bioactivity of chemicals. Its quantitative descriptor (logP), considerably used in the early stages of drug development, indicates the ratio of neutral solute concentrations in the organic (apolar) and aqueous (polar) phase of a two-component (n-octanol/water) mixture under equilibrium conditions. Unfortunately, the experimental procedures for the partition coefficient estimation are basically time- and/or material-consuming and require a high purity of the solute; therefore the alternative lipophilicity descriptors have been provided using mainly *in-silico* predictive models e.g., Hansch's π constant derived for chemical constituents as an additive property. On the other hand, it is possible that some methods for theoretical calculation of lipophilicity might be more or less suitable for specific series of compound analyzed, thus a variety of approaches should be employed and subsequently compared with the empirical data.

However, the routine application of various logP predictors requires a continuous evaluation of their credibility by comparison with empirical data taken as a reference. Partition coefficient can be measured experimentally using at least several procedures ranging from 'shake-flask' technique to popular thin-layer (TLC) or high-performance liquid (HPLC) chromatographic methods. The determination of the partition coefficient by direct measurement using the 'shake-flask' faces issues such as poor reproducibility; therefore the advantageous application of non-polar stationary phase and polar mobile phase in so called reverse phase TLC (RP-TLC) or *vice versa* in normal phase TLC is an attractive and reliable alternative to troublesome procedures.

Apart from the purely structural design and synthesis, the additional objectives of the presented investigation was the experimental determination of the lipophilic profiles of the amides offspring and subsequent critical assessment of the relationship between the retention parameters and the corresponding numerical values. The analyzed compounds were coded using SMILES line-notation while the spatial energy-minimized geometry was stored in Sybyl MOL2 file format. The molecular lipophilicity profiles for the entire ensemble of molecules were calculated using different software packages (clogPS, Molinspirations, OSIRIS, HyperChem 7.0, Sybyl X) for predicting logP value whereas the physicochemical properties were specified with DRAGON generator. The experimental logP values were related with the corresponding calculated values and physicochemical properties using MATLAB programming environment.

The chromatographic data were determined for the investigated set of the amides derivatives by RP-TLC method and related with theoretical partition coefficient calculated by means of *in-silico* procedures. Statistically, significant correlation was found between experimental R_{MO} values and the quantitative descriptor of lipophilicity (logP) specified by OSIRIS and Sybyl predictors. The impact of the calculated physicochemical and structural descriptors on the retention parameters was elucidated by variable elimination procedure IVE-PLS, indicating the involvement of various factors on hydrophobic forces.

18.

Testing of complementarity of PDA and MS detectors using chromatographic fingerprinting of genuine and counterfeit Viagra®

D. Custers[1,2], B. Krakowska[3], P. Courselle[1], M. Daszykowski[3], S. Apers[2], E. Deconinck[1]

[1] *Division of Food, Medicines and Consumer Safety, Section Medicinal Products, Scientific Institute of Public Health (WIV-ISP), J. Wytsmanstraat 14, B-1050 Brussels, Belgium*
[2] *Research group NatuRA (Natural products and Food - Research and Analysis), Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium*
[3] *Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland*

Counterfeit medicines pose a huge threat to public health worldwide. Their safety, efficacy and quality cannot be guaranteed. High amounts enter the European market, which is why characterization of these pharmaceuticals is a very important issue.

In this study a High Performance Liquid Chromatography – Photodiode Array (HPLC-PDA) and a High Performance Liquid Chromatography – mass spectrometry (HPLC-MS) were developed for the analysis of genuine and generic products of Viagra® and counterfeit samples. The acquired fingerprints were included in the chemometric data-analysis which aimed to test whether PDA and MS are two complimentary detection techniques. The MS data comprise both MS1 and MS2 fingerprints; the PDA data consist of fingerprints measured at 254nm, 270nm and 290nm. The applied chemometric techniques are Partial Least Squares – Discriminant Analysis (PLS-DA) and k Nearest Neighbours (kNN).

Chemometric analysis of all three single wavelengths showed that the best model was obtained by kNN for the 254nm data with a correct classification rate of 97,37% for cross validation and 96,55% for external validation. Combining all three single wavelengths did not result in an improvement of the model. A perfect model was obtained by PLS-DA for the MS data when both MS1 and MS2 were included in the analysis. This model resulted in a perfect prediction of all three sample classes (genuine – generic – counterfeit). Both PLS-DA (98,25% cross validation and 93,10% external validation) and kNN (97,37% and 96,55%) resulted in good prediction model for the combination of 254nm and MS1 fingerprints.

This study shows that a good discrimination between three groups of samples can be obtained by kNN for PDA data and PLS-DA for MS data. However, when combining PDA and MS data, both PLS-DA and kNN are capable to discriminate between genuine, generic and counterfeit Viagra® samples.

19.

Characterizations of *Ipomoea reptans* extracts through HPLC and HPTLC fingerprint development, phytochemical profiling and in vitro cytotoxicity and antioxidant activity determination

M. Hefny Gad[1,3], N. Elsawi[2], E. Elmewafy[3], S. Younes[2], Y. Vander Heyden[1], K. Demeyer[4] and D. Mangelings[1]

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

2 *Department of Chemistry, Faculty of Science, Sohag University, Sohag, Egypt*

3 *Department of Medicinal and Aromatic Plants Research, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt*

4 *Department of Toxicology, Dermato-Cosmetology and Phamacognosy, Center for Pharmaceutical Research, Vrije Universiteit Brussel VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium*

For several centuries, natural products have played a very important role in our daily life. The secondary metabolites of medicinal plants form a main source of natural antioxidants compound. The present study aims developing and finding the best conditions to separate bioactive compounds from various *I. reptans* fractions by both high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) approaches. The results revealed that the HPLC fingerprint analysis produces more peaks and a better separation than HPTLC, while HPTLC analysis helped identifying the classes of the active compounds in some fractions and confirming the similarity between fractions. The evaluation of phytochemical bioactive constituents of the *I. reptans* fractions was performed using standards methods. The results showed the presence of carbohydrates, alkaloids, phenolics and flavonoids in the ethyl acetate-methanol and methanol fractions. Terpenoids and cardiac glycoside constituents were found in hexane-dichloromethane, dichloromethane-ethyl acetate, ethyl acetate, ethyl acetate-methanol, methanol and aqueous fractions. The cytotoxicity of different *I. reptans* fractions was tested using the brine shrimp assay and the results revealed the ethyl acetate-methanol and methanol fractions as the more active fractions. Furthermore, the antioxidant scavenging activity of several fractions was determined by DPPH and ABTS assays. The DPPH results revealed the ethyl acetate-methanol and methanol fractions as the most potent, and this was confirmed by the ABTS results. In addition, the aqueous fraction possessed a higher ABTS radical scavenging activity. Current work focuses on the separation and purification of the bioactive compounds from the active fractions using reversed phase (RP-18) open column chromatography (OCC), thin layer chromatography (TLC) and HPLC according to the same method that was used to develop the chromatographic fingerprints. In the final stage, the chemical structures of isolated pure compounds will be elucidated by several techniques, like Nuclear Magnetic Resonance (1D-NMR & 2D-NMR), Infrared Spectroscopy (IR) and Mass Spectrometry (MS).

POSTGRADUATE STUDENT SECTION

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Maja Natić and Adam Voelkel

20.

How to read and to write scientific publications: a personal view on an important piece of our individual scientific lives

Huba Kalász

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

One of the most important activities in our every-day scientific carrier is reading and writing. Well-planned and properly done activities greatly facilitate our lives. Reading, writing and understanding scientific and other sources are of importance. The "freshness" of any material may range from up-to-date to as old as several decades.

The validation of a **protocol** is generally an up-to-date instruction and, what is more, an order, unless otherwise instructed. It is an obligatory statement, irrespective of its author. A well-constructed protocol should contain the possibility of suggestions concerning its validity period and other conditions.

Scientific papers give an account of either original research or a review-type activity of the author(s). Accelerated and letter-type publications usually have a one-to-several months delay from their submission and acceptance. Regular papers give an account of the stage of a discipline, three-to-twelve months before publication. Special permissions (such as keeping ethical requirements, source of financial support, etc.) must be stated. It is the author's responsibility (in the first place) to keep the copyright law, that is not to borrow a text, a figure or a table without a written permission of the publisher.

Printed comprehensive **materials** are books or proceedings depending on the origin of materials. Delay in their topicality can be from zero to several years.

The evaluation of a manuscript has several basic points of view. Formal requirement (typing instructions) is a must. Understandable, clear, fluent and correct English (or the required language of publication) is an important aspect of decision for acceptance. The manuscript should correspond with the scope of the given journal or book. These requirements are normally controlled by the Managing Editor. Reviewers are authorized to express their opinions on the value of the manuscript, as well as to give a definite evaluation, such as accepted, accepted with minor/major revision, to be rejected. Reviewers' statements mean guiding, and the decision is solely made by the Editor. Schedule and actual of publication is the publisher's responsibility.

The form of scientific publications has shifted from printed copies to electronically accessible materials. In general, both forms exist in parallel for the time being. It is essential to know, that writing is the duty and privilege of the author(s). Success achieved and fame earned by scientific work through publications are primarily up to the author.

21.

Inverse liquid chromatography as a tool for biomaterials surface characterization

K. Adamska, K. Kadlec, A. Voelkel

Poznań University of Technology, Institute of Chemical Technology and Engineering,

ul. Berdychowo 4, 60-965 Poznań, e-mail: celdak@gmail.com

The linear free energy relationship (*LFER*) proposed by Abraham is a well-known mathematical correlation used for surface characterization via chromatographic analysis. It combines the value of retention parameter with the force of interaction taking place in all systems occurring in liquid chromatography, i.e. solute – solvent, solute – stationary phase and solvent – stationary phase interactions. The materials being investigated in that way are most often commercially available stationary phases. The aim of our investigation was to prove that liquid chromatography might be applied as a tool for biomaterials surface characterization.

Two ceramic biomaterials applied as a bone tissue substitute were examined by inverse liquid chromatography. Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA) and β-tricalcium phosphate $Ca_3(PO_4)_2$ (β-TCP) were pelletized and in the form of crushed pellet were introduced to the stainless steel tube. Such prepared material served as a stationary phase which the surface properties were examined by inverse liquid chromatography method (ILC). Few types of mobile phase systems were used in these investigations. First, acetonitrile and the mixture of acetonitrile and water had been chosen to determine the physicochemical properties of biomaterials surface. The addition of water enabled to observe the influence of mobile phase polarity on test solutes retention and thereby a change of biomaterials surface properties. Additionally the attempts were made to investigate the physicochemical properties of HA surface in simulated body fluid as a mobile phase. It should help to estimate HA surface behavior in real system when being implanted into human body. Results of these experiments forced us to characterize the HA surface by using two mobile phases revealing much lower ionic strength than SBF – water and 0,1M Na_2HPO_4

22.

Determination of pesticides in herbs by gas chromatography

Patrycja Marczewska[1], Dariusz Szeremeta[1], Marek Mucha[2], Józef Rzepa[1], Mieczysław Sajewicz[1]

[1]*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland*

[2]*Department of Chemistry, Faculty of Science, University of Ostrava, 22 30 Dubna Street, 701-03 Ostrava 1, Czech Republic*

The pesticides constitute a plentiful group of chemical compounds intended to destroy or incapacitate the organisms which are dangerous for humans or their surroundings. Application of pesticides has resulted in a rise of food production and simultaneously posed a serious threat to natural environment and people's health. A widespread using of pesticides in order to preserve herbal cultures makes the control of their residues an obvious duty [1].

As far as herbal matrixes are concerned and due to an occurrence of lipids, fatty acids, ether oils, etc. therein, they characterize by an unusual complexity. The presence of these compounds can lead to difficulties in pursuing analysis, so that the selection of a proper research procedure, a right sample preparation and selection of the extraction method and working conditions are important. The analysis of pesticides is challenging due to the low concentrations distributed in complex botanical matrices, so that the process of pesticide isolation from the matrix and enrichment for the chromatographic analysis is necessary. The main aim of the research is optimization of sample preparation, the extraction and the purification of extract for the qualitative and quantitative determination of pesticides' residues in medicinal plants, including the so-called research of homogeneity [2,3].

Gas chromatography is the most often applied technique in pesticide analysis, which allows to achieve verifiable qualitative and quantitative results with an adequately selected column (i.e. DB 5, HP-5 MS, DB-XLB) and detector (i.e. Electron Capture Detector – ECD, Flame Ionization Detector – FID, and Mass Spectrometry Detector). Monitoring pesticide residua is a tool which facilitates an estimation of the consumer exposure to pesticide residues in medicinal plants. Moreover, the results of such monitoring can spur modification of a scope of using the chemical plant protection products and change the value of acceptable levels of the pesticide residues [4].

References:

[1] M. Bziuk, A. Przyjazny, , J. Chromatogr. A., 1996, 754, s. 103
[2] C. Ferrer, M. J. Gomez, J. Chromatogr. A., 2005, 1069, s. 283-194
[3] Y. He, Y-H Liu, Chromatographia, 2007, 65, s. 581-590
[4] J. F. Garcia-Reyes, C. Ferrer, Trends Anal. Chem., 2007, 26, s. 828-841

23.

Investigation of the peptide nanofibers and nanospheres formation by chromatographic and microscopic techniques

Agnieszka Godziek[1], Anna Maciejowska[1], Ewa Talik[2], Teresa Kowalska[1], and Mieczysław Sajewicz[1]

[1]*Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland*
[2]*Department of Physics of Crystals, University of Silesia, 4 Uniwersytecka Street, 40-007 Katowice, Poland*

In our earlier studies, we have proved that the low molecular weight chiral compounds (e.g., amino acids), can undergo spontaneous oscillatory condensation. This kind of reactions is characteristic of single compounds [1], or the mixtures of compounds [2] in aqueous or non-aqueous solvents. In our previous research on the pair of amino acids (*L*-Pro– *L*-Phe), it was shown that the investigated amino acids characterize with an oscillatory instability, which consists in spontaneous oscillatory oligopeptidization, i.e., in sequential formation and decay of homo- and heterooligopeptides as the products of spontaneous peptidization process. In our other research, it was found out that the amino acids not only undergo spontaneous peptidization, but also self-assemble to form nanostructures [3]. For example, the pair of amino acids (*L*-Pro-*L*-Phe) forms peptide nanofibers, while *L*-Cys forms peptide nanospheres.

In this study on three pairs of amino acids (*L*-Pro-*L*-Cys, *L*-Phe-*L*-Cys, and *L*-Phg-*L*-Cys), it was found out that *L*-Cys determines the formation speed and the shape of nanostructures. To prove that the obtained structures are peptides and have nanostructure characteristics, we used LC-MS and the scanning electron microscopy (SEM). To prove that this process has an oscillatory nature, we used HPLC-ELSD and turbidimetry.

The obtained results demonstrate that the investigated amino acids can undergo an oscillatory chiral conversion and condensation, with a consequence that these compounds may form peptide nano- and microstructures in an abiotic system.

Acknowledgement

One author (A.G.) acknowledges the financial support of the DoktoRIS project, co-financed by the European Union within the European Social Found.

References:

[1] A. Godziek, A. Maciejowska, E. Talik, M. Sajewicz, T. Kowalska, "Thin-Layer Chromatographic Investigation of *L*-Cysteine in Solution", *Journal of Planar Chromatography*, **28,** 144–151, (2015)

[2] M. Sajewicz, A. Godziek, A. Maciejowska, T. Kowalska, "Condensation dynamics of the L-Pro–L-Phe and L-Hyp–L-Phe binary mixtures in solution", *Journal of Chromatographic Science,* **53**, 31-37, (2015)

[3] A. Godziek, A. Maciejowska, E. Talik, R. Wrzalik, M. Sajewicz, T. Kowalska, " On spontaneously pulsating proline-phenylalanine peptide microfibers", *Current Protein and Peptide Science* (accepted).

24.

Investigation of peptide structures resulting from spontaneous oscillatory reactions of biogenic amino acids

Anna Maciejowska[1)], Agnieszka Godziek[1)], Ewa Talik[2)], Mieczysław Sajewicz[1)], Teresa Kowalska[1)]

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

2) Department of Physics of Crystals, Institute of Physics, University of Silesia, 4 Uniwersytecka Street, 40-007 Katowice, Poland

The phenomenon of spontaneous oscillatory chiral conversion was for the first time reported with profen drugs [1]. Further research has shown that the oscillatory reactions are specific of hydroxy acids [2] and amino acid [3].

Our research also showed that the amino acids dissolved in aqueous organic solvents undergo two parallel processes of chiral conversion and peptidization. As a result of peptidization, amino acids are formed giving the nano- and microstructures.

In the reported experiment, we focused our attention on two pairs of amino acids (*L*-His-*L*-Thr and *L*-Met-*L*-Ser). The choice of these amino acids was dictated by their important functions in living organisms. *L*-Thr is essential in the synthesis of proteins, contributing to the growth and development of the muscles. *L*-His acts as a precursor of histamine and it is often present as a key amino acid in active centers of many enzymes. *L*-Met and *L*-Ser are necessary for the translation of proteins and they are important reagents in the synthesis of taurine and glutathione.

Observations of the oscillatory peptidization reactions of amino acids were possible with use of HPLC. We also checked the amino acid structures by means of HPLC-MS bcause with time, there appeared additional structures in both binary amino acid samples, which did not belong to the amino acid monomers. With HPLC-MS, we confirmed the presence of peptides in the solutions as a result of spontaneous peptidization. Moreover, we studied peptide nanostructures by means of SEM.

The obtained results expose the oscillatory changes of the monomeric amino acid concentrations in solutions, are indicative of spontaneous peptidization reactions, and they were confronted with predictions of the theoretical model proposed by Epstein et al. [4].

References

1. M. Sajewicz, R. Piętka, A. Pieniak, T. Kowalska; Acta Chromatogr, **15**, 131- 149 (2005)
2. M. Sajewicz, D. Kronenbach, M. Gontarska, M. Wróbel, R. Piętka, T. Kowalska, J. Planar Chromatogr., **22**, 241-248 (2009)
3. M. Sajewicz, D. Kronenbach, M. Gontarska, T. Kowalska, J. Liq. Chrom. Rel. Technol., **31**, 1047-1057 (2010)
4. M. Sajewicz, M. Dolnik, T. Kowalska, I. R. Epstein, RSC Adv., **4**, 7330–7339 (2014)

25.

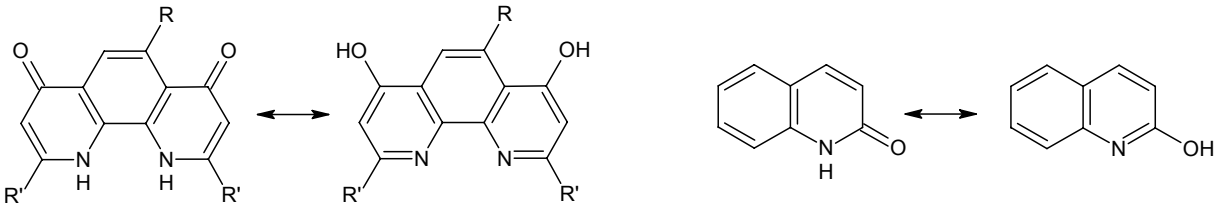
The GC-MS investigation of hydrogen–deuterium exchange among selected 1,10-phenanthroline-4,7-diones

Marcin Szala, Karolina Czyż, Jacek E. Nycz

Department of Chemical Physics, Institute of Chemistry, University of Silesia, Katowice, Poland

1,10-Phenanthrolines are one of the most significant compound among *N*-heterocyclics [1]. 1,10-Phenanthroline was first synthesized by F. Blau in 1898 [2]. Nowadays, 1,10-phenanthroline and their derivatives have wide range of applications that encourage many synthetic chemists to explore and plan more productive experiments to minimize the side-reactions and increase the yield of target products. New compounds can be precursors for innovative technologies in medicine or dying [3, 4]. Many of them are ligands in coordination chemistry as N, O or N, N atom donors for chelating with metal ions.

The presence of the hydroxyl group in the –ortho or –para position in the pyridine ring in phenantroline constitution generates tautomerism (Scheme 1).



Scheme 1. Tautomerism among 1,10-phenanthroline-4,7-diones and quinolin-2-one.

Our studies are focused on hydrogen/ deuterium (H/D) exchange reactions. We compare the GC-MS and NMR (^{1}H and ^{13}C) techniques. The predominant hydrogen–deuterium exchange reaction is observed for ortho position in phenol ring similarly to reference compounds (quinolin-2-one and quinolin-4-one). On presentation we will present the GC chromatograms and MS spectra comparing with NMR (^{1}H and ^{13}C).

Literature:

[1] R.R. Ye, C.P. Tan, Y.N. Lin, L.N. Ji, Z.W. Mao; *Chem. Comm..,* **2015**, DOI: 10.1039/c5cc02354h
[2] F. Blau; *Monatsh.*, **1898**, 19, 647.
[3] R. Musioł, J. Jampilek, J.E. Nycz, M. Pesko, J. Caroll, K. Kralova, M. Vejsova, J. O'Mahony, A. Coffey, A. Mrozek, J. Polański; *Molecules,* **2010**, 15, 288-304.
[4] W. Cieślik, R Musioł, J. E. Nycz, J. Jampilek, M. Vejsova, M. Wolff, B. Machura, J. Polański; *Bioorg. Med. Chem*., **2012**, 20, 6960-6968.

SESSION V

FRIDAY, MAY 29th, 2015

CHAIRPERSONS: Debby Mangelings and Agnes Moricz

26.

Distribution of selegiline in the rats and rabbits

Kornélia Tekes and Huba Kalász

Semmelweis University, Budapest, Hungary

Selegiline is the generic name of L-Deprenyl, that is (R)-N-methyl-N-(1-phenylpropan-2-yl)prop-1-yn-3-amine, a selective and irreversible inhibitor of the MAO-B enzyme. It has been registered worldwide under the names of Anipryl, Apo-selegiline, Atapryl, Carbex, Eldepryl, Emsam, Jumex, Selgene, Zelapar, Zydis as a medicine to treat Parkinsonian patients. Its indication has been recently expanded for treatment of human depression, however Anipryl is a formulation for animal use (e.g. treats Cushing's Disease & Cognitive Dysfunction Syndrome in dogs).

Tissue distribution of selegiline was studied by a variety of methods, however no systemic examination of tissues with high MAO-activity was carried out. In our present study rats were treated intraperitoneally and per os by selegiline and/or by methyl-C^{14} radiolabelled selegiline and selegiline was administered also to rabbits. Time-dependence of tissue-concentrations were determined both by HPLC and liquid scintillation methods. Following isolation of tissues and appropriate clean up of the samples was developed and a validated RP-HPLC method was used applying Zorbax Rx-C18 octadecyl silica column stationary phase and a phosphate buffer (pH 3.7) with ion-pairing agent (sodium 1-decanesulfonate) and acetonitrile (organic modifier) was used as mobile phase. UV absorbance was determined at 255 nm, and amperometric detection was at 0.9 V. Radioactivity of samples was determined following solubilization with Soluene 350.

Both methods gave unanimous experimental evidence about the fast onset (a maximum at 15 minutes post-treatment) and relatively fast offset of selegiline both in rats and rabbits. The high levels of selegiline in the lacrymal gland, in the parotid gland and in the testes at the first half an hour following treatment is a striking new result.

Our results definitely suggest a rapid detoxifying intention of the animal organisms for selegiline even by hitherto not studied organs. Tissue binding, metabolism and excretion following “invasion” with such a xenobiotic agent as selegiline, needs further studies for better understanding the possible side effects/new indications of the compound. The RP-HPLC method used can be preferentially used for adequate monitoring of tissue concentrations.

Acknowledgements: This work was financially supported by OTKA 100155 of the Hungarian National Granting Agency. Technical help of Ms. Zita Pöstényi and Mrs. Györgyike Guth are highly appreciated. Animal experiments were done according to 86/ 509/EEC regulation on the well-being of experimental animals and protocol was approved (permission number: 1810/003/2004 ANTSZ, Budapest, Hungary).

27.

Determination of Drugs Loaded to Silica Nanoparticles in Brain Tissue

Michal Oravec[1], Josef Jampílek[2]

[1]*Global Change Research Centre AS CR, Bělidla 986/4a, 603 00 Brno, Czech Republic*

[2]*Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 612 42 Brno, Czech Republic;*

e-mail: josef.jampilek@gmail.com

The blood–brain barrier (BBB) represents a structure with complex cellular organization that separates the brain parenchyma from the systemic circulation. The BBB is the last, critical and serious obstacle for the permeation of central nervous system (CNS) acting agents. The BBB properties result in strong selection of permeating drugs depending on their physicochemical properties, such as molecular weight, molecular volume, lipophilicity, ionisation state and/or their affinity to specific transporters (uptake/efflux transporters).

To circumvent the BBB and allow an active CNS compound to reach its target, various strategies have been developed. They can be sorted with respect to the BBB as either invasive or non-invasive such as the use of alternative routes of administration, inhibition of efflux transporters, chemical modification of drugs or encapsulation of drugs into nanocarriers (e.g., liposomes, polymeric nanoparticles and solid lipid nanoparticles).

This contribution discusses difficulties faced at determination of the concentration of drug bulk substances and drug substances in silica nanoparticles that permeated through the BBB to the brain tissues in rat brain perfusion experiments. Various extraction techniques for isolation of the drugs from the tissue and nanocarriers are discussed. The concentration of the substances in the brain was determined by means of UHPLC-DAD/HRMS LTQ Orbitrap XL.

This study was supported by the EfCOP—IPo project ENVIMET (CZ.1.07/2.3.00/20.0246), CzeCos/ICOS (LM2010007) and by GACR P304/11/2246.

28.

Determination of interactions of drug and alcohol, caffeine, smoking and their clinical relevance with different bioanalytical technics

Imre Klebovich

Semmelweis University, Department of Pharmaceutics Budapest, Hungary

Nowadays different types of drug interactions (drug, acid-stimulating and inhibiting drugs, food, alcohol, caffeine, smoking, drug transporter), as new discipline of pharmacokinetics, has been appreciated both in the original and generic drug development. It is proven by numerous new regulatory guidelines (FDA, EMA, WHO), as well.

The presentation summarizes, but not limited to interactions required to verify in today's modern pharmaceutical research, as well as the pharmacokinetic changes of drugs due to the effect of alcohol, caffeine and smoking. In the second part of the lecture a various mechanisms of interactions of drugs belonging to different pharmacological groups with alcohol, caffeine and smoking will be presented, as well. The enhanced effect of interactions in the case of the concomitant use of pleasure-giving materials, the different clinical effects of acute and chronic alcohol consumption and the effect of caffeine and smoking on various drug interactions of clinical relevance are also discussed. The role of cytochrome P450 detoxification isoenzymes involved in different types of interactions is also summarized.
The different bioanalytical technics with high sensitivity and selectivity of Headspace-GC, GC-MS/MS, LC-MS and LC-MS/MS (with different ion sources) applications are of high importance in the pharmaceutical drug-alcohol, -caffeine, and -smoking interaction research. Bioanalytical methods play an important role in the original, supergeneric and generic drug development.

The message of this lecture is to highlight the importance of the clinically relevant, frequently unexpected interactions of medicines with alcohol, caffeine, tobacco and drugs illustrating with several examples. The different adequate bioanalytical methods will also presented.

POSTER SESSION I

WEDNESDAY, MAY 27th, 2015

CHAIRPERSONS: Josef Jampilek and Jim Van Durme

1.

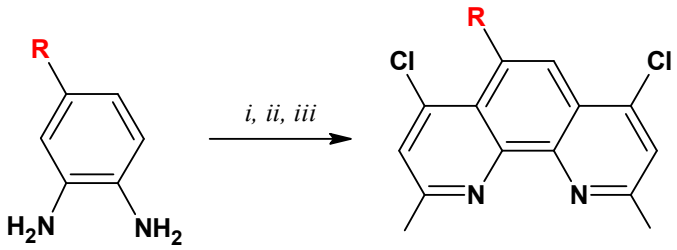
Synthesis of 1,10-phenantroline derivatives; the GC-MS investigation

Karolina Czyż, Marcin Szala, Jacek E. Nycz

Department of Chemical Physics, Institute of Chemistry, University of Silesia, Katowice, Poland

Phenantrolines (*o*-phenantrolines) is one of the most important class of compounds among *N,N*-heterocyclic organic compounds. 1,10-Phenantroline was first synthesized by F. Blau in 1898 [1]. This class of compound has been widely use as bidentate nitrogen donating ligands in coordination chemistry with numerous applications [2]. They found broad range of applications as ligands in transition metal catalyzed reactions, such as iron(II) and copper(I) [3]. They formed five-membered rings with metal cations [4].

This class of compounds can be received on various methodologies, including the classical Skraup, Friedlander, Doebner-Miller or Pavarov reactions [5]. Our research was based on a literature methodology [6] (Scheme 1).



R = F, Cl, Br, CH_3, COOEt

Scheme 1. Synthesis of selected 1,10-phenantroline derivatives. Reagents and conditions: *i* = Meldrum's acid, trimethyl orthoacetate, reflux; *ii* = diphenylether, reflux; *iii* = phosphoryl chloride.

The aforementioned novel 1,10-phenantroline derivatives were synthesized and analyzed by GC-MS techniques. On our oral presentation, we will present the GC chromatograms and MS spectra.

Literature:

[1] F. Blau, *Monatsh.*, **19**, 647 (1898).
[2] H. Saggadi, D. Luart, N. Thiebault, I. Polaert, L. Estel, C. Len; *RSC Adv.*, **4**, 21456–21464 (2014).
[3] P. Belser, S. Bernhard, U. Guerig, *Tetrahedron*, **52**, (1996) 2937-2944.
[4] P. G. Sammes, G. Yahioglu, *Chemical Society Reviews*, (1994) 327-334.
[5] A. Takahashi, Y. Hirose, H. Kusama, N. Iwasawa; *Chem. Commun*., 609–611 (2008).
[6] A.F. Larsen, T. Ulven; *Organic Letters*., **13**, 3546-3548 (2011).

2.

Analysis of Vitamin C (Ascorbic Acid) in Raw, Pasteurized and Ultra High Temperature Cow's Milk Using HPLC – PDA – ESI (+) - MS as a highly sensitive and Highly Confirmatory Analytical Tool

Walid A. S. Gafour[a], Abeer F. Zayn[a] and Omar AL sayed Omar[b] .

a-Food Technology Research Institute, Agricultural Research Center, Giza., Egypt.
b- Drug and Food Quality Control Laboratories, Ministry of Health, Kuwait.

Here our research is related to quantification of vitamin C (Ascorbic Acid ,AA) content in raw , pasteurized and ultra high temperature (UHT)Cow's milk .For that purpose analytical procedure based on high performance liquid chromatography –photo diode array detector combined with a single quadruple mass analyzer interfaced with electro spray ionization operated in positive ion mode [HPLC- PDA – ESI (+)- MS] has been developed and validated for separation of AA , identification and quantification. AA was extracted from milk samples using 2.5 % solution of meta- phosphoric acid with extraction recoveries ranged 94 – 104 %. Separation of the analyte was carried out using C-18 column and isocratic elution of mobile phase consisting of 0.1 M acetic acid: acetonitrile (98: 2, v/v). Confirmatory identification for presence of AA in investigated milk samples were achieved using UV and MS data obtained from reference standard and sample at the same retention time .For quantification analysis ,HPLC – PDA – ESI (+) – MS (PDA mode) was used and the calibration curves for AA were constructed and was linear with R^2=0.9998 .Limit of detection(LOD) and limit of quantification(LOQ) were 0.052mg /L and 0.1 mg / L respectively .Obtained validation parameters showed that the method appears sensitive , accurate , precise ,specific and relatively simple in both sample preparation and equipment .The procedure provides a very useful tool for rapidly determination of AA in Cow's milk and has been successfully applied for study the effect of storage conditions on its content and the data will be in discussion .

3.

The preliminary studies of the selected aqueous mosses using HPLC and TLC fingerprint methods

Anna Hawrył, Mirosław Hawrył, Aleksandra Turula and Grzegorz Jóźwiak

Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Witolda Chodźki 4A, 20-093 Lublin

More than 14 000 *Bryophyta* (mosses) species are known, but only low percentage of them have been analyzed using chromatographic methods. It was ascertained based on the literature that mosses have complex chemical composition. The different compounds like monoterpenoids, sesquiterpenoids, diterpenoids, steroids, triterpenoids, bibenzyl derivatives, coumarins, flavonoids were found in their chemical composition.

In our work the extraction procedures were presented. The raw material was dried and it has been extracted by three days using dichloromethane and next the methanol as solvents using the Soxhlet apparatus.

The obtained extracts have been analyzed by HPLC and TLC methods to make the fingerprints of particular extracts. In HPLC method the selected gradient with the methanol-water as mobile phase and the phenyl-hexyl chromatographic column have been used.

The TLC analysis was performed on the silica gel in one-dimensional thin layer chromatographic systems with non-aqueous mobile phases. The methanolic extracts were also analyzed using two-dimensional chromatographic method with the RP-18 chromatographic plates in orthogonal systems with the application of aqueous and non-aqueous solvents.

Moreover the biological activity with DPPH reagents has been studied.

Obtained results have been subjected the chemometric and the PCA analysis to find some differences or similarities between analyzed species of *Bryophyta*.

4.

The fingerprint analysis of selected *Cirsium* species using the HPLC and TLC methods.

Anna Hawrył, Mirosław Hawrył, Agata Ziobro and Monika Waksmundzka-Hajnos

Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Lublin, Chodźki 4A, 20-093 Lublin

The *Cirsium* species from *Asteraceae* family have been analyzed of this work. The several chemical groups of biological active compounds like flavonoids, phenolic acids, sterols, triterpenes, sesquiterpenes occur in these plants. Based on the literature, it is known that the phenolic acids have various biological activities, especially bacteriostatic, fungistatic, antioxidant, anticancer, choleretic, potential sedative hypnotic, antianxiety and anticonvulsant, activity. The flavonoids display vasoprotective, hepatoprotective, anti-inflammatory, anticarcinogenic and free radical-scavenging properties.

Ten *Cirsium* species have been studied using two chromatographic methods. The powdered raw materials have been extracted in Soxhlet' apparatus and they have been extracted with dichloromethane and next methanol as solvents.

The obtained extracts have been analyzed using HPLC method with the selected methanol-water gradient using the phenyl-hexyl chromatographic column and the fingerprint chromatograms have been obtained. The selected standards have been analyzed in the same chromatographic conditions and the retention times of them were compared with obtained particular *Cirsium* chromatograms.

The TLC analysis was performed using various TLC plates. The silica gel chromatographic plates with selected mobile phase were used to analysis of particular *Cirsium* extracts using one dimensional method. The RP-18, cyanopropyl and diol bonded stationary phases were used in two-dimensional TLC method using aqueous and non-aqueous eluents.

For the better observation of the similarity of the chemical composition of particular *Cirsium* species the results have been subjected to the chemometric and PCA analysis.

5.

TLC isolation of biologically active compounds from extracts obtained from different *Potentilla* species

Grzegorz Jóźwiak, Monika Waksmundzka-Hajnos, Wojciech Zieliński

Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Lublin, ChodŸki 4A, 20-093 Lublin

Experience of many generations and study of plants growing around us, was a cause of medical and pharmaceutical sciences development. Now, natural folk medicine can be often good inspiration for studies of plant material. Plants used as drugs, contain the biologically active compounds or whole groups of compounds which can be isolated and used in various forms of the drug. Isolation of valuable fractions of extract eliminate unnecessary substances and reduce the dose of drug taken by the patient. Because of complicated matrix, preparative thin layer chromatography is a very good method for the raw plant extract separation. Application of biological detection combined with i.e. mass spectrometry should allows to find important bands on chromatogram.

During the works of comparing the chromatographic fingerprints of various *Potentilla* species rhizomes (A. JóŸwiak 2010-2014), one of examined methods was comparing the TLC bioautograms to find the differences among them. Bioautograms of four species: *Potentilla. erecta, P. collina, P. megalantha* and P. *crantzi* showed significant bacteriostatic/antibacterial properties.

The aim of our works was isolation of biologically active compounds/groups of compounds from extracts of examined plants. Extract was prepared from pulverized rhizomes in dichloromethane, evaporated and disolved again to needed volume. Chromatographic separation was performed on TLC systems: silica/mixture of organic solvents, under earlier found conditions. The samples were introduced as narrow bands (automatic applicator with evaporation of solvent) on silica and TLC plates were developed. Two methods: bioatugraphy and spraying with the special visualization agent, to localization bands of chomatogram were performed. The next step was the scraping the important bands and washing out it content from bed. Obtained fractions were rechromatographed in various system and after bioautographic confirmation of its biological properties, prepared to mass spectrometry analysis.

6.

Searching for urine biomarkers of prostate cancer using LC-MS and GC-MS metabolomic approach

Marta Kordalewska[1], Wiktoria Struck-Lewicka[1], Renata Bujak[1], Arlette Yumba Mpanga[1], Marcin Markuszewski[2], Julia Jacyna[1], Marcin Matuszewski[2], Roman Kaliszan[1], Michał J. Markuszewski[1]

[1]Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdansk, Al. Gen. J. Hallera 107, 80-416 Gdansk, Poland; [2]Department of Urology, Medical University of Gdansk, ul. M. Smoluchowskiego 17, 80-214 Gdansk, Poland

martike@gumed.edu.pl

Prostate cancer (CaP) is one of the leading causes of cancer deaths in men worldwide. Development and application of new high-throughput and specific diagnostic methods is essential for early detection of CaP. In the present study urine metabolic fingerprinting was performed to determine potential biomarkers that could be useful for understanding and explanation of CaP pathomechanisms at molecular level. Urine samples from CaP patients (n=32) and healthy volunteers (n=32) were analyzed with the use of HPLC-TOF/MS in positive and negative ionization modes as well as GC-QqQ/MS in a scan mode. Afterwards, univariate statistical analysis (t-test or U Mann-Whitney test depending on data distribution) was applied for obtained data to select statistically significant metabolites between studied groups. Next, the advanced multivariate methods such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were carried out in order to determine metabolites that were contributed the most into group classification. The identification of selected metabolites using NIST, HMDB, METLIN, KEGG and CEU Mass Mediator databases allowed for creation of a list of putative biomarkers and related biochemical pathways which they are involved in. As a result, 235, 248 and 28 statistically significant variables were selected for LC-TOF/MS analyses in positive ionization mode, negative ionization mode and for GC–MS analyses, respectively. Altered metabolites were found to be involved in amino acid, purine and glucose metabolism as well as urea and TCA cycles. The obtained results suggest that urine metabolic fingerprinting is a powerful tool which might be useful in research for CaP diagnosis and eventual further pathomechanisms explanation.

Acknowledgements

This project was supported by the National Science Centre grant no. 2012/07/E/NZ7/04411.

The authors thank Shimpol A.M. Borzymowski Company for the opportunity to carry out analysis with the use of GC–MS 8030TQ System.

7.

Identification of tributyltin in environmental water samples supported by means of discriminant models

Barbara Krakowska[1], Michał Daszykowski[1], Karol Fabiańczyk[2], Marcin Korzeń[3]

[1] *Institute of Chemistry, The University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland*

[2] *Polcargo International, 5 Poboznego Street, 70-900 Szczecin, Poland*

[3] *Faculty of Computer Science and Information Technology, West Pomeranian University of Technology, 49 Zolnierska Street, 71-210 Szczecin, Poland*

Tributyltin is a biocide agent which has been used as ingredient of antifouling paint with the aim to prevent the growth of organisms on coated with paint surface. However, proved that TBT is released into the environment and exceeds acute and chronic toxic levels. For this reason, different international regulations have been issued to effectively prohibit further usage of TBT, and thus to reduce progression of water contamination with TBT and its degradation products. Unfortunately, toxic effects are still observed [1]. It was a reason why, determination of TBT in different water bodies, is a subject of a strict on-going monitoring. The carried out research illustrate the usefulness of chemometric tools in an accredited laboratory in the context of the support process, the routine identification of the tributyltin cation (TBC) in environmental samples of water [1]. Water samples (1403) collected from 2011 to 2013 were analyzed according to European Norm PN-EN ISO 17353:2006 and described by means of chromatographic fingerprints using gas chromatography coupled with mass spectrometry. Then, for data sets after and before elimination of the baseline and peaks shifts removal [2], discriminant models were built using discriminant method - partial least squares - discriminant analysis [3]. Validated PLS-DA models for raw and preprocessed data sets allows correct discriminate 80.5% and 79.5% of the samples, respectively. Its sensitivity is 81.9% and 80.0% and specificity is 79.1% and 79.0%, respectively.

[1] B. Antizar-Ladislao, Environ. Int., 2008, 34, 292–308
[2] M. Daszykowski, B. Walczak, Trends in Analytical Chemistry, 2006, 25, 1081-1096
[3] M. Barker, W. Rayens, J. Chemometrics, 2003, 17, 166-173

Barbara Krakowska is grateful for the financial support within the framework of the DoktoRIS program—scholarship program for innovative Silesia co-financed by the European Union under the European Social Fund.

8.

THE ENCOUNTER OF MACROFUNGHI RESEARCH AND DIRECT BIOAUTOGRAPHY

Dániel KRÜZSELYI[1], János VETTER[2], Péter G. OTT[1], Ágnes M. MÓRICZ[1]

[1]*Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*

[2]*Department of Botany, Faculty of Veterinary Science, Szent István University, Budapest, Hungary*

*E-mail addresses: *moricz.agnes@agrar.mta.hu, ott.peter@agrar.mta.hu, vetter.janos@aotk.szie.hu, kruzselyi.daniel@agrar.mta.hu,*

Keywords: direct bioautography, mushrooms, HPLC-ESI-MS, TLC, antibacterial effect

Fungi are the most diversified group of organisms; nearly one million species are estimated. Their environmental and morphological diversity and interactions are remarkable. They produce compounds that also reflect this diversity. Among the basidiomycete mushrooms, large numbers of species produce different bioactive compounds (antioxidant, anti-microbial, anti-tumor, etc.). Many of these substances are derived from secondary metabolism. Our research focuses on those basidiomycete species that are suitable for human consumption, such as a variety of cultivated mushrooms (*Agaricus spp.*, *Pleurotus spp.*) as well as some wild mushrooms (*Flammulina velutipes*, *Agrocybe cylindracea syn. Agrocybe aegerita, Leccinum duriusculum*). The antibacterial activity of certain compounds of the fruiting body, capskin, hat meat, gills and stalk extracts was examined by thin-layer chromatography-direct bioautography using different Gram-negative and Gram-positive bacteria (X*anthomonas euvesicatoria, Pseudomonas syringae* pv. *maculicola*, *Aliivibrio fischeri, Bacillus subtilis,* etc.). During the test the developed dried chromatoplates were immersed in the bacterial cell suspension, and the inhibition zones of the antibacterial substances (chromatographic spots) were visualized with vital dyes or by bioluminescence detection. All examined mushrooms showed antibacterial effect against all tested bacteria. More characteristic inhibition zones were revealed with direct bioautography. Utilizing different reagents *in situ* in the adsorbent layer the antibacterial substances were characterized mainly as fatty acids (lipophilic) or phenolic substances. The major active component of all mushrooms has been identified by HPLC-DAD-ESI-MS as linoleic acid. The MS characterization of the minor active components is under process. Further investigations are planned such as classical bacteriological tests (eg. MIC), MS/MS and NMR examinations.

This work was supported by OTKA grant PD83487, and Á.M. Móricz was supported by Bolyai grant.

9.

Optimization of the extraction of total heme from meat for determination by HPLC

Hannelore De Maere[1,3], Eveline De Mey[1], Martyna Baca[2], Mieczys³aw Sajewicz[2], Teresa Kowalska[2], Ilse Fraeye[1], Hubert Paelinck[1]

[1]*KU Leuven, Department of Microbial and Molecular Systems, Research Group for Technology and Quality of Animal Products, member of LFoRCe, Technology Campus Ghent, Belgium*
[2]*Department of General Chemistry, Institute of Chemistry, Katowice, Poland*
[3]*Food Quality Laboratory, Groupe ISA, Lille-Cedex, France*

Myoglobin is the most important red pigment in meat, but also the content of hemoglobin and cytochrome C partially influence the meat colour [1]. Myoglobin is a compact globular protein consisting of globin and an iron containing heme group (Fe-protoporphyrin IX) as chromophore. The colour of myoglobin is determined by the redox state of heme and by the type of ligand bound at its sixth' coordination place, and is found in meat in three main forms: deoxymyoglobin (MbFe(II)), oxymyoglobin ($MbFe(II)O_2$) and metmyoglobin (MbFe(III)) [2]. For the production of meat products, traditionally nitrite or nitrate is added, whereby nitrosomyoglobin (MbFe(II)NO) is formed. As a result the typical red cured colour of meat products is attained [3]. Colour, a crucial factor for consumer's buying decision of meat and meat products, can be affected by several factors [1, 2]. In order to investigate the colour formation in meat and meat products in detail, it is of primary interest to have a reliable method for the determination of the total heme content.

In this study, the method for extracting heme from meat samples, more specific dry fermented sausages, has been optimized and validated prior to further analysis by HPLC-UV. Chromatographic analysis was based on the method described by Wakamatsu et al. (2009) [4]. The separation was carried out by isocratic elution using methanol/ammonium acetate (80:20, v/v, pH = 5.16) at a flow rate of 1 mL/min. Forty microliters of each sample was injected. Detection of heme was carried out at the wavelength of 400 nm.

The influence of different factors was investigated to find suitable conditions for the heme extraction. The procedure of extraction was based on the method described by Lombardi-Boccia et al. (2002) using acidified acetone as extraction solvent. This ensures the extraction of heme from all heme proteins, in the form of acid hematin (hemin) [5]. In this study the effects of extraction solutions (combinations of water, HCl and acetone) and operational parameters, such as homogenization time (30 s versus 5 min using an ultra-turrax T25 homogenizer, IKA®, Staufen, Germany) and shaking (0 h versus 1 h using a nutating mixer, VWR International, West Chester, PA, USA) were studied. Also double and single extraction of the meat samples was examined.

The optimal extraction of heme was obtained with an acidified acetone solution of 78% acetone and 1.375% HCl, 5 min homogenization and no additional shaking. The recoveries obtained after double extraction were better than the values yielded after single extraction, indicating that a double extraction is more acceptable for the determination of heme in meat products. The optimized extraction method is a valuable tool for assessing the total heme concentration in salami samples by HPLC.

Acknowlegments

This investigation was done in the framework of an European Interreg IV a 2 seas program. The research project $MeCagrO_2$ involves safe products, sustainable processes and employment increased attractiveness for companies from the 2 Seas agro-food area. Note: "The document reflects the author's views. The interreg IVA 2 Seas Program Authorities are not liable for any use that may be made of the information contained therein." E. De Mey is a postdoctoral fellow supported by the Postdoctoral Mandate (PDM) from KU Leuven.

References

[1] R.A. Mancini, M.C. Hunt (2005). *Meat Science 71: 100-121.*
[2] G. Lindahl (2005). *Thesis Colour characteristics of fresh pork.*
[3] K. -O. Honikel (2008). Meat Science 78: 68-76.
[4] J. Wakamatsu, H. Odagiri (2009). *Meat Science 82: 139-142.*
[5] G. Lombardi-Boccia, B. Martinez-Domingez, et al. (2002). *Food Chemistry 78: 505-510.*

10.

Detection of ovarian cancer based on metabolomic data obtained from liquid chromatography coupled with mass spectrometry

Paweł Matuszewski, Michał Daszykowski

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

Ovarian cancer remains as one of the major causes of deaths among different gynecological malignancies. Only in 2008, in US, it claimed 15,520 lives out of 21,650 new ovarian cancer cases mostly as a consequence of a late diagnosis. Over the last years, a rapid development of metabolomic approaches and advanced analytical platforms opened new possibilities of efficient and nearly non-invasive diagnosis based on tracing of low molecular compounds (metabolites). It is expected that certain metabolite(s) found in examined body fluids can be an indication of a developing disease. In order to enable discrimination between metabolite profiles obtained from healthy and diseased group of patients, different chemometric and machine learning data modeling approaches are used [1,2]. They take into account selected metabolites or even entire chemical fingerprints trying to discover unique pattern of metabolites differentiating healthy and diseased patients. In practice, multivariate modeling approaches are preferred over univariate ones because in most cases a single metabolite (a biomarker) has a little discrimination power.

We aim to build multivariate discriminant model that can assist in diagnosis of papillary serous ovarian cancer based on the chemical content of selected metabolites determined in serum samples of healthy and diseased women using the LC-MS technique. In contrast with the previous study [1], to discriminate the two groups of samples, a relatively simple linear multivariate model is considered - partial least squares discriminant analysis, PLS-DA [3]. Each sample has been described by 360 and 232 peak areas determined using the positive and negative detection mode. A detailed description of experiment (including cohort description), sample pretreatment, peak detection, etc. can be found in reference [1].

Due to a limited number of available samples, the PLS-DA model is validated in the course of the bootstrap procedure [4]. It consists of multiple drawing at random a predefined number of samples that serve as a model set used to build a model, whereas the remaining samples test model performance. For a given bootstrap sample, a number of PLS-DA models with increasing complexity are built and are characterized by the number of incorrectly recognized samples. When the number of bootstrap samples is reasonably large, it is possible to estimate prediction errors and associated uncertainty.

For the studied data set the best PLS-DA model, constructed for two groups of metabolites identified using positive and negative detection mode, leads to mean values of correct classification rates, estimated using the bootstrap procedure, approaching 80%.

References

[1] W. Guan, M. Zhou, C.Y. Hampton, B.B. Benigno, L.D. Walker, A. Gray, et al., Ovarian cancer detection from metabolomic liquid chromatography/mass spectrometry data by support vector machines, BMC Bioinformatics. 10 (2009) 259.

[2] J. Trygg, E. Holmes, T. Lundstedt, Chemometrics in metabonomics, J. Proteome Res. 6 (2007) 469–479.

[3] M. Barker, W. Rayens, Partial least squares for discrimination, J. Chemometrics. 17 (2003) 166–173.

[4] R. Wehrens, H. Putter, L.M.C. Buydens, The bootstrap: a tutorial, Chemometrics and Intelligent Laboratory Systems. 54 (2000) 35–52.

11.

Between batch differentiation of stamping inks from the same manufacturer by means of Thin Layer Chromatography

A. Menżyk[1] , M. Sajewicz[1], G. Zadora[1,2]

[1]*Institute of Chemistry, University of Silesia, 40-006 Katowice, Poland*

[2]*Institute of Forensic Research, 31-033 Krakow, Poland*

Analysis of ink is an important part of forensic investigations which very often regards questioned documents with significant values, such as contracts or insurance claims. Even though writing inks are very common subject of research, greater attention should undoubtedly be paid on the stamping inks examination.

Hence, the aim of this study was to propose an approach allowing to compare black and red stamping inks and determine whether the developed method could discriminate between closely related formulas, that is inks from three different batches of the same manufacturer. Due to its importance in forensic ink analysis (despite destructive character), Thin Layer Chromatography (TLC) was applied.

The chromatographic separation of inks was performed on Merck TLC silica gel 60 plates (without fluorescent indicator) with use of following mobile phase: ethyl acetate/ ethanol/ water 70:35:30 (v/v/v). Development of the chromatograms was carried out to distance of 145 mm in normal CAMAG chamber. In order to investigate the influence of the substrate on the chromatographic separation of the dyes, the TLC analysis of ink extracted from the paper with 100 μl of methanol/ water 1:1 (v/v) was also conducted. The obtained chromatograms were inspected in UV light and densitometrically scanned (λ=418 nm for black and λ= 495 nm for red inks). On this basis, R_f values were calculated for each band, allowing to compare investigated inks more precisely.

The obtained results demonstrated the inability of differentiating examined stamping inks by means of Thin Layer Chromatography, when procedure described above was applied, as the products from different batches exhibit the same dye composition. A reliability of comparison process could be probably improved by introducing proper calibration standards and applying algorithms for the comparison [1], as the environmental and analytical factors (such as the influence of TLC plate) can impact ink chromatograms, resulting in misleading conclusions.

[1] C. Neumann, R. Ramotowski, T. Genessay, *Forensic examintaion of ink by HPTLC- The United States Secret Service Digital Ink Library;* Journal of Chromatography A 1218 (2011), 2793-2811.

12.

Optimization of chromatographic condition for separation of ziprasidone and its impurities by TLC

Darija Obradović[1], Slavica Filipić[1], Katarina Nikolić[1], Marija Čarapić[2], Danica Agbaba[1]

[1]*University of Belgrade -Faculty of Pharmacy, Department of Pharmaceutical Chemistry*
[2]*Medicines and Medical Devices Agency of Serbia, Belgrade, Serbia*

Ziprasidone is a second generation antipsychotic drug used for the treatment of schizophrenia and the acute maniac or mixed episodes associated with bipolar disorder. Five recognized ziprasidone impurities originating from the synthesis as the precursor impurities (impurity I and IV) or representing degradation products of ziprasidone (impurities II, III, and V) are compounds significantly different in polarity and therefore represent a challenge for the simultaneous separation. The main objective of this wark was to optimize thin-layer chromatografic condition for simultaneous separation of ziprasidone and its five impurities.

According to preliminary study, central composite face centered design was chosen to examine the influence of four factors: the developing distance, the amount of toluene in the mobile phase, the amount of acetic acid in the mobile phase, and the spot band size, on the retention behaviour of examined compounds with special emphasis on critical pairs, i.e., impurities III and I as well as ziprasidone and impurity II. The optimal separation conditions were achieved on chromatographic plates precoated with silica gel 60 F_{254} and using toluene-methanol-acetic acid 7.5:0.5:0.5 (v/v/v) as mobile phase in ascending development mode to the distance of 110 mm. The reproducibility of separation by use of the selected TLC method was confirmed with low relative standard deviation of migration distances for all examined compounds (MD ± RSD): 22.47 mm ± 3.66 %, 30.70 mm ± 2.18 %, 40.87 mm ± 1.77 %, 46.37 mm ± 1.34 % , 76.72 mm ± 0.81 %, and 91.65 mm ± 1.39 %, for impurities III, I, II, ziprasidone, impurities V and IV, respectively. According to the obtained results the proposed TLC method can be used as reliable method for simultaneous separation of 6 compounds highly different in polarity with calculated logP values in the range 2.24 (impurity II) to 8.51 (impurity III) and can be further subjected to the validation process.

13.

Bioautography as a method of determination of antibacterial properties with selected thyme species

M. Orłowska[1], W. Jesionek[2], B. Majer-Dziedzic[3], I.M. Choma[2], G. Szymczak[4], M. Waksmundzka – Hajnos[5], T. Kowalska[1], M. Sajewicz[1]

[1]*Department of General Chemistry and Chromatography, University of Silesia, Katowice, Poland*
[2]*Department of Chromatographic Methods, Maria Curie – Sklodowska University, Lublin, Poland*
[3]*Department of Veterinary Microbiology, University of Life Sciences, Lublin Poland*
[4]*Botanical Garden, Maria Curie-Skłodowska University, Lublin, Poland*
[5]*Department of Inorganic Chemistry, Chair of Chemistry, Medical University of Lublin, Lublin, Poland*

Thyme is an aromatic evergreen plant with strongly pronounced curative properties, and with an increasing importance both for the economy and gardening in Europe. Its natural habitat includes vast territories of Europe, North Africa, and Asia. Thyme belongs to the family of *Lamiaceae* (formerly known as *Labiatae*), which embraces ca. 350 different species. The most common among these species is common thyme (*Thymus vulgaris* L.) [1,2]. Based on extensive phytochemical studies on chemical composition of this plant, the presence of several bioactive compound classes was revealed such, as phenolic acids, flavonoids, terpenoids, and essential oils [3]. Representatives of all these botanical classes are responsible for different positive pharmacological properties such, as antiseptic, antioxidant, antibacterial, and expectoral property [4,5].

Thin-layer chromatography combined with different biological and chemical detection methods is a particularly efficient and cheap analytical technique, well suited for studying herbal extracts. Coupling of thin-layer chromatography (TLC) with biological detection carried out by means of direct bioautography (DB), results in a novel TLC-DB technique, which enables a robust screening of herbal material in the search for those plants with strongly pronounced biological activity. Among pharmacologically important properties of herbal material is their antibacterial activity and this can easily be assessed with use of TLC-DB [6]. Characteristic feature of the TLC-DB method is that one observes the growth of bacteria directly on a chromatographic plate [7,8]. DB is the most frequently used one of all bioautographic methods.

Eighteen thyme (Thymus L.) species grown in Botanical Garden of the Maria Curie-Skłodowska University in Lublin underwent phytochemical analysis. All plant species were harvested in July, 2012, and dried under proper working conditions. Extracts from these plants were obtained, which were then analyzed by means of TLC-DB. The main goal of these investigations was to evaluate antibacterial properties of all the thyme species studied and to select those with the strongest pronounced antibacterial activity. The results obtained demonstrate considerable diversity of antibacterial properties of the eighteen investigated thyme species.

Acknowledgement

One author (M.O.) acknowledges the financial support of the DoktoRIS project, co-financed by the European Union within the European Social Found.

References:

1. S.Y. Oh, J.W. Ko, S.Y. Jeong, J. Hong, *Journal of Chromatography A*, **1205** (2008) 117-127.
2. I. Fecka, S. Turek, *Food Chemistry*, **108** (2008) 1039-1053.
3. L. Rubió, A. Serra, A. Macià, X. Borràs, M.P. Romero, M.J. Motilva, *Journal of Chromatography B*, **905** (2012) 75-84.
4. S.J. Lee, K. Umano, T. Shibamoto, K.G. Lee, *Food Chemistry*, **91** (2005) 131-137.
5. B. Boros, S. Jakabová, Á. Dörnyei, G. Horváth, Z. Pluhár, F. Kilár, A. Felinger, *Journal of Chromatography A*, **1217** (2010) 7972-7980.
6. C. A. Simões-Pires, B. Hmicha, A. Marston, K. Hostettmann, *Phytochemical Analysis*, 20 (2009) 511-515.
7. Ch. Weins, H. Jork, *Journal of Chromatography A*, **750** (1996) 403-407.
8. G. Eberz, H. G. Rast, K. Burger, W. Kreiss, C. Weisemann, *Chromatography* **43** (1996) 5-9.

14.

Qualitative evaluation of excise duty marker Solvent Yellow 124 in diesel oil samples

Joanna Orzel, Michal Daszykowski

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

Diesel oil is widely used for transport, heating and driving of agricultural machinery. According to its end use, the level of imposed excise duty on diesel oil is different. A rebated tax fuel (meant for heating purposes and driving agricultural machinery) is deliberately spiked with additives that can be visually recognized (for instance changing the color from yellow to red). In all EU countries two additives are introduced into diesel oil: a marker and a dye compound. Their choice and concentration levels may vary from country to country. Solvent Yellow 124 (SY124) is a fuel marker, and its concentration levels are found within 6.0 $mg \cdot L^{-1}$ and 9.0 $mg \cdot L^{-1}$. A dye compound changes fuel color to red (e.g. Solvent Red 164 and Solvent Red 19, etc.) [1,2]. Decreased concentration levels of excise duty components in diesel oil or their absence are a direct indication of a possible counterfeiting. Substantial differences in applied tax levels encourage illegal removal of excise duty components. Thus, by illegal changing designation of fuel, it gains artificially value on the market.

The reference method for the qualitative evaluation of SY124 in diesel oil is based on the HPLC analysis [3]. We have developed analytical procedure extended with the chemometric modeling to determine the content of SY124 as an alternative to reference method. Bearing in mind low levels of residual SY124 found after 'laundered' of samples a sensitive technique should be used. As illustrated in our study this objective can be achieved with excitation-emission fluorescence technique. To quantify SY124 fuel samples have been characterized by their excitation-emission spectra and further modeled using the three-way partial least squares [4]. Proposed procedure is compared with the reference method and its validation parameters are reported.

References:

[1] European Council Directive 95/60/EC.
[2] European Commission Decision 2003/900/EC.
[3] T. Lisinger, G. Koomen, et.al., Energy Fuels 18 (2004) 1851–1854.
[4] J. Orzel, M. Daszykowski, et.al., Talanta, 101 (2012) 78-84.

MD wishes to acknowledge the support of the National Science Centre, Poland (research grant no. 2014/13/B/ST4/05007), JO appreciates the DoktoRIS scholarship.

15.

Determination of acrylamide in coffee samples using the fluorescence spectroscopy extended with chemometric modeling approaches as a simple and cost-effective alternative to separation-based methods

Agnieszka Psiuk, Joanna Orzeł, Michał Daszykowski

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

Frying or baking of food products increases their taste, but on the other hand many dangerous for health compounds are formed – for instance acrylamide (AA). Its presence was found for the first time in 2002 in food products rich with carbohydrates [1]. AA is formed as a product of the Maillard reaction between amino acids (mainly asparagine) and reducing sugars (glucose or fructose) initiated during frying, roasting or grilling processes at temperature above 120°C [2].

It is proven that AA binds to hemoglobin and DNA [3], and thus it has neurotoxic and carcinogenic properties. For this reasons its content must be monitored. Usually it is done using different chromatographic techniques including high performance liquid chromatography, gas chromatography and liquid chromatography coupled with mass spectrometry or tandem mass spectrometry detection [4]. However, these techniques are in general expensive, time-consuming and laborious.

In this study the possibility of AA quantification in selected food products is examined using the fluorescence spectroscopy - a simple, cost-effective and very sensitive technique. It is known that presence of AA decreases fluorescence intensity of tryptophan (serving as a fluorescent probe). This property will be used for the construction of advanced chemometric calibration models [5] enabling quantification of analyte under the presence of additional fluorescence interferents. Ground coffee samples will be characterized by their fluorescence excitation-emission fingerprints and modeled with the second-order calibration methods [6].

References

[5] M. Friedman, D.S. Mottram, *Chemistry and safety of acrylamide in food*. New York: Springer, 2005.

[6] H. Jaeger, A. Janositz, D. Knorr, The Maillard reaction and its control during food processing. The potential of emerging technologies, *Pathologie Biologie,* 58 (2010) 207–213.

[7] M. Friedman, Chemistry, Biochemistry, and safety of acrylamide. A Review*, J. Agric. Food Chem.,* 51 (2003) 4504-4526.

[8] I.S. Arvanitoyannis, N. Dionisopoulou*,* Acrylamide: Formation, Occurrence in food products, detection methods, and legislation*, Critical Reviews in Food Science and Nutrition* 54 (2014) 708–733.

[9] A. Garszyńska, *Wykrywanie akrylamidu w wybranych rodzajach żywności*, Kraków 2011.

[10] A. Olivieri, G.M. Escandar, *Practical three-way calibration*, Elsevier, 2014.

[11]

16.

Identification and separation of TNF-α after infection of three genospecies of *Borrelia burgdorferi* sensu lato by the use of chromatographic techniques. Preliminary results

B. Rozwadowska[1], J. Gabor[2], M. Łężniak[2], T. Flak[2], H. Okła[1,2], D. Wcisło-Dziadecka[1], U. Mazurek[3], K. Jasik[1], A.S. Swinarew[2*]

[1]*Department of Skin Structural Studies, Medical University of Silesia, 41-200 Sosnowiec, Poland*
[2]*Institute of Materials Science, University of Silesia, 40-007 Katowice, Poland*
[3]*Chair and Department of Molecular Biology, Medical University of Silesia, 41-200 Sosnowiec, Poland*
**andrzej.swinarew@us.edu.pl*

Lyme disease is caused by spirochetes of *Borrelia burgdorferi sensu lato* complex. *B. burgdoferi s.l.* is divided into 19 genospecies of which *B. afzelii, B. garinii* and *B. burgdorferi sensu stricto* are the most important human pathogens.

The spirochete *B.burgdoferi s.l.* stimulate the immune system to cytokine production like tumor necrosis factor TNF-α. It is a pro-inflammatory cytokine which cause plejotropic effects on various cell types. This cytokine is playing a key role in apoptosis, inflammation and immunity. Using Affymetrix oligonucleotide microarray (HG-U133A) analysis demonstrated that 17 ID mRNA from 93 ID mRNA is changing independently from genospecies *B. burgdorferi* s.l. 15 ID mRNA is dependent from the presence of infections only *B. garinii*, 13 ID mRNA exclusively for *B. burgdorferi* s.s., and 9 ID mRNA is specific for *B. afzelii*. This study is an attempt of quantitative and qualitative (QA/QC) identification of TNF-α, after infection of normal human dermal fibroblasts (NHDF) with *B. garinii, B. afzelii* and *B. burgdorferi s.s.* with microarray techniques supported by UHPLC and GPC.

17.

Determination of resveratrol in fruit juices and herbal infusions

Marta Skorek[1], Katarzyna Pytlakowaska[2], Karina Kocot[2], Teresa Kowalska[1], Mieczysław Sajewicz[1]

[1]*Department of General Chemistry, Institute of Chemistry University of Silesia, Katowice, Poland*
[2]*Department of Analytical Chemistry, Institute of Chemistry University of Silesia, Katowice, Poland*

Fruit juices and infusions prepared from dried herbs are widely used in pharmaceutical industry and as traditionally home-made curative preparations. The most common are those which are intensely coloured, i.e., juices and infusions prepared from blueberries, chokeberries, raspberries, and pomegranates. All these intensely coloured raw materials are rich in polyphenols, and resveratrol is one of their constituents. To one of the resveratrol isomers, *trans*-resveratrol (*trans*-3,5,4'-trihydroxystilbene), a broad spectrum of therapeutic properties are ascribed. The most significant are antioxidant, anti-inflammatory, detoxificating, antibacterial, and antifungal properties. Based on the results of clinical investigations, *trans*-resveratrol is considered as responsible for suppressing of lipid levels in the blood serum and it is renowned for an anticancer activity.[1]

Currently, herbal raw materials rich in *trans*-resveratrol have attracted wide attention from the side of manufacturers of dietary supplements and cosmetics. An intense search has started for different botanical materials which might serve as a rich source of resveratrol and an alternative to the grape vine.

In this study, fruit juices (obtained from raspberries, chokeberries and pomegranates) and infusions made of dried fruits (blueberries and chokeberries) were investigated. Infusions made of dried fruits and aqueous solutions of fruit juices underwent the solid phase extraction (SPE) treatment. Then polyphenols (including resveratrol) were extracted with ethyl acetate from the SPE cartridge sorbents and analyzed by means of HPLC. Qualitative analysis of resveratrol with use of an inner standard (IS) was performed.[2,3]

It was demonstrated that the contents of the phenolics (inclusing resveratrol) strongly differ and depend on the plant species and an anatomical part of the plant.

References

1 Kolouchova-Hanzlıkov I., Melzoch K., Filip V., Smidrkal J., Rapid method for resveratrol determination by HPLC with electrochemical and UV detections in wines, Food Chem. 87 (2004) 151–158

2 Jimenezsanchez J.B., Crespo Corral E., Santos Delgado M.J., Orea J.M., Gonzalez Urena A., Analysis of trans-resveratrol by laser ionization mass spectrometry and HPLC with fluorescence detection. Comparison between both techniques, J. Chromatogr. A, 1074 (2005) 133-138

3 Malovaná S., Garcia Montelongo F.J., Pérez J.P., Rodrıguez-Delga M.A. Optimisation of sample preparation for the determination of trans-resveratrol and other polyphenolic compounds in wines by high performance liquid chromatography, Anal. Chim. Acta 428 (2001) 245–253

18.

Determination of hexachlorocyclohexane (HCH) isomers and their biodegradation products in environment samples by GC

Dariusz Szeremeta[1)], Patrycja Marczewska[1)],Magdalena Knaś[1)], Józef Rzepa[1)], Mieczysław Sajewicz[1)]

1) University of Silesia, Institute of Chemistry, Department of General Chemistry and Chromatography,
40-006 Katowice, Szkolna Street 9, Poland

The majority of organochlorine pesticides (OCP), including hexachlorocyclohexane isomers (HCH), have been banned in many countries due to their mutagenic and carcinogenic properties [1]. However, due to their persistency and lipophilicity, these compounds and their degradation products are still present in the environment [2]. Therefore, it is necessary to monitor the soils which are contaminated with OCP, as well as to apply and refine the methods which enable soil remediation. Among the methods used to purify the polluted soils we can distinguish also the bioremediation technics. A crucial element favouring these methods is their natural character. As non-invasive methods which do not destroy the structure of soil, they offer a possibility of ground recultivation without its significant and costly transformation.

An important element of our research was chromatographic estimation of an effectiveness of biodegradation of the HCH isomers by the bacteria strains which have been preliminarily isolated from the grounds polluted by these pesticides. In these studies, the methods of preparation and analysis of samples contaminated with HCH isomers and their biodegradation products have been developed. For isolation of target compounds, solid-phase extraction (SPE) was used. The analysis of the obtained extracts was carried out by means of capillary gas chromatography with mass spectrometric (MS), as well as electron capture detector (ECD). Usage of the GC/MS technique enabled identification of the products acummulated in soil through biodegradation of organochlorine pesticides. These products were identified on the basis of the acquired mass spectra.

One author (D.Sz.) is the scholarship recipient within the framework of the "DoktoRIS Scholarship Programme for the Innovative Silesia", subsidized by the European Social Fund of the European Union.

References

1. G.R. van der Hoff, P. van Zoonen; J. Chromatogr. A, **843**, 301-322 (1999).
2. M.T. Gonullu, et. Al.; Fresen. Environ. Bull., **12**, 1457-1464 (2003).

19.

Application of smoke condensates in smoking process

Agata Włosowicz[a,]; Olivier Goemaere [b], Ann De Winne [b], Jim Van Durme [b]

[a] *University of Silesia Institute of Chemistry, 9 Szkolna, 40-006 Katowice, Poland*

[b] *KU Leuven – Technology campus Ghent, Research Unit Molecular Odor Chemistry,Gebroeders De Smetstraat 1, 9000 Ghent*

Food smoking processes are an important source of Volatile Organic Compound (VOC) emissions of which some are hazardous pollutants. An alternative to traditional wood smoking, is to use purified smoke condensates. The goal of this study is to compare the emissions to the air between both a traditional and innovative smoking process using mackerel as a case.

Two batches of identical amounts of salted mackerel were each subjected to a specific optimized smoking processes: beech wood smouldering and by smoke condensate atomization. After smoking, homogenized mackerel samples obtained from both processes were compared using sensory and chemical-analytical method (HS–SPME–GC–MS). Sensory analyses revealed no significant organoleptical differences ($p = 0.05$) between two types of fish products. However, aroma profiling revealed significant differences in aroma properties, indicating that also VOC emissions might be different. Therefore, two experiments were done to evaluate VOC emissions to the environment using identical smoking programs, but different smoke generating methods as discussed earlier (at 60 □C). After the smoking cycle valves were opened and 4 sequent air samples were collected in the exhaust during first 3 minutes. The VOCs composition of the emitted gases was objectively evaluated by means of chemical-analytical (TD-GC-MS).

The chemical compositions of residual smoke emissions proved to be different.The overall VOC concentrations for different compound classes proved to be mostly higher in the smoke condensates exhausts. Moreover, despite high initial concentrations, a strong descending trend was observed for VOCs emissions within the first 3 minutes of smoking processes. Interestingly, several hazardous compounds from the BTEX (Benzene, Toluene, Ethylbenzene, Xylene) and polycyclic aromatic hydrocarbon groups were measured only in the wood smoke: benzene (0.03 mg/m^3), ethylbenzene (0.01 mg/m^3), toluene (0.05 mg/m^3), xylene isomers (0.03 mg/m^3), styrene (0.01 mg/m^3) and naphthalene (0.01 mg/m^3).

Innovative smoking technique proved to be an effective method for producing smoked food product with organoleptic properties equal to those of traditionally smoked one. However, the post – smoking emissions during processing require further studies to verify the risk of carcinogenic compound accumulation and human respiratory system damage when exposed for prolongued periods (e.g. workplace environment).

20.

LC-MS and GC-MS based untargeted metabolomics to study urogenital tract cancer heterogeneity

A. Yumba Mpanga[1], M. Markuszewski[2], R. Kaliszan[1], M. J. Markuszewski[1]

[1]*Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Al. Hallera 107, 80-416 Gdańsk, Poland*

[2]*Department of Urology, Medical University of Gdańsk, M. Smoluchowskiego 17, 80-214, Gdańsk, Poland*

laetitiayumba@gumed.edu.pl

Metabolomics is a complex study of small molecules that represent the end point of biological processes in cells, tissues or biofluids such as blood or urine. A comparative analysis of the metabolic profile of urine from 30 patients with cancer of the genitourinary system (bladder (n=10), kidney (n=10) and prostate (n=10)) and 30 healthy volunteers as a control group was provided by LC-TOF-MS and GC-QqQ-MS. The LC-MS analysis were provided with a gradient elution of mobile phase consisted of 0.1 % formic acid in water and 0.1% formic acid in methanol on an 150 mm x 4.6 mm x 2.7 µm Ascentis Express C-18 column (Supelco analytical, USA). For GC-MS analysis, helium was used as a mobile phase in a gradient elution on an 30 m × 0.25 mm × 0.25 µm ZB-5MS column (Phenomenex, USA). The data analysis was provided by the use of U-Mann Whitney test or student's t-test, principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). 247 compounds from LC-MS analysis (dataset A) and 27 compounds from GC-MS analysis (dataset B) was found to be statistically significant different in healthy group compared to diseased patients. The PLS-DA was used to form two models (A and B) from dataset A and B, respectively. A relatively high sensitivity which means correctly classified patient (81.81 % for model A and 100 % for model B) and specificity meaning correctly classified healthy volunteers (71.43 % for model A and 100 % for model B) were obtained. The overall classifications to a specific group were 88.1 % for model A and 89.2 % for model B. The combination of chromatographic, spectrometric analyses and chemometric techniques can allow the identification of potential biomarkers based on the differences in metabolites level.

The project was supported by the National Science Center granted on the basis of the decision number DEC-2012/07/N/NZ75/00081.

References

[1] Zhang T, Wu X, et al., Clin Chim Acta 2012, *413*, 861-868.

21.

ANALYSIS OF INSECT SIGNALLING USING ION-TRAP MASS SPECTROMETRY A CASE OF DENDROLIMUS PINI

D. Staszek, K. J. Rudzinski, M. Asztemborska, M. Cieślak, J. Raczko and R. Szmigielski

Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warszawa, Poland

Understanding of insect signalling provides effective tools for pest control. For instance, artificial sexual attractants can be used to lure male insects to traps rather than let them find the calling females. Ion-trap mass spectrometry coupled with a capillary gas chromatography is an efficient tool for analysing blends of volatile substances such as sex pheromones. This work shows the analysis of sex pheromone of pine-tree lappet moth (*Dendrolimus pini*), a serious pest of pine forests in Europe and Asia. This species belongs to a large family of Lepidoptera that comprise the second largest insect pest of coniferous trees.

(a) (b)

Fig. 1. (a) Male and (b) female of pine-tree lappet moth (*D. pini*).

Caterpillars of pine-tree lappet moth are the most dangerous defoliators of pine trees in Poland. On average, each caterpillar consumes 900-1000 needles, which destroys the assimilation apparatus and weakens the trees making them vulnerable to secondary pests. The straightforward consequence of this damage is the death of pine forests. One of the efficient and environment-friendly methods for the forest protection against *Dendrolimus pini* pest takes advantage of disturbing the mating flight with synthetic sex pheromone lures. The pheromone lures available so far are based on substances discovered in the early 1980s. They were tested in many countries and appeared rather inefficient in the forest protection.

Thus, a project was started to discover the full composition of the sexual pheromone of pine lappet moth (*Dendrolimus pini*) and to provide an improved analogue of this pheromone for better forest protection. After a challenging search for an effective method of sample collection (see a companion poster by Rudziński et al.), a variety of SPME samplers was used in a stationary sampling system. Namely, the following SMPE fibres were evaluated: polidimethylsiloxane (PDMS), carboxen-polidimethylsiloxane (CAR/PDMS), divinylbenzene-polidimethylsiloxane (DVB/PDMS), polyethylene glycol (PEG) and polyacrylate (PA). In the first phase of the study, the fibres were evaluated in a static headspace mode against the authentic standards of 5E, 7Z-C12-OH and 5E, 7Z-C12-CHO that are the recognized components of the sex pheromone of *D. pini*. Then, the pheromone samples were collected from living calling females of *D. pini*, with SPME fibers placed not more than 4 mm from the extruded ovipositor of each insect. The GC/MS-IT analyses of adsorbed analytes were carried out using a Thermo 1300 GC gas chromatograph coupled with ITQ 700 ion-trap mass spectrometer with 70eV EI ion source. Our results show which of the SPME cartridges used are appropriate for identifying the individual components of sex pheromone blend that the female pine-tree lappet moth emits.

POSTER SESSION II

THURSDAY, MAY 28th, 2015

CHAIRPERSONS: Andrzej Bąk and Andrzej Swinarew

1.

Synthesis and physicochemical properties of terephthalamides

Roksana Biegun[a], Karina Bochenek[a], Agnieszka Jędrzejowska[a], Violetta Kozik[a]

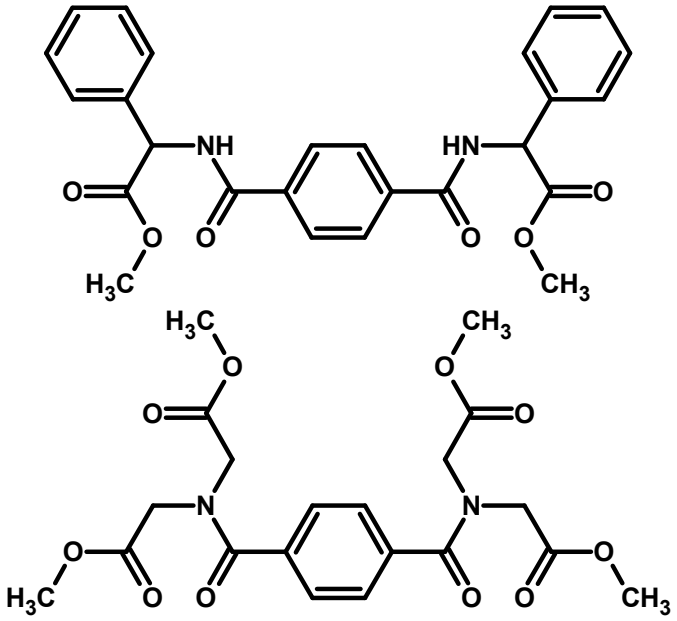
[a]*University of Silesia, Institute of Chemistry, Szkolna 9 street, 40-006 Katowice, Poland*

e-mail: biegun.roksana@gmail.com; karina.bochenek@interia.pl;

Key words: terephthalamides, spectroscopy, crystallographic

Abstract:

The aim of this study was to obtain new diamide of terephthalic acid and derivatives of phenylglycine and iminodiacetic acid. In the first step of the synthesis, the methyl ester hydrochlorides of these amino acids were obtained by reaction with methanol and thionyl chloride. Then, the hydrochlorides were carried out into diamides by reaction with terephthalic acid chloride and triethylamine in chloroform solution. After purification diamides were examinated, the melting point and the solubility were determined. Their chemical structure was confirmed by defining their crystallographic structure and by using spectroscopic methods: ^{1}H, ^{13}C NMR, ESI-MS and IR.



References:

[1] J. Tauchman, I. Cisarova, P. Stepnicka, *Eur. J. Org. Chem.*, **2010**, *22*, 4276 – 4287

[2] E. Armelin, E. Escudero, L. Campos, J. Puiggali, *Acta Cryst.*, **2001**, *C57*, 172 – 173

2.

Top 100 drug bestsellers are getting older

Jacek Bogocz, Aleksandra Tkocz, Jarosław Polanski

Institute of Chemistry, University of Silesia, PL-40006 Katowice, Poland

Drug design and development is an extremely complex technological and economic problem. The complications are clear when we explore recent controversy in pharmaceutical R&D. There are sufficient arguments and evidence to support a hypothesis that R&D productivity is steadily declining [1]. This decline is so important that new designs for Phase II trials were suggested to increase the productivity gap [2]. On the other hand, other arguments are based on evidence of no productivity crisis [3] or that "productivity rides again" [4].

We analyzed the top 100 bestselling drug list as a struggling market for FDA approvals. Our analysis showed that the time from drug (FDA) approval, if used as a measure of drug age for probing the data of the top drugs, indicated a clear increasing effect. In our opinion, this reflects the stalled launch of new drugs into the market in recent years. We probed drug-likeness MW, log P and topological polar surface (TPSA). Interestingly, the lowest MW central nervous system drugs also appeared to be the winners on the list.

References

1. Scannell, J. W. et al. Diagnosing the decline in pharmaceutical R&D efficiency. Nature Rev. Drug Discov. 2012, 11, 191-200.

2. Lindborg, S. R. et al. Statistical refocusing in the design of Phase II trials offers promise of increased R&D productivity. Nature Rev. Drug Discov. 2014, 13, 638-640.

3. Pammolli, F. The productivity crisis in pharmaceutical R&D. Nature Rev. Drug Discov. 2011, 10, 428-438.

4. Lendrem, D. et al. R&D productivity rides again? Pharmaceutical Statistics. 2015, 14, 1-3.

3.

Synthesis of a new nanographene model containing seven-membered ring

[1]Paulina Dybał, [2]Irene Rodríguez Márquez, [2]Araceli González Campaña, [2]Juan Manuel Cuerva Carvajal, [1]Viotetta Kozik

[1]Uniwersytet Śląski, Wydział Matematyki Fizyki i Chemii, Zakład Syntezy Organicznej, ul. Szkolna 9, 40-006 Katowice, paulinadybal@tlen.pl

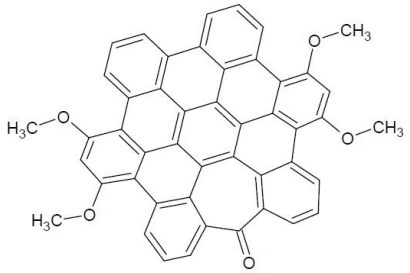
[2]Uniwersytet w Granadzie, Wydział Nauki, Zakład Chemii Organicznej C. U. Fuentenueva, s/n 18071 Granada,

One of the interesting feature of our new nanographene model is that forced into nonplanar structure may lead to changes in the optical and electrical properties. Nanographene containing in its structure a five- or seven-membered rings is more soluble than the hexagonal counterparts[1].

The aim of this study was obtaining a new model of nanographene, in which one of the six-membered rings has been replaced by the seven membered ring[2]. The distorted nanographene was synthesized by Sonogashira coupling, cyclotrimerization with cobalt complex $Co_2(CO)_8$, Scholl oxidation[3] and the ring closure was performed using iron chloride (III)[4].

The chemical structure of the obtained compounds was confirmed using ^1H and ^{13}C NMR. Attempts have been made to obtain a crystal structure.

Synthesized compounds have potential application in electrochemistry as organic semiconductors, in molecular electronics and in nanotechnology.



Scheme 1. The obtained model of nanographene.

[1] K. Kawasumi, Q. Zhang, Y. Segawa, L. T. Scott, K. Itami, Nature Chemistry, 2013, 5, 739-744

[2] J. Luo, X. Xu, R. Mao, Q. Miao, J. Am. Chem. Soc., 2012, 134, 13796−13803

[3] L. Zhai, R. Shukla, R. Rathore, Organic Letters, 2009, 5, 3474−3477

[4] S. H. Wadumethrige, R. Rathore, Organic Letters, 2008, 10, 5139-5142

4.

Mercapto-modified graphene oxide for determination of divalent metal ions and arsenic species

Paulina Janik[1], Beata Zawisza[1], Ewa Talik[2], Eva Margui[3], Ignasi Queralt[4], Rafał Sitko[1]

[1] Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland
[2]Institute of Physics, University of Silesia, Uniwersytecka 4, 40-007 Katowice, Poland
[3]Department of Chemistry, University of Girona, Spain
[4]Labolatory of X-ray Analytical Application, Institute of Earth Science Jaume Almera, Barcelona

Direct determination of trace and ultratrace amounts of heavy metal ions in samples with complex composition is a difficult task. Therefore, the application of an additional separation and/or preconcentration step before the measurement is necessary. Solid phase extraction (SPE) is one of the most commonly applied sample preparation technique due to its rapid phase extraction and low consumption of organic solvents. Another advantage of this technique is its easiness in combining with different spectroscopic techniques, both online and offline mode. The selection of the proper sorbent material is an essential step allowing to obtain high enrichment factor values and good selectivity of the procedure. The most popular solid sorbents used in SPE are silica gel, cellulose, chelating resins and, most of all, carbon nanomaterials, such as carbon nanotubes, graphene and graphene oxide (GO).

The aim of this study was to apply mercapto-modified graphene oxide (GO-SH) as solid sorbent in dispersive micro-solid phase extraction (DMSPE) for the determination of heavy metal ions and arsenic species with the use of total-reflection X-ray fluorescence spectrometry (TXRF) [1]. GO-SH was prepared by grafting 3-mercaptopropyl trimethoxysilane on a graphene oxide surface [2]. The sorption of Co(II), Ni(II), Cu(II), Cd(II), Pb(II), As(III) and As(V) ions on GO-SH was investigated. The parameters affecting the extraction process like pH, sample volume, or contact time between the analytes and sorbent were thoroughly evaluated. Proposed procedure allows obtaining high recoveries, detection limits and enrichment factors. The proposed methodology was successfully applied for determination of ultratrace amounts of metal ions in water samples. It is also noteworthy, that proposed DMSPE/TXRF procedure meets all requirements of green analytical chemistry [3].

References

1 R. Sitko, P. Janik, B. Zawisza. E. Talik, E. Margui, I. Queralt, *Anal. Chem.* **87** (2015) 3535–3542.
2 M. Z. Corazza, B. F. Somera, M. G. Segatelli, C.R.T. Tarley, *J. Hazard. Mater.* **243** (2012) 326-333.
3 F. Pena-Pereira, I. Lavilla, V. Bendicho, *Anal. Chim. Acta* **669** (2010) 1-16.

5.

Synthesis, properties and applications of thioterephthalamides

Agnieszka Jędrzejowska[a], Marek Matussek*[a], Violetta Kozik[a], Andrzej Bąk[a], Łukasz Pieszczek[a], Piotr Kuś[a]

[a]*University of Silesia, Institute of Chemistry, Szkolna 9 street, 40-006 Katowice, Poland*

e-mail: a.jedrzejowska@o2.pl

One of the characteristics of symmetric thioamides is a self-assembly process, due to secondary effects. Forming a supramolecular structure of the nano-chair is used for the preparation of nanomaterials.[1,2] The study of crystal structures is an important contribution to the understanding of the interaction of NH···O hydrogen type, appearing in diamides terephthalic acid.[3,4,5] Presence of sulfur atoms deepens the characteristic effects of the electron, compared with the spectral properties of their oxygen counterparts.[6,7] Of particular note are thioamides forming hydrogen bonds type NH···S.

The studies relate to the synthesis of new sulfur derivatives of terephthalic acid substituted methyl esters of selected amino acids. Acquiring of target compounds consists of synthesis with the use methyl ester hydrochlorides of chosen amino acids, terephthalic acid chloride, phosphorus pentasulfide supported on Al_2O_3 as a thionation agent.[8]

The chemical structure of the obtained compounds was confirmed using ^{1}H and ^{13}C spectra, and ESI-MS spectra. For each of the thioamide was examined physico-chemical properties, and attempts have been made to obtain crystal structures.

Presented class of compounds can be used as a precursor for the synthesis dithiazolidines which can be used as pharmaceuticals and orexin receptor antagonists.[9]

---

[1] D. Jang, H. Y. Lee, M. Park, S. R. Nam, J-I. Hong, *Chem. Eur. J.*, **2010**, *16*, 4836 – 4842
[2] S. Ray, R. P. Hegde, A. K. Das, N. Shamala, A. Banerjee, *Tetrahedron*, **2006**, *62*, 9603 – 9609
[3] P. G. Jones, J. Ossowski, P. Kuś, *Z. Naturforsch.*, 2002, *57b*, 914 – 921
[4] P. Kuś, J. Borek, P. G. Jones, *Acta Cryst.*, **2010**, *C66*, 093 – 096
[5] F. Garcia-Tellado, S. J. Geib, S. Goswami, A. D. Hamilton, J. *Am. Chem. Soc.*, **1991**, *113*, 9265 – 9269
[6] H. T. Flakus, W. Smiszek-Lindert, B. Hachuła, *J. Phys. Chem. A,* **2011**, *115,* 7511 – 7520
[7] W. Smiszek-Lindert, O. Lindert, M. Nowak, J. Kusz, *Acta Cryst.*, **2008**, *C64*, 0599 – 0603
[8] V. Polshettiwar, M.P. Kaushik, *Tetrahedron Lett.*, **2006**, *47*, 2315 – 2317
[9] H. Aissaoui, Ch. Boss, J. Hazemann, R. Koberstein, R. Siegrist,T. Sifferlen (Actelion Pharmaceuticals Ltd), WO 2010/004507 A1, **2010**

* Mark Matussek is co-financed by the European Social Fund, the project DoktoRIS.

6.

Investigation of alcohols and polyols oxidation products over Au_{NPs} and Pd_{NPs} catalysts using ^{1}H, ^{13}C NMR and 2D techniques

Maciej Kapkowski[a,b], Judyta Popiel[a], Kamila Czerny[a], Monika Słota[a], Jarosław Polański[a]

[a] University of Silesia in Katowice, Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-007 Katowice, Poland

Interest in the production of commodity chemicals from biomass feedstocks continues to grow as the biological and chemical transformations of carbohydrates becomes more economical. Conventional resources, mainly fossil fuels, are becoming limited for the rapid increase in energy demand. Biomass is one of renewable resources and can be used to produce various chemicals and fuels. Using renewable biomass for the synthesis of chemicals is greatly highlighted in chemical research field. Among the most promising of these processes is the aqueous-phase oxidation of alcohols and polyols, which utilizes environmentally friendly reagents and can be occured under mild conditions [1–4]. The resulting ketone, ester, aldehyde, and acid products are highly valued intermediates in the fine chemical, pharmaceutical, and agrochemical sectors [5].

Understanding the way of alcohols and polyols oxidation and modification of reaction conditions allows to control of the process towards the required products with high selectivity. The reaction mechanism research and formed mixture of products were performed by spectroscopic techniques ^{1}H, ^{13}C NMR spectra, in the alternative the two-dimensional correlation COSY and HMQC techniques.

References

[1] Zhou C., Beltramini J.N., Fan Y., Lu G.Q., *Chem. Soc. Rev*. 37 (2008) 527.
[2] Mallat T., Baiker A., *Chem. Rev.* 104 (2004) 3037.
[3] Katryniok B., Kimura H., Skrzynska E., Girardon J., Fongarland P., Capron M., Ducoulombier R., Mimura N., Paul S., Dumeignil F., *Green Chem.* 13 (2011) 1960.
[4] Besson M., Gallezot P., *Catal. Today* 57 (2000) 127.
[5] Vinod C.P., Wilson K., Lee A.F., *J. Chem. Technol. Biotechnol.* 86 (2011) 161.

Acknowledgements

This study was supported by the grant ORGANOMET No: PBS2/A5/40/2014 from the Polish National Research and Development Center

[b] Author received a grant for the project *DoktoRIS - Scholarship program for innovative Silesia* co-financed by the European Union in the framework of the ESF

7.

Application of microextraction procedures in determination of trace elements by spectroscopy techniques

Karina Kocot, Rafał Sitko

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

Miniaturisation in its broad sense and development of environmental friendly methodologies reflect current trends in the modern analytical chemistry [1]. Reduction of the amount of toxic solvents and reagents results, inter alia, in the development of new sample pre-treatment techniques as well as measurement automation. According to the aims of green analytical chemistry (GAC), one of the most universally applied enrichment/separation techniques, i.e. liquid-liquid extraction (LLE) and solid-phase extraction (SPE) have been increasingly replaced with microextraction techniques, that are their miniaturised equivalents [2,3]. Application of both liquid phase microextraction (LPME), and solid phase microextraction (SPME) enables to overcome inherent drawbacks of conventional extraction techniques, i.e. lengthy duration, labour intensity, abundant consumption of reagents and production of large amounts of wastes.

Spectroscopic techniques are considered as a sensitive, reproducible and accurate analytical methods, offering great opportunities for the detection and identification of substances in samples of complex composition. Nevertheless, meeting the requirements imposed on the routine analysis, which are especially demanding when it comes to determination of trace and ultratrace amounts of elements, requires an additional separation and/or preconcentration step prior to the measurement. This work covers recently applied approaches aiming at the development of an effective and efficient sample introduction systems, which do not require extract dilution prior the measurement.

References

4 A. Gałuszka, Z. Migaszewski, J. Namieśnik, *TrAC, Trends Anal. Chem.* **50** (2013) 78–84.

5 V. Andruch, I.S.Balogh, L. Kocúrowá, J. Śandrejová, *J. Anal. At. Spectrom.* **28** (2013) 19–32.

6 O.T. Butler, W.R.L. Cairns, J.M. Cook, C.M. Davidson, *J. Anal. At. Spectrom.* **30** (2015) 21–63

8.

Synthesis of building blocks in the quasi-heterogeneous reactions with nano-Pd/Cu catalyst- monitoring the progress of reaction by TLC

M. Korzec, R. Rzycka, S. Senkała, J. Polański

University of Silesia, Institute of Chemistry, Department of Organic Chemistry

In our investigations, we revealed that the heterogeneous catalyst composed of active catalytic Pd species on the copper support was active enough to perform the Sonogashira reaction under mild conditions. Further studies were focused on the development of more efficient, less expensive and simply formulated catalyst for the chemistry of alkynes. In these studies, TLC was used to monitor the progress of chemical reaction. Chromatographic purification of the final products was not required. Composition of novel solid phase supports, namely monolithic materials with pore dimensions of several micrometers and Pd nanoclusters with improved solubility in organic media, presumably affected reduction of reaction time and facilitated separation of the catalyst.

Literature:

1. M. Korzec, P. Bartczak, A. Niemczyk, J. Szade, M. Kapkowski, P. Zenderowska, K. Balin, J. Lelątko, J. Polanski "Bimetallic nano-Pd/PdO/Cu system as a highly effective catalyst for the Sonogashira reaction". Journal of catalysis 313 (2014) 1.
2. Y. Zheng, P.D. Stevens, Y. Gao „Magnetic nanoparticles as an orthogonal support of polymer resins: Applications to solid-phase Suzuki cross-coupling reactions". The Journal of Organic Chemistry 71 (2006) 537.

Correspondence address:korzec@autograf.pl

Acknowledgements
This study was supported by the Grant ORGANOMET No:PBS2/A5/40/2014from the Polish National Research and Development Center.Mateusz Korzec received a grant for the project DoktoRIS - Scholarship program for innovative Silesia co-financed by the European Union in the framework of the ESF.

9.

Investigation of antioxidant activity of pomegranate juices by means of electron paramagnetic resonance (EPR) spectroscopy and UV-VIS spectrophotometry

V. Kozik, A. Bąk, K. Jarzembek, M. Rotkiewicz, A. Jędrzejowska, P. Dybał, K. Pytlakowska, J. Polak, M. Bartoszek, S. Oślizlok, M. Przybyszewska, A. Stasiuka, N. Niestrój, A. Kurpanik, K. Nowosińska

[1]*University of Silesia, Institute of Chemistry, Department of Synthesis Chemistry, 9 Szkolna St., Katowice, Poland*
[2] *University of Silesia, Institute of Chemistry, Department of Organic Chemistry, 9 Szkolna St., Katowice, Poland*

Pomegranate fruit (*Punica granatum* L.) is a source of numerous phenolic compounds and it contains flavonoids such, as anthocyanins, anthocyanidins, cyanidins, catechins and other complexes of flavonoids, ellagitannins and hydrolyzed tannins. Pomegranate juice shows antioxidant, antiproliferative and antiatherosclerotic properties.

The antioxidant capacity (TEAC) of the pomegranate juices was measured using the EPR spectroscopy method and 1,1-diphenyl-2-picrylhydrazyl (DPPH•) as a source of free radicals, and the total phenolics content (TP) was measured using the UV-Vis spectroscopy. The results obtained are presented in this study.

All the examined pomegranate juices exhibited relatively high antioxidant properties. The TEAC values determined by means of EPR (using trolox, TE, as a free radical scavenger) were in the range of 463.12÷1911.91 μmol TE per 100 mL juice. The total phenolics content (TP) measured by the Folin–Ciocalteu method (using gallic acid, GA, as a free radical scavenger) widely varied in the investigated pomegranate juice samples and ranged from 1673.62 to 5263.87 mg GA per 1 L juice. The strongest antioxidant properties were observed with the fresh pomegranate juices obtained from the fruits originating from Israel, Lebanon and Azerbaijan. Correlation analysis of numerical data obtained by means of EPR (TEAC) and UV/Vis (TP) characterizes with the correlation coefficient $r^2 = 0.81$ ($p<0.05$).

(1) Fischer-Zorn, M., Ara, V. (2007) *Flüssiges Obst.* **08**, 386-393

(2) Gil, M.I., Tomas-Barberan, F.A., Hess-Pierce, B., Holcroft, D.M., Kader, A.A. (2000), *J. Agric. Food Chem.* **48,** 4581-4589

(3) Singh, R.P., Chidambara Murthy, K.N., Jayaprakasha, G.K. (2002) *J. Agric. Food Chem.* **2**, 81-86

(4) Cerdá, B., Espín, J.C., Parra, S., Martínez, P., Tomás-Barberán, F.A. (2004) *Eur. J. Nutr.* **43**, 205-220

10.

Bio-corrrosion in biopuryfication of air from VOC's mixture

Violetta Kozik[1], Andrzej Bąk[2], Damian Kasperczyk[3], Sławomir Kuś[4], Krzysztof Barbusiński[5]

[1]University of Silesia, Institute of Chemistry, Department of Synthesis Chemistry, 9 Szkolna St., Katowice, Poland
[2] University of Silesia, Institute of Chemistry, Department of Organic Chemistry, 9 Szkolna St., Katowice, Poland
[3] Ekoinwentyka sp zoo, Ruda Śląska, Poland
[4] Honeywell House, Arlington Business Park, Bracknell, Berkshire RG12 1EB, United Kingdom
[5] Politechnika Ślaska w Gliwicach. Poland

Continuous biodegradation of poisonous, volatile organic compounds (VOC's), which are released to the atmosphere during many industrial operations, is one of the fastest-growing areas of bio-processes. Biological degradation of VOC's provides a cost-effective and highly-efficient alternative to most of popular air-purification technologies like absorption or high temperature, catalytic oxidation. Considering that growth of bacteria colonies and sustaining of theirs activity are essential for proper operation of any bio-process, it is clear, that such elements like bio-reactors, piping/tubing, pumps etc. which are constructed from popular metallic materials like carbon steels or stainless steels series 300, will operate under high threat of microbiologically influenced corrosion (MIC).

High dynamics of bio-processes that includes several operational cycles like bacterial growth, immobilization and reaction, accompanied by typical operating fluctuations of process parameters like pH, oxygen concentration or fluid composition (chlorides) can significantly alter the general corrosion rate as well as potential for localized corrosion. Additionally, bio-degradation of sulphur-containing VOC's usually involves formation of additional corrosive by-products like H_2SO_4 or H_2S which may significantly accelerate corrosion processes. From such perspective, the on-line, real-time insight on the process fluid corrosivity plays a vital role in the proper corrosion management cycle and further in asset's integrity assessment.

11.

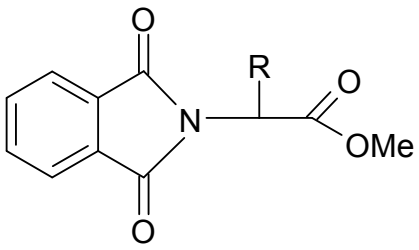
Synthesis of phthalamides obtained from methyl esters of amino acids

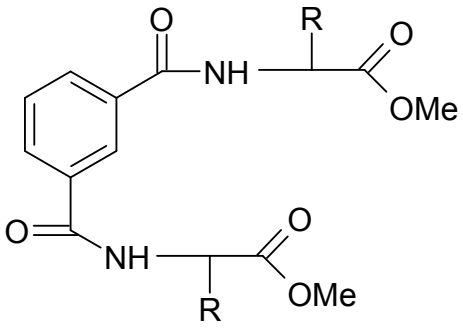
Agnieszka Kruk, Patrycja Sobota, Krystyna Jarzembek, Piotr Kuś

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

The aim of this study was to obtain new derivatives from the reaction isophthaloyl or phthaloyl chloride with hydrochlorides of selected amino acids.

Diamides of isophthalic acid and phthalimides of phthalic acid were produced with the selected aminoacids in two steps of the synthesis. In the first step of the synthesis the methyl ester hydrochlorides of chosen amino acids were obtained by reaction with methanol and thionyl chloride. Then, the obtained compounds were carried out into diamides with acid chloride and triethylamine in chloroform solution.





The usage of isophthalic acid dichloride and methyl ester with one of the selected aminoacids allows to obtain amides disubstituted. In case, when the isomer ortho of phthaloyl dichloride is used the cyclic monoamides of phthalic acid might be received corresponding to the selected methyl ester aminoacids.

The chemical structures of the obtained compounds was confirmed using ^{1}H NMR, ^{13}C NMR and ESI-MS spectra.

12.

Chromatographic and spectroscopic methods for the identification of two new psychoactive substances contained in "designer drugs"

Milena Majchrzak[1)], Marcin Rojkiewicz[1)], Anna Mazurkiewicz[1)], Rafał Celiński[2)], Mieczysław Sajewicz[1)]

1) Institute of Chemistry, The University of Silesia, Department of General Chemistry and Chromatography, 9 Szkolna Street, 40-006 Katowice

2) Toxicology Laboratory ToxLab, 6 Kossutha Street, 40-844 Katowice

„Designer drugs" are groups of substances which are from the structure and mechanism of action point of view similar to illegal psychotropic substances or narcotics like amphetamine, phencyclidine, cannabis. As a result of the closure "legal highs shops" in the last two years, designer drugs are sold on the market as "bath salt", "freshener for toilets", "kindling for the stove", "talisman smile" etc. Lots of these substances are prohibited but some of them still not. The manufactures of these specifics constantly introduced to the market new derivatives. Moreover, these substances are not under consideration of any pharmacological and toxicological studies so we observe rapidly emerging cases of poisoning by unknown substances.

Among these compounds are derivatives of cathinone. It's alkaloide contained in *Catha edulis* – flowering plant native from the Horn Africa and the Arabian Peninsula. Cathinone affect on the central nervous system like amphetamine – stimulating and empatogenic. Selection of appropriate chromatographic and spectroscopic techniques allow to develope rapid methods for identification of psychoactive cathinone's derivatives contained in sold on the market powders or pills.

In this study, we presented method for powdered samples preparation of two new cathinone derivatives and its identification by high performance liquid chromatography coupled with mass spectrometer and by mass spectroscopy MS^n with electrospray ionization.

13.

Differences in polyphenolic and elemental composition of red and white Serbian wines

Maja Natić[a], Milica Pantelić[b], Jelena Mutić[a], Živoslav Tešić[a]

[a] *Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia*
[b] *Innovation Centre, Faculty of Chemistry Ltd., University of Belgrade, Studentskitrg 12-16, 11158 Belgrade, Serbia*

The analysis of wine is very important since this beverage has a great economic and social significance. Fingerprint techniques based on chemical composition and multivariate statistical analysis can be used for classifying wine according to origin, quality, and type. In this study, 40 red wine samples and 17 white wine samples were characterised by elemental composition and polyphenolic profile. Samples were collected from different regions in Serbia: Belgrade, Central Serbia, Vojvodina, East Serbia, South Serbia and Kosovo. The measurements of major elements (calcium, sodium, potasium, magnesium, rubidium, and iron) were carried out in a Inductively Coupled Atomic Emission Spectrometer, ICP-OES (Thermo Scientific, United Kingdom), model 6500 Duo. The other elements were determined using an inductively coupled plasma mass spectrometer (ICP-MS iCAP Q, Thermo Scientific Xseries 2, UK). Quantification of phenolics was done using UHPLC coupled with a diode array detector (DAD) and connected to a triple-quadrupole mass spectrometer. PCA was performed to establish the relationship between the element composition and quantifed phenolics of wines and wine types (black and white). The PCA correlation plots showed clustering of the wines not only according to their types (red and white), but also according to geographical origin. The most influential variables responsible for the clustering were identified using the loading plots.

14.

Elemental composition and antioxidant activity of selected juices

Kinga Pala[1], Katarzyna Pytlakowska[1], Marta Skorek[2], Violetta Kozik[3]

[1]*Department of Analytical Chemistry, Institute of Chemistry, University of Silesia*

[2]*Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia*

[3]*Department of Organic Synthesis, Institute of Chemistry, University of Silesia*

The progressive degradation of the environment has an important impact on human health causing development of many civilization diseases, including different types of cancer, cardiovascular and neurological diseases, and aging-related disorders. In order to prevent these pathologies more and more attention has been paid to watch properly balanced diet. In the nutritional pyramid fruit and vegetable juices play a vital role as good sources of many valuable components, including various types of minerals and organic compounds. Among other chemical constituents they contain antioxidants such as vitamins A, C, and E, carotenoids and phenolic compounds such as flavonoids (anthocyanins, flavonols, catechins, etc.) which display antiradical, antiviral and antimicrobial activity. Moreover, they can also chelate iron, inhibit enzymes (matrix metalloproteinases), regulate gene expression, and significantly improve endothelial function.

The aim of this work was to:

- estimate the antioxidant capacity of selected fruit and vegetable juices (obtained from pomegranates, oranges, apples, pears, pineapple, mandarin, white and red grapefruits, beets, tomatoes and aloe). For this purpose four different methods: DPPH, TEAC, FRAP and CUPRAC were applied. Moreover, total phenolic content was determined using Folin-Ciocalteu reagent;
- determine elemental composition of juices by ICP-OES technique. Prior to analysis samples were mineralized using HNO_3 and H_2O_2.

15.

Analytical techniques used in the synthesis of novel thiosemicarbazones based on 5-bromosalicylaldehyde

M. Rejmund [a], J. Polański [a]

[a] *University of Silesia in Katowice, Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-007 Katowice, Poland*
e-mail of presenting author: rejmund.m@gmail.com

Thiosemicarbazones (TSC) are a class of organic compounds of great pharmaceutical value - they exhibit anticancer, antimicrobial and antiviral activity. TSCs are a versatile ligands due to the potential donor atoms that they possess, among sulfur and nitrogen are of paramount importance in the metal - ligand linkage (especially for transition metal ions). Considering all of these properties, it is important to be able to synthesize new series of thiosemicarbazones which shows biological activity without any side effects.

Microwave – assisted synthesis of TSC allows to obtained pure products in high yields, minimize to use of organic solvents and shorter reaction times [1-3].

During our work, twelve thiosemicarbazides were prepared using a reflux method (2h under reflux in ethanol) and the same quantity of thiosemicarbazones were synthesized using a microwave – assisted methodology, all of them are novel compounds.

For the preparing thiosemicarbazones we used thiosemicarbazides and 5-bromosalicylaldehyde. The reaction mixtures were irradiated in a scientific microwave reactor at 83˚C for 20 minutes at 50W. As the environment of the reaction we used 5 ml of ethanol, and the two drops of acetic acid act as a catalyst.

This method permit to obtain products in high-purity and satisfactory yields after a short time. The thiosemicarbazides and thiosemicarbazones were fully characterized by Liquid Chromatography - Mass Spectrometry, ^{1}H- and ^{13}C-NMR spectroscopy. The progress of the reaction was checked by using TLC – technique.

Acknowledgments:

Marta Rejmund is supported by the Forszt project co-financed by EU from the European Social Fund.

Reference:

[1]. C. Moretto dos Reis, D. Sousa Pereira, R. Paiva, L. Ferreira Kneipp, A. Echevarria, *Molecules.* 16 (2011) 10668-10684.
[2]. S. Cunha, T. Lima da Silva, *Tetrahedron Letters.* 50 (2009) 2090-2093.
[3]. V. Polshettiwar, R.S. Varma, *Acc. Chem. Res*. 41 (2008) 629-639.

16.

One-pot synthesis of 1,4-disubstituted-1,2,3-triazoles using nano-Pd/Cu catalyst

Roksana Rzycka[1], Sandra Senkała[1], Mateusz Korzec[1], Jacek Mularski[1], Jarosław Polański[1]

[1] *University of Silesia, Institute of Chemistry, Department of Organic Chemistry*

Triazoles are useful building blocks in chemistry and pharmacy. The 1,2,3-triazole heterocycles are recognized as biological active (e.g. anti-HIV theraupetics), antiallergic, antifungal and antimicrobial agents. Disubstituted 1,2,3-triazoles are synthesized in 1,3-dipolar Huisgen cycloaddition between organic azides and substituted alkynes. However, this synthesis requires elevated temperature and often produces mixture of the two regioisomers when asymmetric alkynes are used (fig 1.). In addition, separation of these regioisomers by chromatographic method is not straightforward and encounters difficulties. 1,4-Disubstituted-1,2,3-triazoles can be obtained via copper-catalyzed reactions. In our studies, nano-Pd/Cu system exhibited high catalytic activity in the azide-alkyne cycloaddition reaction. Bi-metallic nanocatalysts provided several advantages over the homogeneous catalysts, i.e. they featured easier separation, recovery and recycling or/and improved stability (longer lifetimes).

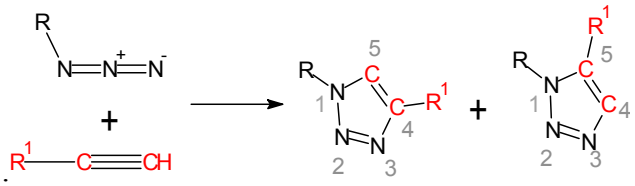


Fig. 1. Two regioisomers 1,2,3-triazole

Correspondence address: r.rzycka @gmail.com

Acknowledgements

This study was supported by the Grant ORGANOMET No: PBS2/A5/40/2014 from the Polish National Research and Development Center. Mateusz Korzec received a grant for the project DoktoRIS - Scholarship program for innovative Silesia co-financed by the European Union in the framework of the ESF.

17.

Studies on the Cadiot - Chodkiewicz coupling reaction in heterogeneous system using chromatographic techniques

Sandra Senkała[1], Roksana Rzycka[1], Mateusz Korzec[1], Jarosław Polański[1]

[1] *University of Silesia, Institute of Chemistry, Department of Organic Chemistry*

The Cadiot–Chodkiewicz reaction and other related methods including coupling of haloalkynes with terminal alkynes (Fig.1.) are the most efficient and widely used synthetic routes to variety unsymmetric diynes.

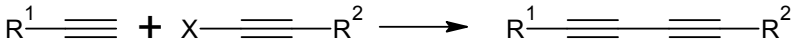


Fig.1. The Cadiot-Chodkiewicz coupling

These coupling reactions have several advantages including relatively high yield, low cost of catalyst, wide substrate scope and mild conditions. To improve the efficiency of the Cadiot–Chodkiewicz coupling, palladium catalysts with Cu(I) salts were employed.

In our investigations, we aimed to study the possibility of using the new catalysts in the Cadiot – Chodkiewicz coupling reaction. In addition, our research was focused on the synthesis of unsymmetrical and symmetrical diynes which were analyzed qualitatively and quantitatively using chromatographic methods: TLC and HPLC.

Correspondence address: senkala@op.pl

Acknowledgements

This study was supported by the Grant ORGANOMET No: PBS2/A5/40/2014 from the Polish National Research and Development Center. Mateusz Korzec received a grant for the project DoktoRIS - Scholarship program for innovative Silesia co-financed by the European Union in the framework of the ESF.

18.

Determination of lipid concentration in liposomal drug formulation

Jan Procek[1], Marek Langner[1,2], Magdalena Przybyło[1,2]

1- Faculty of the Fundamental Problems of Technology, Wroclaw University of Technology, Wroclaw, Poland.

2- Lipid Systems sp. z o.o., Wroclaw, Poland

The use of liposomes as drug carriers has been widely investigated due to their abilities to improve pharmacokinetic and reduce toxicity. Active loading of drug in the liposomes interior is one of the most efficient methods to encapsulate drug molecule. In the method an electrochemical gradient is used to drive the active molecule through the lipid bilayer. The process of liposomes manufacturing require several steps. Briefly, liposomes are extruded in solutions with high salt concentration and then the external part of the salt is substituted by a second isotonic solution, e.g. sucrose. Concentration of lipids on each step has to be precisely controlled because of the potent losses associated with complicated nature of the processes and thermal instability of some lipids. Sample preparation for HPLC is complicated due to high salt content which precipitate in organic solvents. In the presented study we investigated the use of ultrafiltration on MicroKros columns (SpectrumLabs) and Chromabond RP SPE columns (Macherey-Nagel) as a desalting method during sample preparation. Lipids were analyzed on C8-HPLC-ELSD method with the gradient of ammonium acetate in water and methanol. Obtained results shown that both method have good recovery and could be used during sample preparation process.

Acknowledgments

Presented study was co-financed by the European Union as part of the European Social Fund, the financial support of Lipid Systems sp. z o.o. and the National Centre for Research and Development, Warsaw, Poland, grant no. WND-DEM-1-027/00.

19.

An analysis of fragmental drug-likeness topology.

Aleksandra Tkocz, Jacek Bogocz, Jaroslaw Polanski

Institute of Chemistry, University of Silesia, PL-40006 Katowice, Poland

Data mining methods have potential advantages for structural chemical data and have significant impact in chemistry and drug development. Knowledge Discovery in databases allows to find interesting patterns in databases.

In contrast to the traditional drug design methods, polypharmacology is focused on the fact that one drug can interact with multiple targets. Identification of compounds that interact with multiple targets could provide information about potential side effects and improve better results for drug discovery.

Structure-Activity Relationship is an approach designed to find relations between chemical structure and biological activity of studied compounds. It is the concept of linking chemical structure to a chemical property or biological activity. Lipinski's rule of five is used as a filter and a guideline for bioavailability estimation, it defines cutoffs for molecular mass, lipophilicity, number of hydrogen bond donors and acceptors. The concept of drug-likeness has gained wide acceptance as an approach to reduce attrition in discovery and development.

Thiosemicarbazones 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 partial charge, Tanimoto and ADMET properties was used as molecular descriptors in our analyses.

References

1. Beraldo, H. Gambino, D. *The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes.* Medicinal Chemistry 2004, 4: 31-39

2. Keiser, MJ. et al. *Predicting new molecular targets for known drugs.* Nature 2009, 462: 175-181.

3. Cao, T. Jiang, T. Girke, T. *A maximum common substructure-based algorithm for searching and predicting drug-like compounds.* Bioinformatics 2008, 24: 366-374.

20.

Application of high-performance counter-current chromatography for the isolation of coumarins from the non-polar extract of *Mutellina purpurea* L.

Magdalena Walasek*, Krystyna Skalicka-Woźniak*, Tomasz Mroczek*

**Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki St., 20-093 Lublin, Poland*

m.walasek@interia.pl, 508 183 519

In the study, high-performance counter-current chromatography (HPCCC) has been successfully used for the semi-preparative isolation and purification of coumarin derivatives from non-polar extract from the fruits of *Mutellina purpurea* L.

Mutellina purpurea L. is a plant commonly growing on pastures, alpine grasslands halls and greenswards. It is typically seen in the Polish Tatra and Carpathian mountains and is easy to grow. Despite the large accessibility, the literature data contain a small amount of information concerning the detailed phytochemical researches. Previously experiments demonstrated the antibacterial, antifungal, anti-inflammatory, anti-tumor and antioxidant efficacy. As the part of our researches, the modern and innovative method - a high-performance counter-current chromatography (HPCCC) was used. The method allowed to carry out the isolation and purification of 2 simple coumarins (including osthole and its derivative) as well as an angular pyranocoumarin, named hyuganin C, in a short time. For the proper isolation a series of the two-phase systems was tested, which are the mixture of n-hexane, ethyl acetate, methanol and water. Based on the results of the selected partition coefficients, finally the mixture of n-hexane - ethyl acetate - methanol - water (5: 2: 5: 2, v/v/v/v) was chosen. The isolation was performed in the reversed phase system. As a result, in one single, isocratic run 1,7mg osthol and 1,3mg of its derivative, and 2,1mg of hyuganin C from 300 mg of the crude extract in less than 40 minutes were obtained. All compounds were separated with purity in a range between 95-99%. Identification of pure compounds was performed by using of HPLC-DAD, LC/HRMS and 1D and 2D NMR method.

21.

A new similarity measure for comparative analysis of two-way chromatographic fingerprints

Klaudia Drab, Michał Daszykowski

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

Hyphenated techniques, such as liquid chromatography with diode array detection, are frequently used to characterize complex samples (drugs, biological fluids, etc.). However, there are two major difficulties in handling chromatographic fingerprints obtained from hyphenated techniques. Usually, they provide a huge amount of data that require advanced chemometric treatment [1], since sample is characterized by a two-way chromatographic fingerprint with elements corresponding to signal intensities measured at a given wavelength channel and certain elution time. Moreover, the retention process in a given chromatographic system is amenable to small changes of experimental conditions and/or is affected by column ageing. These result in considerable shifts of corresponding peaks in collected signals that make their further chemometric analysis impossible. In the literature different alignment methods have been proposed to compensate peak shifts, but they are rather time consuming and require optimization of various input parameters [2]. Therefore, a real challenge is to enable comparative analysis of two-way chromatographic fingerprints without their prior alignment [3]. We have developed a new similarity measure, based on the correlation coefficient in order to score similarity between two two-way chromatographic fingerprints. Its performance is demonstrated using a simulated HPLC-DAD chromatographic fingerprints that take into account different chemical composition of samples, different level of peaks co-elution and different degree of peaks shifting.

Bibliography:

[1] M. Daszykowski, B. Walczak, Methods for the exploratory analysis of two-dimensional chromatographic signals, Talanta 83 (2011) 1088–1097

[2] G. Tomasi, Practical and computational aspects in chemometric data analysis, PhD thesis, The Royal Veterinary and Agricultural University, Denmark, 2006

[3] M. Daszykowski, R. Danielsson, B. Walczak, No-alignment-strategies for exploring a set of two-way data tables obtained from capillary electrophoresis-mass spectrometry, Journal of Chromatography A 1192 (2008) 157-165

22.

Chromatographic separation of products of 4-bromotoluene nitration

J. Bebłot [a], J. Polański [a]

[a] *University of Silesia in Katowice, Institute of Chemistry, University of Silesia, 9 Szkolna Street,*
40-007 Katowice, Poland
e-mail adress of presenting author: jakubbeblot@gmail.com

Nitroarenes are a class of compounds frequently used in organic synthesis because they can be easily converted into many other derivatives through reduction of nitro group into amine, and subsequent forming of diazonium salts and further displacement of N_2 group. 2-nitrobenzaldehyde can be used for synthesis of indigo, thus 2-nitro-4-bromobenzaldehyde can give tyrian purple (6,6'-dibromoindigo) in analogous way. Electron withdrawing groups such as nitro group in halogenoarenes are advantageus in such reactions as Sonogashira coupling. Our attempt to synthesize 6,6'-substituted indigo derivatives consists of nitration of 4-bromotoluene, oxidation into 2-nitro-4-bromoaldehyde, Sonogashira coupling with terminal alkynes, and synthesis of indigo derivatives by Baeyer-Drewson procedure.

Nitration of 4-bromotoluene in mixture of nitric and sulfuric acid does not lead to the formation of simple mixture of two isomers. During the nitration reaction also the mixture of dinitro derivatives of 4-bromotoluene is formed, thus the resulting mixture consists of six different compounds. Besides the two mononitro derivatives there are four dinitro derivatives formed in the reaction. The column chromatography allowed the identification of each compound on ^{1}H-NMR spectrum of crude product sample. Identification of isolated individual products also allowed to find rapid method to control progress of chromatographic separation of reaction mixture by thin layer chromatography.

References:
[1] F.R. Shaw, E.E. Turner, *J. Chem. Soc.,* **1932**, 1884-1888.
[2] R.D. Kleene, *J. Am. Chem. Soc.,* **1949**, 71, 2259-2259.
[3] J.L. Wolk, A.A. Frimer, *Molecules*, **2010**, 15, 5561-5580.