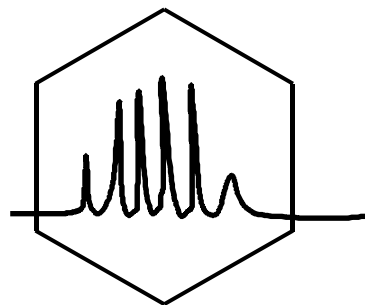


**INSTITUTE OF CHEMISTRY, UNIVERSITY OF SILESIA,
KATOWICE, POLAND**



**THE XXXVIIth
SYMPOSIUM**

**CHROMATOGRAPHIC METHODS
OF INVESTIGATING THE ORGANIC COMPOUNDS**

JUNE 11th -13th, 2014

**KATOWICE – SZCZYRK
POLAND**

PROGRAM

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SESSION I WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS: Monika Waksmundzka-Hajnos and Imre Klebovich

9.25 – 9.30 am OPENING ADDRESS

9.30 – 10.00 am

1. LC and EC-FAPA - novel applications

J. Silberring

10.00 – 10.30 am

2. Methods resulting in specific enrichment of solutes

H. Kalasz

10.30 -11.00 am

3. Application of chromatography in analysis of microtraces – case examples

G. Zadora

11.00 – 11.30 am

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

M. Pietrzyńska

1.00 pm LUNCH

SESSION II WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS: Huba Kalasz and Josef Jampilek

2.00 – 2.30 pm

5. Odor and aroma analysis: combining chemical-analytical and human detection

J. Van Durme

2.30 – 3.00 pm

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

N. Miękus

3.00 – 3.30 pm

7. Fast breath analysis by the use of GC/MS supported with novel porous polymeric materials for bio applications

A. Swinarew

3.30 – 4.00 pm

8. Extraction of tricyclic antidepressants from plasma using supported liquid extraction plates

M. Danch - ABL&E JASCO Polska

POSTER SESSION I WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS: Agnes Móricz and Łukasz Cieśla

4.00 – 5.00 pm

4.00 – 5.00 pm COFFEE BREAK

6.00 pm BONFIRE

SESSION III THURSDAY, JUNE 12th, 2014

CHAIRPERSONS: Jerzy Silberring and Hubert Paelinck

9.30 – 10.00 am

9. Bioanalytics / radiobioanalytics and imaging techniques in drug metabolism research: Overview of modern aspects and novel techniques

I. Klebovich

10.00 – 10.30 am

10. Effect – directed analysis: Bioautography – principle of the method and applications

I. Choma

10.30 – 11.00 am

11. Liquid-chromatography mass spectrometry for food authenticity assessment

Ž. Tešić

10.30 – 11.00 am

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

A. Móricz

1.00 pm LUNCH

SESSION IV THURSDAY, JUNE 12th, 2014

CHAIRPERSONS: Živoslav Tešić and Łukasz Komsta

2.00 - 2.30 pm

13. Preparative Layer Chromatography (PLC) and its use in phytochemistry

M. Waksmundzka-Hajnos

2.30 – 3.00 pm

14. Chromatographic methods for analysis of triterpenoids and phytosterols in plant epicuticular waxes

I. Vovk

3.00 – 3.30 pm

15. Bioactive natural compounds in vegetables: *Scorzonera hispanica* L. (black salsify) and Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip

Ch. Zidorn

3.30 - 4.00 pm

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

P. Stalica - Shim-pol A. Borzymowski

POSTER SESSION II THURSDAY, JUNE 12th, 2014

CHAIRPERSONS: Natalia Vorobets and Grzegorz Zadora

4.00 – 6.00 pm

4.00 – 6.00 pm COFFEE BREAK

6.00 pm DINNER

SESSION V FRIDAY, JUNE 13th, 2014

CHAIRPERSONS: Irena Vovk and Christian Zidorn

10.00 – 10.30 am

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

N. Vorobets

10.30 – 11.00 am

18. Investigation of cholic acid derivatives as transdermal permeation enhancers

J. Jampilek

11.00 – 11.30 am

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

Ł. Cieśla

11.30 am CLOSING REMARKS

12.00 am LUNCH

SESSION I

WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS:

Monika Waksmundzka-Hajnos
and Imre Klebovich

1.

LC and EC-FAPA - novel applications

Marek Smoluch¹, Michał Babij², Przemysław Mielczarek¹, Gary Hieftje³, Jerzy Silberring¹

¹*Department of Biochemistry and Neurobiology, AGH University of Science and Technology, Krakow, Poland*

²*Faculty of Microsystem Electronics and Photonics, Wrocław University of Technology, Wrocław, Poland*

³*Indiana University, Bloomington, IN, USA*

jerzy.silberring@agh.edu.pl

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

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

2.

Methods resulting in specific enrichment of solutes

Huba Kalász¹ and Kornélia Tekes²

¹*Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary*

²*Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary*

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

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

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

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

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

3.

Application of chromatography in analysis of microtraces – case examples

Rafał Borusiewicz¹⁾, Grzegorz Zadora^{1,2)}

1) *Instytut Ekspertyz Sądowych im. Prof. dra Jana Sehna, Kraków*

2) *Zakład Chemii Analitycznej, Instytut Chemii, Uniwersytet Śląski w Katowicach*

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

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

[1] J. Zięba-Palus, G. Zadora, J.M. Milczarek, *J Chrom A*, 2008 (1179) 47-58.

[2] R. Borusiewicz, G. Zadora, J. Zieba-Palus, *Talanta* 2013 (116) 630-636.

[3] R. Borusiewicz, J. Zieba-Palus J., G. Zadora, *For Sci Int*, 2006 160(2-3) 115-126.

[4] G. Zadora, A. Martyna, D. Ramos, C. Aitken, *Statistical Analysis in Forensic Science Evidential Values of Multivariate Physicochemical Data*, John Wiley and Sons, 2014.

4.

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

M. Pietrzyńska, A. Voelkel

*Poznań University of Technology, Institute of Chemical Technology and Engineering,
Pl. M. Skłodowskiej-Curie 2, 60-965 Poznań, e-mail: monikapietrzynska@gmail.com*

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

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

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

[1] M. Pietrzyńska, A. Voelkel, K. Héberger, K. Bielicka-Daszkiwicz, M. Kaczmarek, Effectiveness of in-needle extraction device for liquid samples, *Anal Chim Acta.*, 751 (2012) 182–188

[2] M. Pietrzyńska, A. Voelkel, K. Bielicka-Daszkiwicz, Preparation and examination of Monolithic In-Needle Extraction (MINE) device for the direct analysis of liquid samples, *Analytica Chimica Acta* 776(2013) 50-56

SESSION II

WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS:

Huba Kalasz and Josef Jampilek

5.

Odor and aroma analysis: combining chemical-analytical and human detection

Jim Van Durme*

*Research Group Molecular Odor Chemistry, Department of Microbial and Molecular Systems (M2S),
Research Cluster Food and Biotechnology, KU Leuven Campus Ghent, Technology Campus,
Gebroeders De Smetstraat 1, B-9000 Ghent, Belgium (*corresponding author:
jim.vandurme@kuleuven.be)*

ABSTRACT

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

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

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

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

REFERENCE:

Van Durme, J., Goiris, K., De Winne, A., De Cooman, L., & Muylaert, K. (2013). Evaluation of the Volatile Composition and Sensory Properties of Five Species of Microalgae. *Journal of Agricultural and Food Chemistry*, 61(46), 10881–10890. doi:10.1021/jf403112k

6.

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

Natalia Miękus, Alina Plenis, Piotr Kowalski, Tomasz Bączek

Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Hallera 107, 80-416 Gdańsk, Poland

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

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

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

7.

Fast breath analysis by the use of GC/MS supported with novel porous polymeric materials for bio applications

J. GABOR¹, T. FLAK¹, B. SWINAREW³, E. LATOS¹, A.S. SWINAREW^{1,2*}

¹*Institute of Materials Science, University of Silesia, 40-007 Chorzow, Poland*

²*SHIM-POL A.M. Borzymowski E.Borzymowska-Reszka A. Reszka Spółka Jawna*

³*Institute for Engineering of Polymer Materials and Dyes, Paint and Plastics Department, 44-100 Gliwice, Poland*

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

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

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

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

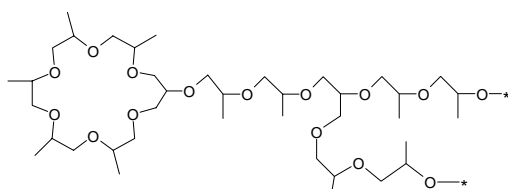


Fig. 1. Macromolecule with two branches (* means an end)

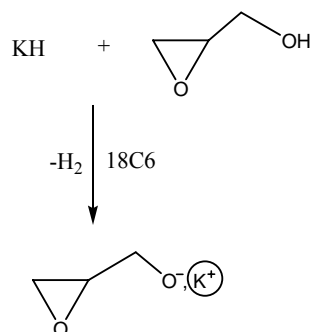


Fig. 2. Initiators synthesis

8.

Extraction of Tricyclic Antidepressants from Plasma Using Supported Liquid Extraction Plates

Mirosław Danch

ABL&E JASCO Polska Sp z o.o.

Abstract

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

SESSION III

THURSDAY, JUNE 12th, 2014

CHAIRPERSONS:

Jerzy Silberring and Hubert Paelinck

9.

**Bioanalytics / radiobioanalytics and imaging techniques in drug metabolism research:
Overview of modern aspects and novel techniques**

Imre Klebovich

*Semmelweis University, Department of Pharmaceutics,
Budapest, Hungary*

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

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

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

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

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

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

10.

Effect – directed analysis: Bioautography – principle of the method and applications

Wioleta Jesionek¹, Edyta M. Grzelak², Barbara Majer-Dziedzic³ and Irena M. Choma¹

¹ *University of Maria Curie-Skłodowska, Lublin, Poland*

² *Institute for Tuberculosis Research, University of Illinois, Chicago, USA*

³ *University of Life Sciences, Lublin, Poland*

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

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

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

References:

[1] M.G. Weller, *Sensors*, **2012**, 12, 9181-9209.

[2] L. Botz, S. Nagy, B. Kocsis, in: Sz. Nyiredy (Ed.), *Planar Chromatography*, Springer, Budapest, **2001**, 489-516.

[3] I. M. Choma, E. M. Grzelak, *J. Chromatogr. A*, **2011**, 1218, 2684–2691.

[4] E.M. Grzelak, W. Jesionek, B. Majer-Dziedzic, I.M. Choma, *JAOAC Int., Sp. Section*, **2013**, 96, 1167-1174.

11.

Liquid-chromatography mass spectrometry for food authenticity assessment

Živoslav Tešić

Faculty of Chemistry, University of Belgrade

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

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

Highly efficient liquid chromatography connected to mass detector for determination of relations between stable isotops is very successful for food authenticity assessment. This method combines chromatographic technique with stable isotope determination. Variations detected in isotopic representation of stable isotops of hydrogen, carbon, nitrogen and oxygen are of great interest for food authenticity studies. Most chemical elements have stable isotops that might be useful in this purpose. All plants are divided into 3 types by way of photosynthesis: C₃ (Calvin-Benson photosynthetic cycle), C₄ (Hatch-Slack cycle) and CAM (Crassulacean cycle) plants. Each photosynthetic way discriminates in a different manner heavier carbon isotope (¹³C), found in the atmosphere. In that case C₃ and C₄ demonstrate different ranges ¹³C, δ¹³C value and so in C₄ plants that value varies from -11 to -14 ‰ while in C₃ plants it varies from -23 to -30 ‰. In this manner relative quantities of C₃ i C₄ ingredients used from their production can be determined. By using this method it is possible to determine counterfeit honey and wine. In addition to that, stable isotops provide us with other important information such as: isotopes H - climate indicator, geographical origin, botanical origin; isotopes O – geographical origin; isotopes C – climate indicator, ways of nutrition, geographical origin; isotopes N – organic food / agricultural practice; isotopes S – geological indicator.

12.

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

Ágnes M. Móricz, Péter G. Ott

*Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences
Herman Ottó Str. 15 H-1022 Budapest, Hungary*

In the fight against various human, animal and plant diseases there is an increasing demand for new bioactive agents, especially antimicrobials, because of the emergence and spread of bacterial resistance to commonly used antibiotics. The plant kingdom possessing an arsenal of secondary metabolites represents an underestimated source of bioactive substances; however obtaining pure active compounds can be time-consuming and expensive, especially when bioactivity is studied only after the isolation process that usually incorporates extraction, fractionation and purification steps. Therefore, to save time and money, the strategy of the isolation process has been changed in the last decade to focus on the isolation of only the components having the desired activity. The bioassay-guided analyses are essential for this purpose, as they lead only the active fractions along the process and discard the uninteresting ones. These procedures require continuous bio-monitoring as guidance, for which planar chromatography hyphenated with bioassays is especially suited, fulfilling such requirements as being high-throughput, rapid, relatively simple and reliable. Its additional advantage is a possibility to analyse single components in various matrices, thus eliminating synergistic and antagonistic effects. The further combination of this system with spectroscopic and spectrometric techniques enables the characterization of the bioactive substances.

In this study we demonstrate through examples the applicability of planar chromatography-direct bioautography as a rapid, easy-to-use tool for monitoring the components having antibacterial effect against the Gram-negative pepper pathogen *Xanthomonas vesicatoria*, the luminescence gene-tagged *Arabidopsis* pathogen *Pseudomonas syringae* pv. *maculicola*, luminescent marine *Vibrio fischeri* bacteria and the Gram positive soil bacterium *Bacillus subtilis*.

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

SESSION IV

THURSDAY, JUNE 12th, 2014

CHAIRPERSONS:

Živoslav Tešić and Łukasz Komsta

13.

Preparative Layer Chromatography (PLC) and its use in phytochemistry

Monika Waksmundzka-Hajnos, Grzegorz Józwiak

Department of Inorganic Chemistry, Medical University of Lublin, Poland

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

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

The use of special development modes (such, as multiple development techniques and multidimensional separations) can increase the separation yields and bring satisfactory results. The goals of preparative layer chromatography (apart from isolation of the pure compounds) are the following ones: an important step of sample preparation, an on-line purification of crude plant extracts, and optimization of column preparative separations.

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

14.

Chromatographic methods for analysis of triterpenoids and phytosterols in plant epicuticular waxes

Irena Vovk^{1,2}, Katerina Naumoska¹, Zoran Kitanovski¹, Alen Albreht^{1,2}, Breda Simonovska¹
Mitja Martelanc¹, Karmen Kapp³, Heikki Vuorela³

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

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

³ *Faculty of Pharmacy, P.O. Box 56, University of Helsinki, FIN-00014 Helsinki, Finland*

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

The aim of our work was to investigate triterpenoids and phytosterols in epicuticular waxes of various vegetables and several Slovenian apple cultivars. Among vegetables we investigated cabbage, eggplant, zucchini, tomato, red pepper, mangold, spinach, lettuce white-colored radicchio di Castelfranco, raddichio Leonardo, white cabbage, red cabbage, savoy cabbage and parsley. Screening of the extracts before and after hydrolysis was performed by silica gel and reversed-phase (C18 RP) thin-layer chromatography (TLC), RP-TLC-MS/MS² and C18 RP high-performance liquid chromatography (HPLC) with UV and mass spectrometric (MS) detection using atmospheric pressure chemical ionization (APCI) and gas chromatography (GC). For all the extracts TLC screening on silica gel (separation of triterpenoids with different functionality) and on C18 RP (separation of isomers) was performed before the HPLC-UV and HPLC-MS analysis. Different developing solvents were used for the separation of triterpenolic acids and neutral triterpenoids on the C18 RP HPTLC plates. For the RP-TLC-MS/MS² confirmation of the presence of the triterpenoids and phytosterols in the extracts we created mass spectra library. Additionally, all the extracts were analyzed by the new HPLC-UV-APCI-MS/MS² and GC-FID methods. Critical evaluation of the results obtained by all the methods confirmed the presence of some of the studied triterpenols (α -amyrin, β -amyrin, lupeol), esterified triterpenol (lupeol acetate) triterpenolic acids (ursolic, oleanolic), lupenone and phytosterols (β -sitosterol, stigmasterol) in the plant extracts, while the presence of cycloartenol, cycloartenol acetate and betulinic acid was not confirmed.

15.

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

Christian Zidorn

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

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

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

16.

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

Paweł Stalica

“Shim-Pol A.M Borzymowski” E. Borzymowska-Reszka, A. Reszka sp.j.

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

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

SESSION V

FRIDAY, JUNE 13th, 2014

CHAIRPERSONS:

Vovk and Christian Zidorn

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

N. Vorobets*, M. Olech**, R. Nowak**

*Department of Pharmacognosy and Botany, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

** Department of Pharmaceutical Botany, Medical University of Lublin

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

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

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

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

18.

Investigation of Cholic Acid Derivatives as Transdermal Permeation Enhancers

Josef Jampilek¹, Lenka Coufalová¹, Lech Mrózek²

¹*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

²*BorsodChem MCHZ s.r.o., Chemická 1/2039, 709 03 Ostrava-Mariánské Hory, Czech Republic*

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

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

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

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

19.

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

ŁUKASZ CIEŚŁA, KAROLINA WOJTUNIK & MONIKA WAKSMUNDZKA-HAJNOS

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

Lublin, Poland

e-mail: lukecarpenter@poczta.onet.pl (Łukasz Cieśla)

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

POSTER SESSION I

WEDNESDAY, JUNE 11th, 2014

CHAIRPERSONS:

Agnes Móricz and Łukasz Cieřła

1.

Co-clustering of chromatographic data

Klaudia Drab, Michał Daszykowski

Instytut Chemii, Uniwersytet Śląski, Katowice 40-006

Separation and determination of individual components in a complex mixture is a necessary and important step in chemical and biological sciences. In everyday laboratory practice, chromatographic methods are frequently applied to accomplish these fundamental goals. They are well suited for the compositional analysis of drugs, biological fluids, food samples, cosmetics, etc. However, with the increasing number of samples and parameters being analyzed, useful chemical information can be hidden in analytical data. Recently, there is a trend to uncover groups of samples by means of clustering with respect to their specific chemical features. It can be done with the so-called co-clustering or biclustering techniques [1]. Co-clustering is a useful tool when a particular subset of samples should be related with meaningful subset of parameters. The main advantage of such a methodology is that samples and parameters are clustered simultaneously. As a result, individual samples can belong to several clusters, and detected clusters can overlap to each other [2].

In this study, theoretical aspects of co-clustering are discussed, and as an example a real data set of 572 samples of olive oil is used. For each sample concentrations of eight fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, eicosanoic, linolenic, and eicosenoic) were determined by gas chromatography [3]. Olive oil samples have been collected in nine different regions in Italy (North Apulia, Calabria, South Apulia, Sicily, Inland Sardinia, Coast Sardinia, East Liguria, West Liguria, and Umbria).

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2.

Identification of geographical origin of Indian opium on the basis of amino acid profiles

Joanna Orzel, Michal Daszykowski

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

The Opium poppy (*Papaver somniferum*) is cultivated mainly in Turkey, Indian subcontinent, China, Spain, France and Australia. Dried latex from this plant is called opium. Due to its narcotic properties, illicit cultivation of Opium poppy, its further processing and distribution is strictly prohibited. Attempts have been made to develop an analytical tools facilitating identification of geographical origin of opium. It is well known that the mineral nutrition of plants can be related with place of their cultivation. On the other hand, link between the mineral nutrition of plants and their amino acid composition was reported [1]. Thus, the possibility to assess geographical origin of opium samples on the basis of their amino acid profiles was evaluated.

A total of 124 samples of opium collected from three states of India were examined according to their amino acid composition. 14 amino acids (i.e. aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, and arginine) were quantitated using liquid chromatography coupled with fluorescence detection [2]. On the basis of obtained profiles chemometric models discriminating samples according to their origin were constructed. In order to build models, discriminant variant of partial least squares algorithm, D-PLS [3], was used.

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3.

Detection of counterfeit medicines based on chromatographic impurity profiles

B. Krakowska^a, I. Stanimirova^a, D. Custers^{b,c}, E. Deconinck^b, M. Daszykowski^a

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

^b*Division of Food, Medicines and Consumer Safety, Section Medicinal Products, Scientific Institute of Public Health (WIV-ISP), J. Wytsmanstraat 14, B-1050 Brussels, Belgium*

^c*Laboratory of Pharmacognosy and Pharmaceutical Analysis, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium*

Quality control of drugs is of great importance in the protection of human health. Counterfeit medicines are a global concern because they are not subjected to quality control, and thus their safety and effectiveness cannot be guaranteed. Safety assessment of drugs is not based solely on the identification and quantification of the active substances but also on the analysis of secondary components which might exhibit toxic effects (e.g. residual solvents, impurities). In this work the samples of counterfeit and authentic Viagra medicine were analyzed by means of High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). The obtained impurity profiles were used as fingerprints and analyzed using chemometric methods. In order to distinguish between counterfeit and authentic samples of medicine the discriminant variant of partial least squares, PLS-DA method was used [1]. Whereas to uncover differences in chemical composition variable importance in projection criterion VIP, was applied [2]. By means of this discriminant approach 90% of samples was correct assigned to the appropriate classes.

Literatura

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4.

**Probing a chemical space for fragmental topology-activity landscapes (FRAGTAL):
Application for diketo acid and catechol HIV integrase inhibitor offspring fragments**

Andrzej Bak¹, Tomasz Magdziarz¹, Violetta Kozik¹, Krystyna Jarzembek¹, Marcin
Rojkiewicz¹, Agata Kurczyk¹, Katarzyna Serafin and Jaroslaw Polanski¹

¹ *Institute of Chemistry, University of Silesia, 40-006 Katowice, Poland*
e-mail: Andrzej.Bak@us.edu.pl

The nature of the drug-target interactions ruled by inter- and/or intra-molecular forces is a tremendously complex and divergent process that still lacks a universal approach. Hence, several approaches to drug discovery have been developed in the quest to identify the differences between drug- and non-drug molecules (chemicals). Most often, these methods are related to the drug-likeness concept originating from the idea that drug compounds differ from other molecules in their physicochemical properties.

In reality, molecular descriptors quantifying drug-like properties (DP) are easily available from molecular formulae, e.g. the number of hydrogen bond donors (HBD) or acceptors (HBA). The emergence of the quantitative property-based Lipinski Ro5 ‘sieve’ is focused on the importance of restricting the chosen *in silico* molecular parameters of preclinical drug candidates in the pharmacokinetic-friendly property space. The discrimination Ro5 model restrains the ‘drug-like property space’ through a set of threshold values which are necessary but are not sufficient boundary guidelines of the preferred ‘drug architecture’ that is especially useful in the computer-driven process of filtering chemical libraries; however, this concept is still elusive and ambiguous. Fragment-based drug design or fragonomics, which has recently been getting more and more widespread appeared to offer an alternative way for identifying advantageous drug structures. This methodology assumes the existence of a preferred molecular ‘core’, namely a framework that works as an ‘anchor’ for the ‘ornamenting’ blocks – privileged motifs. Generally, the problem of identifying relevant molecular fragments is a complex issue that is related to the so-called frequent subgraph mining (FSM). More practical FSM approaches to drug design are still a matter of future developments that need some sophisticated algorithms for massive *in-silico* database mining and data processing.

Fragmental topology-activity landscapes (FRAGTAL), a new concept for encoding molecular descriptors for fragonomics into the framework of the molecular database records is presented. Thus, a structural repository containing biological activity data was searched in a substructure mode by a series of molecular fragments constructed in an incremental or decremental manner. The resulted series of database hits annotated with their activities construct FRAGTAL descriptors encoding a frequency of the certain fragments among active compounds and/or their activities. Actually, this method might be interpreted as a simplified adaptation of the frequent subgraph mining (FSM) method. The FRAGTAL method reconstructs the way in which medicinal chemists are used to designing a prospective drug structure intuitively. A representative example of the practical application of FRAGTAL within the ChemDB Anti-HIV/OI/TB database for disclosing new fragments for HIV-1 integrase inhibition is discussed. In particular, FRAGTAL method identifies ethyl malonate amide (EMA) as the diketo acid (DKA) related arrangement. Since new molecular constructs based on the EMA fragment are still a matter of future investigations we referred to this as an the DKA offspring.

5.

Chemometric analysis of TLC spots and adsorbents by NIR and ATR spectrophotometry

Joanna Polek, Łukasz Komsta

Department of Medicinal Chemistry, Faculty of Pharmacy

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

The TLC infrared detection is very rarely presented in literature, however it is easy to handle. The spot can be measured by NIR-reflectance spectrophotometry without destroying adsorbent layer and by ATR-FTIR spectroscopy after grinding small fragment of adsorbent from the centre of the spot.

The infrared spectra of pure TLC plates and caffeine/theophylline spots in 14 various concentrations (2 – 56 ug per spot, total 15 distinct concentrations) were collected from silica, alumina, DIOL, NH₂, CN, RP8, RP18, RP2 and cellulose plates. Thermo Nicolet 67000 with diamond ATR or integrating NIR sphere units were used to collect spectra. The wavenumber ranges were: 650 – 4000 (6950 data points) and 4000 – 10000 (1557 data points). The whole dataset consisted of 555 NIR spectra and 135 ATR-FTIR spectra.

The explorative analysis (scaled PCA) of the NIR dataset led to conclusion, that the adsorbents form very distinct clusters and there is an easy ability to identify adsorbent from NIR spectra of the spot, regardless of caffeine concentration. Only slight dependence of caffeine concentration in spot was observed, which was surely not enough to build quantitative supervised chemometric models. Over 80% of variance was included in two first PCs. There is a clustering tendency between caffeine and theophylline NIR spots on silica, however this tendency is also related to variability between plates, therefore trying to find discriminative model would be overoptimistic.

In the case of ATR-FTIR, the spectra of cellulose and alumina were outliers in multivariate space, and first two PCs (95% of variance) is related to differences between them and other adsorbents. Clustering of other adsorbents can be seen in PC3 and PC4 space (about 2% of variability). Removing of outliers changes only slightly clustering of other adsorbents.

The final conclusion from this preliminary study is that quantitative detection in TLC by infrared method cannot be easily applied. However, the NIR spectrum of the plate is quite constant regardless of presence/absence of any substance in measured place. Taking into the account the distinction of spectra of various adsorbents, NIR can be useful tool to identify the adsorbent of the plate, even in the case of used/developed plates.

6.

Quantitative structure-metabolism relationship modeling of new aryl-piperazine derivatives metabolism rates

Szymon Ulenberg¹, Mariusz Belka¹, Marek Król², Franciszek Herold², Tomasz Bączek¹

1. Department of Pharmaceutical Chemistry, Medical University of Gdańsk,

2. Department of Drug Technology, The Medical University of Warsaw

Low metabolic stability of drug derivatives is a factor, that often excludes them from further clinical trials. Therefore, a way to predict this stability in a cheap, fast and efficient manner is valuably considered. In this experiment a half time of 30 potent anti-depressive drugs was evaluated. Several molecular descriptors using Gaussian and Dragon software were also computed. Using Statistica software a transformation and auto-scaling was applied to the whole dataset. Applying multiple linear regression, a model was proposed that quantitatively shows which molecular descriptors possess the most significant effect on metabolic stability of the studied compounds. Metabolic stability test was performed with the use of microsome preparations obtained from rat liver in *in vitro* conditions in the presence of NADPH. The study of the possible first phase metabolic biotransformation catalyzed mostly by cytochrome P450 enzymes was conducted. Separation and quantification of compounds was performed using a Single Quadrupole LC-MS system with C18 column. A relationship between structure of arylpiperazines and their half-time were straight-forward discussed.

7.

**The influence of chaotropic effect on retention of fluoroquinolones in
RP-HPLC, RP-OPLC and RP-TLC system**

Małgorzata Kamińska¹, Marek Studziński¹, Patrycja Cieśla¹, Anna Czajkowska-Żelazko^{1/2},
Mateusz Jasikowski^{1/3}, Karol Pilorz¹ and Irena Choma¹

¹*Chromatographic Method Department, UMCS, Lublin, Poland*

²*Zakład Diagnostyki Hematologicznej, UM, Lublin, Poland*

³*Medicofarma SA, Radom, Poland*

Chaotropic ions are small inorganic compounds causing disruption of the water structure. They are arranged in the so-called Hofmeister series according to ability to cause “chaos” in water structure, what is connected with their polarizability, charge delocalization and symmetry [1]. Chaotropic ions, oppositely to surfactants, show weak interactions with alkyl chains of bonded phase in RP chromatographic systems. However, they influence interaction of basic compounds with alkyl chains increasing their retention. According to Kazakevich and LoBrutto theory, chaotropic anions, as perchlorate or hexafluorophosphate, disrupt hydrogen bridges in water shell surrounding the solute, in this way increasing hydrophobicity of the solute [2,3]. The influence of chaotropic anions on retention of cationic solutes can be also interpreted by ion-pair or dynamic ion-exchange mechanism. Probably the mechanism of retention is complex and related to contribution of all above mentioned models.

Five amphoteric piperazynyl fluoroquinolones, which are bases in acidic conditions, and flumequine, which is neutral at a low pH, were analyzed in HPLC, OPLC and TLC RP systems [4]. The influence of chaotropic ions on retention of these drugs was compared and discussed.

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8.

Solubility parameter used to predict the effectiveness of monolithic in-needle extraction (MINE) device for the direct analysis of liquid samples

Monika Pietrzyńska, Katarzyna Adamska, Magdalena Szubert, Adam Voelkel

Poznań University of Technology, Institute of Chemical Technology and Engineering, pl. M.

Skłodowskiej-Curie 2, 60-965 Poznań, Poland; monikapietrzynska@gmail.com

Combination of extraction and chromatographic techniques opens NEW possibilities in sample preparation area. Macroporous poly(styrene-divinylbenzene) (PS-DVB) monoliths with different proportion of monomers were prepared by in situ polymerization in stainless steel needles [1]. MINE devices were used in the preparation of a series of test water samples for chromatographic analysis. Taking into account possible large flow resistance of monolithic sorbent layer it was necessary to examine the permeability of the in-needle device.

So far in-needle technique was relatively seldom used for direct separation of analytes from liquid samples and most often was combined with head-space (HS) or purge and trap (P&T) techniques. This limited application is associated with a high flow resistance produced by a sorbent layer. New proposal - the application of monolithic filling in the in-needle device should prevent changes occurring in the sorbent layer and increase the efficiency of this sample preparation tool.

The magnitude of interactions between three components: monolithic sorbents, eluents and analytes were estimated by using solubility parameters [2]. The expected calculated results were compared with experimental ones. Therefore, it can be concluded, that based on the solubility parameter concept, it is possible to select a suitable sorbent and solvent for the proper isolation of the examined analytes.

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Chromatographic analysis of defense sprays for forensic purposes

Rafał Borusiewicz¹⁾, Grzegorz Zadora^{1,2)}

1) Instytut Ekspertyz Sądowych im. Prof. dra Jana Sehna, Kraków

2) Zakład Chemii Analitycznej, Instytut Chemii, Uniwersytet Śląski w Katowicach

Defence sprays are pressurised metal cans equipped with a nozzle and containing liquid solution of active ingredient. The active ingredients are having irritant effect on humans, causing burning sensation, troubled breathing and temporary blindness. Natural and synthetic capsaicinoids (CAP's) as well as chlorobenzylidene malononitrile (CS) are most often encountered on Polish market. Sometimes formulations containing obsolete 2-chloroacetophenone (CN) still can be found. Defence sprays which are freely accessible on Polish market are, despite of their name, often used by perpetrators. Items connected with such cases are subjects of chemical analysis in forensic laboratories. Samples are analysed using gas chromatography with mass spectrometry (GC-MS).

If original can is sent to be analysed, preparation is limited to taking a sample of liquid content and diluting it with polar organic solvent e.g. methanol. Even for seemingly empty cans some amount of the original content can be recovered after cutting it. If some other materials are to be analysed, like clothes of victim or suspect, the first step of analysis is to separate and concentrate the traces of active component. CS and CN are volatile enough to be separated using passive adsorption from the headspace of the sample. To separate capsaicinoids (active ingredients of "pepper sprays") liquid extraction with methanol must be conducted.

Details of the isolation procedures and parameters of chromatographic analysis will be presented on the example of two real cases: one with PAVA (colourless synthetic capsaicinoid) and one with the traces of CS gas.

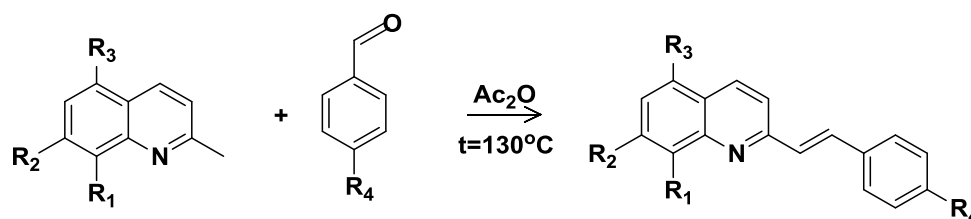
10.

Chromatographic methods of identification and purification of styrylquinoline derivatives

Milena Majchrzak, Wioleta Cieřlik, Mieczysław Sajewicz, Robert Musiol

Institute of Chemistry, University of Silesia

The fungal infections become serious problem of modern medicine. Especially after, the growth of systemic mycoses and the emergence of drug resistance that have been noticed recently. Some simple derivative of quinaldine are effectively applied in the modern pharmacotherapy as 5,7-dichloro-quinolin-8-ol. In this study we are searching for new styrylquinoline derivatives which potentially show antifungal properties. Four synthesis was carried out following the scheme:



- | | |
|----|--|
| 1) | $R_1 = -H, R_2 = -H, R_3 = -H, R_4 = -C_4H_9,$ |
| 2) | $R_1 = -H, R_2 = -H, R_3 = -H, R_4 = -OC_4H_7$ |
| 3) | $R_1 = -H, R_2 = -H, R_3 = -H, R_4 = -OC_6H_{13},$ |
| 4) | $R_1 = -OH, R_2 = -Cl, R_3 = -Cl, R_4 = -C_4H_9$ |

Post- reaction mixtures were analyzed by thin layer chromatography (TLC) in the following conditions of separation- stationary phase: aluminium sheets coated with silica gel 60 F₂₅₄ (DC Kieselgel 60 F₂₅₄, Merck), mobile phase: ethyl acetate/ hexane (1:8, v/v). Visualization was carried out at a wavelength of 254 nm. The TLC chromatograms were the basis for the design of column chromatography separation for each product. The detailed procedure are provided in the table:

| Synthesis | Stationary phase | Mobile phase |
|-----------|---|-------------------------------------|
| 1 | Silica gel 0,035-0,070mm, 60A (Poch S.A.) | ethyl acetate/ hexane (1:8, v/v) |
| 2 | Silica gel 0,035-0,070mm, 60A (Poch S.A.) | ethyl acetate/cyclohexane(1:10,v/v) |
| 3 | Silica gel 0,035-0,070mm, 60A (Poch S.A.) | ethyl acetate/ hexane (1:8, v/v) |
| 4 | Silica gel 0,035-0,070mm, 60A (Poch S.A.) | ethyl acetate/ hexane (1:10, v/v) |

11.

Investigation of glycerol oxidation mechanism over nanogold catalyst using NMR and MALDI techniques

Maciej Kapkowski^{a,c}, Jadwiga Gabor^b, Jarosław Polański^a

^aUniversity of Silesia in Katowice, Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-007 Katowice, Poland

^bInstitute of Materials Science, University of Silesia, 75 Pułku Piechoty 1A Street, 41-500 Chorzow, Poland

Abstract

Glycerol constitutes a potential starting material for various industrially valuable products, such as glyceric acid, tartronic acid, acetic acid and acrolein. Heterogeneous catalysts based on nanoparticulate gold supported by a variety of carriers such as: copper, nickel, silica play a key role in the promotion and control of oxidation reactions. The selectivity of oxidation to specific products is determined by numerous parameters, among others: the metal particles size, the pore size of the support and the pH of the reaction medium [1,2].

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

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Acknowledgements

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

^c 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

12.

Synthesis of thioterephthalamides obtained from methyl esters of chosen aminoacids and thioterephthaloyl chloride

A. Jędrzejowska*, P. Dybał, V. Kozik, M. Matussek, K. Jarzembek, A. Bąk, P. Kuś

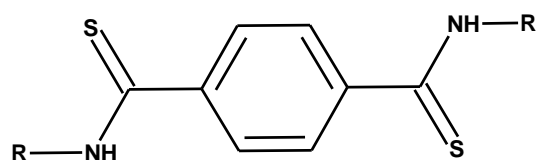
University of Silesia, Institute of Chemistry, Szkolna 9 street, 40-006 Katowice, Poland

* e-mail: a.jedrzejowska@o2.pl

Keywords: thioamides, diamides, terephthalic acid, carrier compound, column chromatography, NMR Spectra

Abstract:

The aim of this study are to synthesizing new thioterephthalic acid diamides (thioterephthalamides) from thioterephthaloyl chloride, phosphorus pentasulfide with carrier compound and methyl esters of chosen aminoacids, investigating the physicochemical properties of the new compounds and trying to find their crystallographic structure. Chemical structure obtained intermediates products and thioterephthalamides was determined by ^1H NMR, ^{13}C NMR spectra. Solubility and melting point were determined for new molecules.



R = methyl ester aminoacid

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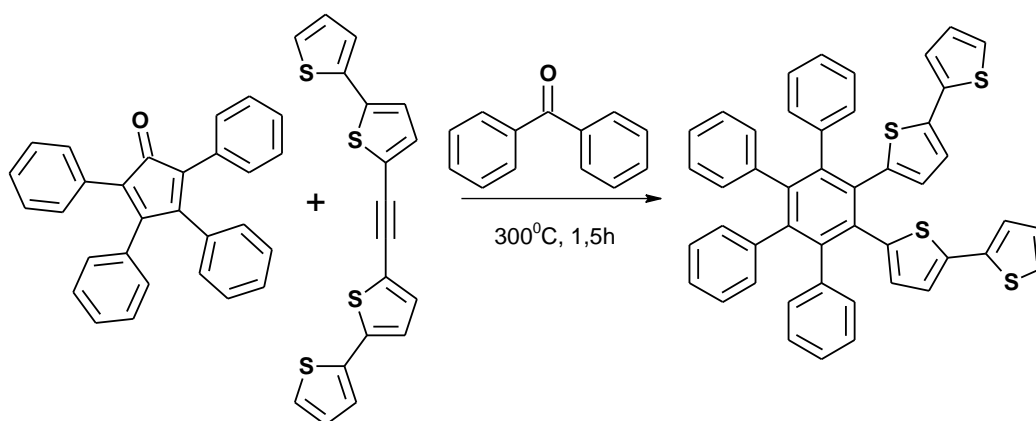
13.

[4 +2] Cycloaddition reactions - new hexasubstituted benzene derivative

Agata Szłapa^a, Sławomir Kula^a, Marek Matussek^a, Michał Filapek^a, Stanisław Krompiec^a

^aInstitute of Chemistry, University of Silesia, 40-006 Katowice, Poland

[4 +2] Cycloaddition reaction (with CO-extrusion) of disubstituted alkynes to selected cyclopentadienones creates the new opportunities in the synthesis of organic compounds [1], [2]. This reaction opens up the possibility to obtain compounds with potential uses in the organic electronics: donor-acceptor systems, π - conjugated derivatives or monomers for conductive polymers, to name a few. In the first step of our research bis(2,2'-bithiophene-5-yl)acetylene (in the Sonogashira coupling) was obtained. This alkyne, enables to obtain a various hexasubstituted benzene derivative containing 2,2'-bithiophene substituents (Scheme 1).



Scheme 1. The synthesis of hexasubstituted benzene derivative (example).

The aim of the study was to obtain 1,2-bis(2,2'-bithiophene-5-yl)acetylene by Sonogashira coupling, followed by [4 +2] cycloaddition reaction (with CO-extrusion) to selected cyclopentadienones leading to the formation of hexasubstituted benzene derivative. The structure of all the obtained compounds was confirmed by ¹H NMR, ¹³C NMR and HRMS.

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Agata Szłapa acknowledge a scholarships from the DoktoRis project co-financed by the European Social.

14.

Synthesis of fluorine and carbazole derivatives as fluorescent materials for organic electronics

M. Matussek*, A. Szłapa, A. Słoddek, I. Grudzka, A. Jędrzejowska, S. Krompiec

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

Keywords: carbazole, fluorene, Sonogashira coupling, TMSA, TBAF, diacetylene.

Abstract: Nowadays, organic fluorescent compounds are very attractive due to their versatile applications in the fields of organic electronics (e. g.: OLEDs - *Organic Light-Emitting Diodes*, OFETs - *Organic Field-Effect Transistors*, OPVs - *Organic Photovoltaics*)^[1], biochemistry, supramolecular chemistry and many others. The large number of organic molecules have been designed and synthesized as potential candidates for optoelectronic materials, such as 9,9-disubstituted fluorene^[2] or N-substituted carbazole derivatives^[3].

Herein, we present the synthesis of fluorene and carbazole derivatives substituted by terminal alkynes in second or third position, respectively. These compounds were prepared *via* Sonogashira coupling using TMSA (trimethylsilylacetylene), and typical catalytic system CuI/[PdCl₂(PPh₃)₂] or [Pd(PPh₃)₄]. Furthermore, the novel conjugated dimers of fluorene and carbazole derivatives (connected by 1,3-butadiynes) were obtained *via* Sonogashira coupling. Conjugated fluorene and carbazole dimers have been obtained in reaction between corresponding iodoarenes and a gaseous diacetylene (which has been generated from 1,4-dichloro-2-butyne). Additionally, we report an alternative synthetic route for the preparation of above mentioned new compounds from 1,4-bis(trimethylsilyl)butadiyne in the presence of TBAF (tetrabutylammonium fluoride) and standard catalytic system CuI/[Pd(PPh₃)₄]. It was found that the second route allowed to obtain the novel compounds in much higher yield.

The new obtained molecules are well soluble in common organic solvents. Besides, all the new compounds in the present study are fluorescent. The optical and electrochemical properties of new derivatives were investigated using UV-Vis and luminescence spectroscopy. Chemical structures of all products were characterized using HRMS, ¹H NMR and ¹³C NMR spectroscopy.

These compounds will be further used as ligands for transition-metal complexes. The novel compounds are very promising and interesting materials which can be applied in organic electronics.

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* Correspondence address: matussekmarek@gmail.com

Marek Matussek acknowledges a scholarship from the DoktoRis project co-financed by the European Social.

15.

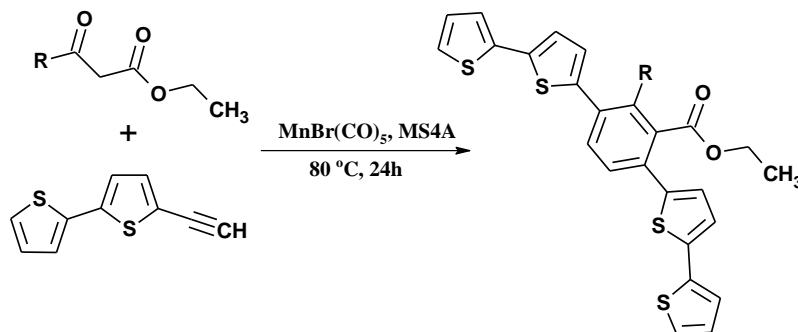
New tetrasubstituted benzene derivative - [2+2+2]cycloaddition reactions

Sławomir Kula^a, Agata Szłapa^a, Angelika Bugaj^a, Aleksandra Tkocz^a, Marek Matussek^a,
Stanisław Krompiec^a

^a*Institute of Chemistry, University of Silesia, 40-006 Katowice, Poland*

Thiophene derivatives are very important group of organic materials used in electronics. Therefore, there are continuously ongoing developments in the new methods of obtaining both molecules and macromolecules with thiophene motif. A completely new perspective for the synthesis of thiophene derivatives is the [2+2+2] cycloaddition reactions of terminal alkynes to β -keto esters catalyzed by manganese complex i.e. $[\text{MnBr}(\text{CO})_5]$ [1], [2]. It make possible to obtain a derivatives with potential usage in the organic electronics, that contains heteroaromatic substituents in position 1 and 4, respectively.

The aim of the study was to obtain 5-ethynyl-2,2'-bithiophene by Sonogashira coupling [3], followed by the [2+2+2] cycloaddition to selected β -keto esters, leading to the formation of tetrasubstituted benzene derivatives (Scheme 1). The structure of all the compounds obtained was confirmed by ^1H NMR, ^{13}C NMR, HRMS.



Scheme 1. [2+2+2] cycloaddition reactions of 5-ethynyl-2,2'-bithiophene to β -keto esters.

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16.

Derivatives of Tetra(4-bromo)phenylporphyrin and 5-Aminolevulinic Acid as Potential Drugs in Photodynamic Therapy

K. Mońka^a, V. Kozik^a, K. Jarzembek^a, M. Rojkiewicz^a, A. Bąk^a, M. Szczotka^a, M. Angeles Castro^b, E. Valles Martin^b, A. Nobis^c, P. Kuś^a

^a *Department of Organic Synthesis, Institute of Chemistry, University of Silesia, , 40-006 Katowice, Poland*

^b *Department of Pharmaceutical Chemistry. CIETUS. IBSAL. Faculty of Pharmacy.*

Campus Miguel de Unamuno. 37007 Salamanca. Spain

^c *i-Petrol Sp. z o.o., 40-600 Katowice, Poland*

Photodynamic therapy (PDT) is a medical treatment that utilizes a photosensitizing molecule (frequently a drug that becomes activated by light exposure) and a light source to activate the applied drug. Very thin superficial skin cancers called actinic keratoses and certain other types of cancer cells can be eliminated this way.

PDT essentially has three steps.

First, a light-sensitizing liquid, cream, or intravenous drug (photosensitizer) is applied or administered. Occasionally, a photosensitizing molecule that is already part of the body can be activated.

Second, there is an incubation period of minutes to days.

Finally, the target tissue is then exposed to a specific wavelength of light that then activates the photosensitizing medication.

5-Aminolevulinic acid is gaining the increasing acceptance in medicine as effective photosensitizer in 5-aminolevulinic acid – based photodynamic therapy (ALA-PDT).

Another substances which can also be used as photosensitizers are porphyrins derivatives of Protoporphyrin IX.

To functionalize porphyrins and ALA some molecules were selected with the known and proven effect on the human organism.

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17.

**Sorption of heavy metal ions on graphene oxide modified with
(3-mercaptopropyl)-trimethoxysilane**

Anna Baranik, Paulina Janik, Urszula Porada, Rafał Sitko

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

Graphene oxide (GO) is a novel two-dimensional carbon nanomaterial which is considered one of the most interesting materials in recent years. GO can be synthesized through the strong oxidation of graphite with the use of potassium permanganate or potassium dichromate. Properties of the GO such as presence of reactive functional groups, hydrophilic nature and large surface area are very important from the standpoint of the sorption process [1].

The aim of present work was to perform a synthesis of GO by Hummer method [2] and functional modification of its surface by (3-mercaptopropyl)trimethoxysilane (GO-SH) [3]. The sorption of selected metal ions (Cd(II), Co(II), Cu(II), Ni(II), Pb(II) and Zn(II)) on GO-SH was investigated. The results of experiment indicate that maximum adsorption can be achieved in broad pH ranges: 5-10 for Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II). The maximum adsorption capacities of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) on GO-SH at pH = 5 are 20.9, 20.8, 40.9, 22.2, 36.4, 108.0 mg g⁻¹, respectively. Adsorption isotherms suggest that sorption of metal ions on GO-SH nanosheets is monolayer coverage and adsorption is controlled by chemical adsorption involving the strong surface complexation of metal ions with the sulfur-containing groups on the surface of GO-SH.

Sulfonic-modified graphene oxide in adsorption of metal ions

Paulina Janik, Urszula Porada, Anna Baranik, Rafał Sitko

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

Graphene and graphene oxide (GO) have received much attention for their many potential applications in analytical chemistry mainly due to high adsorptive properties. Unmodified and modified GO can be applied as a sorbent in solid-phase extraction (SPE) in preconcentration and further determination of trace metal ions in water samples [1,2]. The oxidation process introduces large quantities of oxygen atoms on the GO surface in the form of hydroxyl, epoxy, carboxyl or ester groups. Introduced groups can efficiently bind the metal ions by sharing an electron pair.

The aim of this study was to investigate the sorption capacities of sulfonic acid functionalized graphene oxide (GO-SO₃H) towards heavy metal ions, such as Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II). GO-SO₃H was prepared as follows. First step of GO-SO₃H preparation was synthesis of GO by the oxidation of graphite powder according to Hummers method [3]. Subsequently, the functional groups introduced onto the GO surface reacted with (3-mercaptopropyl)trimethoxysilane (3-MPTMS) in order to obtain GO-SH [4]. In the last step thiol groups were oxidized to the sulfonic acid groups using H₂O₂ [5]. Langmuir and Freundlich models were applied to calculate the maximum adsorption capacities of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) ions on the GO-SO₃H surface. The concentration of metal ions after experiments were determined using inductively coupled plasma emission spectrometry (ICP-OES). The study shows that GO-SO₃H can be very attractive as sorbent in analytical chemistry due to its unique adsorption capacities. Obtained results revealed that GO-SO₃H is an attractive and efficient sorbent in developed methodology.

Dispersive micro solid phase extraction for preconcentration of selected metal ions of graphene

Damian Stefański, Klaudyna Czech Katarzyna Pytlakowska, Violetta Kozik

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

Dispersive micro-solid phase extraction (DMSPE) is one of a new technique used for separation and preconcentration of trace amounts of elements. The main advantages of this method are: rapidity, simplicity and ease of combination with various instrumental techniques.

Selection of sorbent is the most important factor in the extraction process. First of all, sorbent should be selective and cheap. It should provide high preconcentration factor and total recovery.

In the last few years, carbon nanomaterials such as fullerenes (C₆₀), nanotubes, oxidized nanotubes, graphene and oxidized graphene have generated a lot of attention. [1-3] Graphene is a new allotropic form of carbon. It consists of sp²-hybridized carbon atoms organized in regular hexagonal layers, which make it chemically stable, flexible, corrosion resistant and durable. Moreover, graphene is characterized by a large surface area providing high adsorption capacity towards various molecules due to the fact that both sides of planar sheets are responsible for molecules adsorption. Sorption properties are explained also by great delocalized π -electron system which plays a significant role in the formation of π - π stacking interaction with carbon-based ring structures. In the procedures based on sorption of metal ions on graphene the using of chelating agent is very important, because it allows the formation of hydrophobic complexes that can be adsorbed on the nanomaterial surface through the Van der Waals forces and hydrophobic interactions.

In this work, graphene as sorbent and cupferron or 8-hydroxyquinoline as chelating agent were proposed for preconcentration of selected metal ions (Fe²⁺, Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺) from liquid samples. The proposed methodologies are based on combination of dispersive micro-solid phase extraction (DMSPE) with energy-dispersive X-ray fluorescence spectrometry (EDXRF). The various parameters including pH, amount of chelating agents, Triton X-100, sample volume, mass of sorbent, and sorption time were optimized in order to obtain the best recoveries. The developed procedures were applied to the analysis of wine and instant coffee samples.

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“Sila”-Sonogashira coupling in a heterogeneous system - investigations using chromatographic techniques

M. Korzec^{1,3}, Jadwiga Gabor², J. Polański¹

¹ *University of Silesia, Institute of Chemistry, Department of Organic Chemistry, 9 Szkolna Street, 40-006 Katowice.*

² *University of Silesia, Institute of Materials Science, 75 Pułku Piechoty 1A Street, 41-500 Chorzów*

Numerous reports describe the coupling of trimethylsilylacetylene with aryl halides in Sonogashira-type reactions. The C(sp)–Si bond is generally not affected by these reaction conditions. The silyl group can, therefore, if desired, subsequently be removed to furnish a structurally modified terminal alkyne. The trimethylsilyl group is thereby used as a protective group. This process avoids totally the formation of the alkyne homocoupling Glaser-type product. In "Sila"-Sonogashira reaction, aryl halides can be coupled directly with alkynylsilanes via the Si–C bond activation by using the Pd/Cu catalyst system. In our study, we wanted to check the possibility of the "sila"-Sonogashira reaction in a heterogeneous systems using Pd/Cu catalysts. Various chromatographic methods were used for the purpose of this study. This includes: TLC, HPLC and HPLC-ESI MS, MALDI TOF. These techniques provide qualitative and quantitative specification of the reaction products.

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Acknowledgements

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

³ 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

POSTER SESSION II

JUNE 12th, 2014

CHAIRPERSONS:

Natalia Vorobets and Grzegorz Zadora

1.

***Dendrolimus pini* – first results of analytical and behavioral studies**

Dorota Staszek, Rafał Szmigielski, Krzysztof J. Rudziński, Monika Asztemborska,
Marek Cieślak and Jerzy Raczko

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

e-mail: dstaszek@ichf.edu.pl

Caterpillars of pine-tree lappet moth, *Dendrolimus pini*, are the most dangerous defoliators of pine trees in Poland. Feeding on pine needles, they destroy the assimilation apparatus and weaken the trees which become vulnerable to secondary pests. The straightforward consequence of the damage is death of pine forests. In the post-war Poland, there were 8 gradations of this folivore. The two largest took place in the mid 90-ties and from 2007 to 2008. To prevent the damage, about 105 000 ha of pine forest were sprayed with chemical pesticides in 1993-1995, and 90 000 ha – in 2007-2008. A new large gradation of pine-tree lappet moth started in 2011, so in 2012 more than 56 000 ha of pine forest were preventively sprayed.

The aim of the project is to improve a synthetic analogue of a sexual pheromone of pine lappet moth (*Dendrolimus pini*) and to develop a method for its use in forest protection. Pheromone lures available so far are based on substances discovered in the early 1980s. They were tested in many countries and appeared rather inefficient. Using modern analytical and bioanalytical techniques, such as gas chromatography hyphenated to ion trap mass spectrometry, FID detection and electroanthography, we have started to determine an effective composition of the pheromone mixture, and later to synthesize all its stereocomponents. Then, we intend to determine: a) the optimal doses of the lure components by laboratory and field tests, b) the best type and form of a dispenser, c) the most effective trap type, and d) the optimal height for traps location. Finally, we plan to define: a) how to use the optimal trap-attractant combination for detecting the presence of *D. pini* in pine stands, and b) the methodology of determining the culmination of *D. pini* swarming period that can be used in the forest protection practice.

2.

Insight into chemistry of atmospheric aerosol by liquid chromatography with tandem mass spectrometric detection

Anna Malinowska¹, Krzysztof J. Rudziński¹, Grzegorz Spólnik², Dorota Staszek^{1,3}
and Rafał Szmigielski¹

¹*Institute of Physical Chemistry, Polish Academy of Sciences, 44/52 Kasprzaka Street, 01-224 Warsaw, Poland*

²*Institute of Organic Chemistry, Polish Academy of Sciences, 44/52 Kasprzaka Street, 01-224 Warsaw, Poland*

³*Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia,
9 Szkolna Street, Katowice, Poland*

e-mail: ralf@ichf.edu.pl

Atmospheric aerosol is defined as a complex mixture of solid and liquid particles of minute diameters ($d < 10 \mu\text{m}$) suspended in the air. They significantly influence the Earth's climate by scattering or absorbing the sunlight and deteriorate the human health. The adverse effect of aerosol particles results from their inhalation into the respiratory tract and easy deposition therein which causes asthma and cardiovascular diseases. According to data from the World Health Organization, the air pollution reduces by a year or more the life expectancy for people living in European cities [1]. On the other hand, aerosols play an important role in the chemistry of the atmosphere and significantly contribute to the climate change through an array of physicochemical processes the understanding of which is still in its infancy [2] and warrants continuous multidisciplinary research.

One of relevant pathways of the atmospheric aerosol formation is the oxidation of volatile organic compounds, emitted by living vegetation, with the atmospheric radical oxidants (i.e., ozone, OH, NO₃, SO₄⁻), followed by further processing of low-volatility products. In the presented study we have evaluated the role of the crotonic acid in the formation of aerosol particles in the laboratory framework. The crotonic acid is one of the unsaturated C₄ plant volatiles playing an important role as an allelopathic agent. On the other hand, the crotonic acid bears the C=C-C=O moiety, which increases its chemical reactivity towards radical species. This makes the crotonic acid a likely source of atmospheric aerosol particles, so far unrecognized.

In this work we applied a self-designed simulation chamber to evaluate the fate of crotonic acid in aqueous-phase solutions containing sulphydryl radicals and to follow it by an online monitoring using a mass spectrometric detection. In the poster, we will present the first results of the chemical characterisation of the unknown product from the crotonic acid oxidation obtained using a reversed-phase liquid chromatography/electrospray triple quadrupole mass spectrometry, and additionally, MRM modes.

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3.

Application of gas chromatography in a lab-scale coal gasification system simulating use of High Temperature Reactor excess heat for synthesis gas production

Smoliński A.¹⁾, Cempa-Balewicz M.²⁾, Howaniec N.¹⁾

Central Mining Institute,

¹⁾ *Department of Energy Saving and Air Protection*

²⁾ *Department of Post-Industrial Sites and Waste Management*

Plac Gwarków 1

40-166 Katowice, Poland

Gasification as an alternative to combustion offers increased efficiency, lower negative environmental impact and wider application range of the main product – synthesis gas – in power generation and chemical synthesis. Nuclear energy may also be considered as one of the ways of mitigation of global warming resulting from GHG emission from energy systems. Highly efficient fossil fuel utilization in energy sector may be also combined with an application of excess heat from nuclear reactors within a coal-nuclear synergy concept.

The paper presents the experimental study of application of simulated High Temperature Reactor (HTR) excess heat in allothermal coal gasification to synthesis gas. The heat was applied in pre-heating of gasification agents, such as oxygen, air and steam in a laboratory scale installation of the Laboratory of Advanced Energy Technologies of the Department of Energy Saving and Air Protection, Central Mining Institute. The main element of the test stand is a fixed bed reactor of a volume of approximately 0.8 L heated with a resistance furnace. The installation is also equipped with an additional heating system for gasification agents, simulating the utilization of the excess HTR heat.

Qualitative and quantitative analyses of dry and cooled product gas were performed with application of a two-channel gas chromatograph Agilent 3000A and a flow meter, respectively. In the gas chromatograph a PLOT U column ($8 \cdot 10^{-3} \times 0.32 \cdot 10^{-3}$ m) with thermal conductivity detector (TCD) for determination of content of carbon dioxide and other compounds of 2÷5 carbon atoms in a molecule and analytical column MS5A PLOT ($10 \times 0.32 \cdot 10^{-3}$ m) with thermal conductivity detector (TCD) for determination of hydrogen, nitrogen, carbon monoxide and methane content in gas analyzed were applied.

4.

Gas chromatography application in steam co-gasification of coal and biowaste

Howaniec N.¹⁾, Smoliński A.¹⁾

¹⁾ *Central Mining Institute, Department of Energy Saving and Air Protection, Plac Gwarków 1, 40-166 Katowice, Poland*

Waste biomass is a significant element of renewable energy balance in many countries. Research efforts are, however, still required to make its utilization more efficient. This may be achieved for example with an application of gasification technologies of higher efficiency and lower emission of contaminants than combustion technologies, most commonly applied in thermal processing of biomass today. And yet, at present only approximately 0.5% (373 MW_{th}) of synthesis gas production worldwide is based on biomass and waste. This results basically from low energy density of biomass, local shortages in biomass supplies and operating problems inherently combined with biomass utilization in gasifiers designed for fossil fuel processing. The process of co-gasification of coal and biowaste offers solutions to some of the above mentioned problems. It gives the benefits of stable supplies of a primary energy resource – coal and utilization of a zero-emission, waste material (i.e. agriculture waste, sewage sludge, etc.) with higher process efficiency and lower environmental impact than in biomass and coal gasification, respectively.

The study presented concerns co-gasification of coal and biomass to hydrogen-rich gas, as a prospective clean energy carrier. The experiments were performed with an application of a laboratory scale fixed-bed reactor installation of the Laboratory of Advanced Energy Technologies, Department of Energy Saving and Air Protection, Central Mining Institute. Gasification product gas was analyzed with the two-channel gas chromatograph Agilent 3000A. A column PLOT U (8m x 0.32mm), with helium as a carrier gas, was used for separation of CO₂ and C₂-C₅, whereas a backflush injector module with a pre-column PLOT U (3 m x 0.32 mm) and an analytical column MS5A PLOT (10 m x 0.32 mm), with argon as a carrier gas, was used for separation of H₂, N₂, CO and CH₄. The temperature of an inlet, injector and the columns was 60°C. The injection time was $50 \cdot 10^{-3}$ s for both columns and the run time and post run time were 150 s and 10 s for PLOT U and MS5A PLOT, respectively, whereas the backflush time for column PLOT U was 12 s.

5.

**Optimization of sample preparation workflow by use of an automated platform –
acceleration of clinical proteomic studies**

Natalia Miękus^{1,2}, Fernando García Martínez², Javier Muñoz², Tomasz Bączek¹

¹*Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Hallera 107, 80-416 Gdańsk, Poland.*

²*Centro Nacional de Investigaciones Oncológicas C/ Melchor Fernández Almagro, 3, E-28029 Madrid*

Clinical proteomics possesses a huge potential in understanding pathological processes in human body by identifying the specific proteins that are altered only during the disease. These analyses rely on the extraction of proteins from clinical samples and its subsequent enzymatic digestion in peptides prior to mass spectrometric analysis. This process is mostly hand-made and time consuming, making parallel processing of clinical samples prone to errors and thus compromising the reproducibility needed for confident comparative analyses.

Here, the new Agilent's Bravo Automated Liquid Handling Platform (AssayMAP Bravo) is introduced. Bravo is intended for high-throughput sample preparation proteomic workflows. This allows the analysis of multiple samples in parallel and reduces the experiment time considerably which is the intention of nowadays clinical research.

Herein, the optimization of digestion protocol owing to trypsin-containing cartridges and clean-up workflow by using C18 solid-phase extraction/desalting cartridges was performed for test samples. The digestion parameters (e.g. protein concentration, buffer composition, temperature, washing cycles and flow rates) were adjusted by using both bovine serum albumin and a six protein mixture sample. Resulting peptides were analyzed by LC-MS/MS employing a LTQ Orbitrap Velos mass spectrometer.

Using the AssayMAP Bravo system, the time for protein digestion completion was significantly decreased to 40 minutes when compared to the standard 16 hours in-solution digestion. Sequence coverage of test samples ranged from 14% for lysozyme to 65% for myoglobin which is similar to those obtained by standard procedures.

Based on the above optimized protocol, further processing of archival clinical tumor samples will be carried out. In this case, protein extraction from formaldehyde fixed-paraffin embedded tissues will be carried out following a previously published protocol, using 6M guanidine hydrochloride.

Presented, automated platform evaluated here will contribute to establishing robust and sensitive proteomic strategies that enable high-throughput analyses in clinical environments, especially in the field of cancer.

6.

Thin – layer chromatography - bioassays

Wioleta Jesionek¹, Edyta M. Grzelak², Barbara Majer-Dziedzic³ and Irena M. Choma¹

¹ *University of Maria Curie–Skłodowska, Lublin, Poland*

² *Institute for Tuberculosis Research, University of Illinois , Chicago, USA*

³ *University of Life Sciences, Lublin, Poland*

Bioassays (biological activity tests) are based on a measurement of an effect emerging in a given biological system as a result of biological action of the substances under investigation. They are often hyphenated with chromatographic methods, preferably with thin layer chromatography (TLC), which enables separation of many samples in the same run and, what is very important, evaporation of a mobile phase. The situation is ideal when TLC-bioassay are followed by spectroscopic methods giving full information about targeted analytes [1]. Thin-layer chromatography–direct bioautography (TLC-DB) usually gives information on antimicrobial properties of separated compounds [2]. The developed and dried HP(TLC) plate is immersed in a suspension of bacteria in a nutrient broth, incubated and sprayed with a reagent (usually tetrazolium salt MTT). Around spots of antibacterials, inhibition zones are formed as pale areas on a purple background. The information about antimicrobial properties of analyzed substances can be complemented with other bioassays as antioxidant activity, enzymatic or estrogenic tests. TLC-DB can be treated as a screening or even semi-quantitative method. The relationship between the diameter or area of inhibition zone plotted against the logarithm of the concentration of the antimicrobial applied is linear or exponential depending on the range of concentrations. Because of the lack of commercially available TLC-DB tests, two direct bioautography assays were developed in our laboratory to be used after TLC separation: one based on Gram (-) bacteria, *Escherichia coli*, and another one based on Gram (+) bacteria, *Bacillus subtilis* [3,4]. These tests were used with success to determine antibiotics at their MRL (maximum residue level) in milk as well as to establish antimicrobial properties of plant extracts and pharmaceuticals.

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7.

GC/MS spectra for 8-hydroxy-2-methylquinoline derivatives

Marcin Szala¹, Jacek E. Nycz², Jan G. Małecki¹, Karolina Czyż², Józef Rzepa³

¹ Department of Crystallography, Institute of Chemistry, University of Silesia, Katowice, Poland

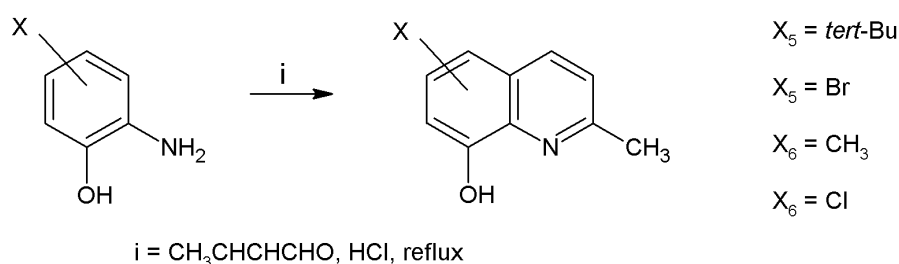
² Department of Organic Chemistry, Institute of Chemistry, University of Silesia, Katowice, Poland

³ Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice, Poland

Quinoline was first isolated by F.F. Runge in 1834 during extraction of coal tar [1]. They are widely seen in a number of natural products and have attracted considerable attention due to their biological activities such as anti-malarial, anti-fungal, anti-bacterial, anti-asthmatic, anti-hypertensive, anti-inflammatory, and trichomonal [2, 3]. They received many synthetic protocols and applications both in academia and industry.

Many of them are ligands in coordination chemistry as a N and/or O atom donors for chelating with metals, such as ruthenium metalloantimalarials and are used for the identification of metals. Additionally quinolines have been used in components for molecular electronic devices such as OLED displays (Organic Light-Emitting Diodes) [4].

We reported GC/MS spectra of new aforementioned compounds. Our studies were carried out to compare selected quinolines [5, 6]. On our poster presentation, we will present the GC chromatograms and MS spectra with some discussion.



Scheme: Synthesis of derivatives 8-hydroxy-2-methylquinoline.

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8.

Solid Phase Microextraction as an efficient method for determination of organochlorine pesticides in soil samples

Klaudia Pszczolińska, Marlena Płonka

*Plant Protection Institute – National Research Institute, Sośnicowice Branch, Gliwicka 29,
44-153 Sośnicowice, Poland*

One of the major soil contaminants are organochlorine pesticides (OCPs), which could cause serious problems for crops, soil organisms and humans. They were widely used worldwide in last century and large amounts of these OCPs were piled up in various storage facilities which nowadays are more or less damaged. Today OCPs are banned according to the Stockholm Convention due to their very persistent and bioaccumulating nature.

Recent trends in samples preparation have focused on a development of simpler, faster, more reliable and cost-efficient methods by reducing analysis time and solvent consumption. Solid phase microextraction (SPME) is a technique which combines extraction and concentration processes into one step without using solvent. SPME is a simple, selective and efficient method, based on the redistribution of analytes between microextraction fiber and sample matrix i.e. on the selective sorption of target analytes in the active layer of the fiber and direct thermal desorption in the chromatograph injector (GC).

The aim of the present study is to investigate the applicability of SPME to the determination of organochlorine pesticide in soil samples. The variables involve fiber selection, temperature effect and absorption time. The fibres used (Supelco) were 100 µm and 7 µm polydimethylsiloxane (PDMS). The soil was air dried and sieved (2 mm) before using. The analyses were carried out on a gas chromatograph (Agilent 7890A) equipped with electron capture detector (ECD) and headspace automatic injector (Agilent 1888), using an HP-5MS UI (30 m × 0,32 mm × 0,25 µm) capillary column.

The proposed method can be employed in the field for quick turnaround methods. This method is convenient, and reliable for the purity control of soil samples. The results obtained confirm that the SPME is high precision and accurate; it is also effective in formal quantitative analyses.

9.

5,10,15,20-Tetra(4-hydroxyphenyl)porphyrin as a surrogate for the recovery study of porphyrins in Parma ham

Hannelore De Maere^{1,3}, Eveline De Mey¹, Martyna Baca², Mieczysław Sajewicz², Hubert Paelinck¹, Ilse Fraeye¹, Teresa Kowalska²

¹*KU Leuven, Department of Microbial and Molecular Systems, Research Group for Technology and Quality of Animal Products, member of Leuven Food Science and Nutrition Research Centre (LFoRCe), Ghent, Belgium*

²*Department of General Chemistry, Institute of Chemistry, Katowice, Poland*

³*Food Quality Laboratory, Groupe ISA, Lille-Cedex, France*

Porphyrins are compounds which are naturally occurring in prosthetic groups of eukaryotic cells such as hemoglobins, myoglobins and cytochromes. All compounds which belong to this group contain porphin, an organic compound consisting of four pyrrole rings joined together by four methine (=CH—) groups to form a larger macrocycle ring¹. Due to that, all these molecules are flat, stable, strongly coloured, and prompt to form complexes with metal ions. Porphyrins absorb light and have characteristic absorption spectra both in the visible and ultraviolet wavelength range². In meat tissue, porphyrins play an important role in metabolism of living organisms and can also be found in intermediates of the heme synthesis. Protoporphyrin IX plays a specific role among all tetrapyrroles, as it is a kind of a template for a wide variety of naturally occurring compounds, e.g., for the investigated Zn-protoporphyrin IX and hemin. All these compounds affect the colour properties of meat, and moreover, Zn-protoporphyrin IX contributes to the formation of a characteristic stable red colour of Parma ham³.

In this study a high-performance liquid chromatography (HPLC) method was developed for the determination and quantification of three porphyrins, i.e., protoporphyrin IX, Zn-protoporphyrin IX, and hemin in Parma ham. Because all compounds are naturally present in meat, no appropriate blank material is available for the recovery study. Therefore, the in-house synthesized porphyrin 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin, which is not present in meat, was used as a surrogate in the recovery study.

Prior to HPLC analyses, the porphyrins of interest were extracted from Parma ham samples according to the method of Wakamatsu³, with some minor modifications. High-performance liquid chromatographic analysis was performed with use of the C18 type stationary phase, in the isocratic mode with use of the following mobile phase: A + B, 9:1 (v/v). A: MeOH + DCM, 9:1 (v/v); B: H₂O + CH₃COOH, 97:3 (v/v). A mobile phase flow rate of 0.8 mL min⁻¹ was used. The retention times (*t_R*) for hemin, 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin, Zn-protoporphyrin IX, and protoporphyrin IX were 2.6, 2.8, 5.5 and 8.2 min, respectively. All analytes were detected by diode array detector (DAD) at a wavelength (λ) of 414 nm, while the surrogate analyte was detected at $\lambda = 444$ nm to avoid interference with hemin.

The recovery study with the metal-free surrogate, 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin showed practically total recovery (above 94%) at all spiked levels (10.0, 20.0 and 30.0 $\mu\text{g g}^{-1}$). As a consequence the standard calibration curves of hemin, Zn-protoporphyrin IX, and protoporphyrin IX can be used for the quantification of these target analytes in meat samples of Parma ham.

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10.

Determination of trans-resveratrol in wine and the extracts from grape vine (*Vitis vinifera*) by means of TLC

Skorek M¹, Biernat N¹, Gawlińska K¹, Kozik W², Konopka E², Kowalska T¹, Sajewicz M¹

¹*Department of General Chemistry, Institute of Chemistry University of Silesia, Katowice, Poland*

²*Department of Organic Synthesis, Institute of Chemistry University of Silesia, Katowice, Poland*

Extracts from grape vine (*Vitis vinifera*) are widely used in pharmaceutical and cosmetic industry. They characterize with anti-oxidative, anti-inflammatory, anti-cancer, detoxifying, antibacterial, antifungal and other properties. In fact, the trans-resveratrol isomer (trans-3,5,4'-trihydroxystilbene) and the stilbene derivatives which are present in vine, exert a wide range of therapeutic activities. Clinical studies have shown that trans-resveratrol can lower lipid level in human blood serum and is effective in anti-cancer prophylaxis. It has been experimentally proved that adding trans-resveratrol to the diet of yeasts, nematodes, fish and mice results in prolongation of medium life expectancy for these organisms^[1].

Nowadays, application of wine and the grape vine extracts in cosmetics and cosmetology attracts a lot of interest. In the anti-ageing cosmetic creams, extracts from the grape seeds, grape skin, vine shots, vine leaves, and even wine itself is applied. In cosmetology, therapeutic rites like "wine therapy" or "wine SPA" are becoming increasingly more popular, with wine applied in place of water.

The contents of the phenolics (resveratrol included) are not constant, but they change depending on the part of the grape vine which has been extracted. Also the wines characterize with different contents of resveratrol, depending on their origin, colour, or sweetness (the taste). In this study, samples of wine and the aqueous or aqueous ethanolic extracts from the grape vine underwent the procedure of solid phase extraction (SPE). Then the trapped polyphenols were eluted from the adsorbent with ethyl acetate. Further, extracts containing trans-resveratrol were analyzed by means of high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). Quantification of resveratrol was performed with use of an inner standard, IS^[2,3,4].

Trans-resveratrol was quantified in the French red and white dry wines, and in the home-made fruit wines (made of white and red grapes, pomegranates, and raisins). Moreover, resveratrol was quantified in the extracts from wine, grape seeds and grape skin, which are used for manufacturing of cosmetics.

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11.

Determination of hexachlorocyclohexane (HCH) isomers in water samples by gas chromatography

Dariusz Szeremeta, Magdalena Knaś, Mieczysław Sajewicz, Teresa Kowalska

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

An extensive use of pesticides to improve agricultural productivity played an important role in the last century. These compounds have been applied for decades in preventing, repelling or mitigating the effects of pests. Although most of organochlorine pesticides (OCPs) including hexachlorocyclohexane (HCH) isomers, have been banned in many countries because of mutagenic and carcinogenic effects [1,2], they and their metabolites are still present in the environment, especially in soil, water and sediment, owing to their persistence and lipophilic properties [3,4]. Most of these organic compounds have a tendency to bioaccumulate and present low rates of biodegradation and consequently they could represent a risk to environmental and human health. The European Environmental Agency (Directive 76/464/EEC and its daughter Directives) has drawn up a list of pollutants for priority monitoring, which need to be analysed with sensitive instrumental methods. Therefore, a rapid, convenient, accurate, and sensitive method is required to monitor pesticide residue concentrations in water, soil sediments and biotic samples.

Chromatographic techniques have been considered as the best methods to determine OCPs in varied sample matrices. At present, more than 60% of registered pesticides and/or their metabolites can be analyzed by using gas chromatography (GC) [5]. In our research, as determination technique we also used GC equipped with different detectors. Chromatographic analysis usually follows the tedious sample preparation to extract the pollutants from environmental matrices (i.e. soil, water, sediment, plant material). For the isolation of target compounds from matrices various extraction and clean-up procedures can be proposed. In our studies we used traditional method as liquid–liquid extraction (LLE). This conventional extraction method gave us efficient and precise results.

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12.

**Self-assembled peptide nanofibers
(L-proline and L-phenylalanine)**

Agnieszka Godziek¹, Anna Maciejowska¹, Mieczysław Sajewicz¹, Ewa Talik²,
Roman Wrzalik³, and Teresa Kowalska¹

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*

³*Department of Biophysics and Molecular Physics, University of Silesia, 1A, 74 Pulku Piechoty Street, 41-500
Chorzów, 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, or the mixtures of compounds 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 [1].

In this study on the same mixture of amino acids (L-Pro and L-Phe), it was found out that the amino acids not only undergo a spontaneous peptidization reaction, but also self-assemble to form nanostructures. To prove that the obtained structures have nanostructure or microstructure characteristics, we used LC-MS, scanning electron microscope (SEM), optical microscope, and IR spectroscopy.

The obtained results confirm the presence of both, nano- and microfiber structures in the solution. In that way, it was additionally confirmed that amino acids in 70% acetonitrile can undergo oscillatory peptidization, forming particles of considerable molar masses, and have an ability to self assemble in nanostructures without an addition of any catalyst.

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13.

Anti-oxidative properties of different thyme species checked by means of different analytical techniques involving reduction of DPPH radical

M. Orłowska¹, J. Antczak¹, W. Czarnecka¹, K. Gęborska¹, M. Seroka¹, M. Bartoszek²,
J. Polak², K. Pytlakowska³, G. Szymczak⁴, M. Waksmundzka-Hajnos⁵, T. Kowalska¹,
M. Sajewicz¹

¹Department of General Chemistry and Chromatography, Institute of Chemistry, University of Silesia, Katowice

²Department of Materials Chemistry and Chemical Technology, Institute of Chemistry, University of Katowice

³Department of Analytical Chemistry, Institute of Chemistry, University of Silesia, Katowice

⁴Botanical Garden, The Maria Curie-Skłodowska University, Lublin

⁵Department of Inorganic Chemistry, Chair of Chemistry, Lublin Medical University, Lublin

Botanical raw materials are rich sources of bioactive substances, which exert positive effects on human health. Scientific investigations reveal a significant preventive influence of phytochemicals as protection against the civilization health problems. Nowadays, pharmaceutical and cosmetic companies turn toward phytochemicals with growing interest and on the basis thereof produce multi-active preparations. Currently, anti-oxidants attract their greatest attention and polyphenols belong to this particular group.^[1-3]

Phenolic acids belong to the herbal secondary metabolites, which characterize with diverse structures and properties. They exert a number of biological activities such, as anti-oxidant, anti-allergic, anti-inflammatory, or anti-bacterial. Phenolic acids include in their structures an aromatic ring, which is connected with one or more hydroxyl groups. Number and placement of these groups determine anti-oxidant performance of phenolic acids. Phenolics of plant origin include phenolic acids, flavonoids, lignins, stilbenes, etc.^[4-6]

Anti-oxidants are the compounds able to neutralize the oxygen and nitrogen free radicals present in a given system. Participating in numerous metabolic processes, they exert positive effect on various different physiological functions. Owing to that, anti-oxidants exert prophylactic, or even curative effect and they mainly appear in edible parts of the plants.^[7]

Common thyme (*Thymus vulgaris* L.) originates from the Mediterranean zone, with characteristic morphology of leaves and purple flowers. It is widely utilized in medicine, cosmetics, and cuisine. Common thyme contains up to 2.5% essential oils, up to 3% tannins, moreover flavonoids, phenolic acids, triterpenes, aluminium salts and carbohydrates.^[8]

Phytochemical analysis was performed for the eighteen thyme (*Thymus* L.) species originating from Botanical Garden of the Maria Curie-Skłodowska University in Lublin. Specially derived thyme extracts were analyzed with use of thin-layer chromatography (TLC), electron paramagnetic resonance spectroscopy (EPR), and UV-Vis spectrophotometry. In order to assess anti-oxidative properties, the characteristic reagent DPPH (2,2-diphenyl-1-picrylhydrazyl) was used. As a result, a differentiated total anti-oxidant potential of different thyme species was shown.

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14.

Chromatographic investigation of spontaneous reactions of sulphur amino acids

Anna Maciejowska, Agnieszka Godziek, Marta Wszolek, Joanna Klimek,

Anita Kulosa, Mieczysław Sajewicz, Teresa Kowalska

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

Oscillatory reactions are among the most fascinating ones. They are the non-equilibrium processes in which intermediates undergo periodic changes. Oscillatory reactions are commonly encountered in chemical and physical, biotic and abiotic, systems. *L*-Methionine and *L*-cysteine are essential for normal development of the organisms' functions. Firstly, these compounds play a significant role due to the presence of the sulphur atom in a side chain. *L*-Met is needed for translation of proteins and it is an important reagent in the synthesis of taurine and glutathione. The other amino acid (*L*-Cys) exerts an influence on cardiovascular system. *L*-Met and *L*-Cys possess therapeutic properties as well, so they can be used as components of numerous pharmaceuticals and cosmetics.

Low molecular weight chiral compounds (like profen drugs, amino acids and hydroxyacids) can undergo spontaneous oscillatory chiral inversion and oscillatory peptidization [1]. Present research contributes to the search for the new compounds which can undergo spontaneous chiral inversion and peptidization. The main tool for studying this phenomenon was high performance liquid chromatography with diode array detection (HPLC-DAD), evaporative light scattering detector (HPLC-ELSD) or liquid chromatography with mass spectrometric detection (LC-MS).

The obtained results demonstrate the oscillatory instability of *L*-Met and *L*-Cys, which consists in spontaneous oscillatory chiral inversion and oligopeptidization.

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References: Graphene as a novel solid sorbent in dispersive micro-solid phase extraction of Se

Karina Kocot*, Riccardo Leardi**, Beata Walczak*, Rafał Sitko*

**Institute of Chemistry, Analytical Chemistry Department, University of Silesia, Katowice.*

***Department of Pharmaceutical and Food Chemistry, University of Genova, Italy*

Se is an essential trace element, responsible for the proper work of the human organism. However, its excess or deficiency may cause serious health effects [1]. The most popular forms present in water samples are selenites (SeO_3^{2-}) and selenates (SeO_4^{2-}), wherein selenates are more toxic than selenites [2,3]. The difference between the toxic dose and the nutritional requirement is very small and exceeding the tolerable upper intake level of 400 μg per day may lead to selenosis. For that reason, there is a need to develop effective analytical procedures enabling not only determination of total Se amount but also speciation analysis [4].

In the present work a dispersive micro-solid phase extraction (DMSPE) with graphene as a solid sorbent and ammonium pyrrolidinedithiocarbamate (APDC) as a chelating agent was applied for the determination of selenium by energy-dispersive X-ray fluorescence spectrometry (EDXRF). APDC reacts preferentially with Se(IV), while other Se species remain in the analyzed solution. In DMSPE, graphene particles are dispersed through the analyzed solution, therefore the reaction between Se(IV)-APDC and graphene particles occurs immediately. The central composite face-centered design (CCFD) with 3 center points was performed in order to optimize conditions and to study the effect of four variables (pH of the sample, concentration of APDC, concentration of Triton-X-100, and sample volume). Under optimized conditions Se ions can be determined with very good recovery ($97 \pm 2.5\%$), precision (RSD 2.6%) and detection limit (0.032 ng mL^{-1}). The proposed method was applied for the determination of Se in mineral water and biological samples (Lobster Hepatopancreas and Pig Kidney).

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