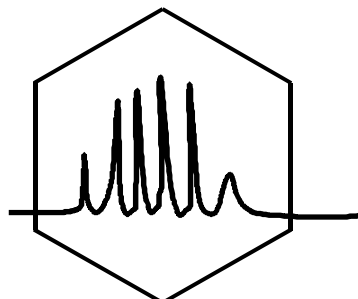


**INSTITUTE OF CHEMISTRY, UNIVERSITY OF SILESIA,  
KATOWICE, POLAND**



**THE XXXIII<sup>rd</sup>  
SYMPOSIUM**

**CHROMATOGRAPHIC METHODS  
OF INVESTIGATING THE ORGANIC COMPOUNDS**

**MAY 25<sup>th</sup> – 27<sup>th</sup>, 2010**

**KATOWICE – SZCZYRK**

**POLAND**

**PROGRAM**

## **SCIENTIFIC COMMITTEE OF THE SYMPOSIUM**

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Grzegorz Zięba (Katowice, Poland)

**SESSION I TUESDAY, MAY 25<sup>th</sup>, 2010**

**CHAIRPERSONS:** F. Geiss and Y. Vander Heyden

**9.25 – 9.30 am OPENING ADDRESS**

**9.30 – 10.00 am**

1. Application of cyclodextrins as chiral selectors in capillary electrophoresis:  
Recent studies on enantioseparation and chiral recognition mechanisms  
B. Chankvetadze

**10.00 – 10.30 am**

2. Use of chlorinated polysaccharide-based chiral stationary phases to update generic separation strategies for capillary electrochromatography  
A. Hendrickx, D. Mangelings, B. Chankvetadze, Y. Vander Heyden

**10.30 -11.00 am**

3. Comparison of different chromatographic supports for liquid chromatography applied to the separation of josamycin and erythromycin  
L. Van den Bossche, A. Van Schepdael, J. Hoogmartens, E. Adams

**11.00 – 11.30 am**

4. Separation of high polar glycosidic compounds from *Verbascum* sp. flower extracts by means of hydrophilic interaction thin-layer chromatography  
Ł. Cieśla, M. Ł. Hajnos, M. Waksmundzka-Hajnos

**11.30 – 12.00 am COFFEE BREAK**

**SESSION II TUESDAY, MAY 25<sup>th</sup>, 2010**

**CHAIRPERSONS:** B. Chankvetadze and J. Polański

**12.00 – 12.30 am**

5. Drug impurity profiling. A methodology for method development

Y. Vander Heyden

**12.30 – 1.00 pm**

6. Thin-layer chromatography for fingerprinting and screening the biological activity of plant extracts

M. Waksmundzka-Hajnos, Ł. Cieśla

**1.00 – 1.30 pm**

7. On alternative fingerprinting solutions in phytochemical research

T. Kowalska, M. Waksmundzka-Hajnos, M. Sajewicz

**1.30 – 2.00 pm**

8. Approaching the quality assurance of artesunate

A. Koch

**2.00 pm LUNCH**

**POSTER SESSION I TUESDAY, MAY 25<sup>th</sup>, 2010**

**CHAIRPERSONS:** W. Verschelde and Ł. Komsta

**3.30 – 5.30 pm**

**6.00 pm BONFIRE**

**SESSION III WEDNESDAY, MAY 26<sup>th</sup>, 2010**

**CHAIRPERSONS:** D. Agbaba and M. Waksmundzka-Hajnos

**9.00 – 9.30 am**

9. Teaching computers chemistry: on-line technologies in chemoinformatics

J. Polański

**9.30 – 10.00 am**

10. The renaissance of additive schemes for evaluation of retention indices in gas chromatography

I. G. Zenkevich

**10.00 – 10.30 am**

11. Hansen solubility parameters – their determination by IGC and Applications

A. Voelkel, K. Adamska, B. Strzemiecka

**10.30 – 11.00 am**

12. Modeling of the chromatographic retention from organic modifier content by different equations- theoretical comparison and practical aspects

Ł. Komsta

**11.00 -11.30 am COFFEE BREAK**

**SESSION IV WEDNESDAY, MAY 26<sup>th</sup>, 2010**

**CHAIRPERSONS:** I. Zenkevich and A. Voelkel

**11.30 -12.00 am**

13. Mapping drug architecture by MoStBioDat - rapid screening of catechol motifs

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

**12.00 -0.30 pm**

14. Drug metabolism development concepts and its bioanalytical technics

I. Klebovich

**0.30 – 1.00 pm**

15. Evaluation of methacrylate-based monolithic stationary phases for the analysis of drug molecules in the capillary format

I. Tanret, D. Mangelings, Y. Vander Heyden

**1.00 – 1.30 pm**

16. Multidimensional SHIMADZU chromatography

M. Szklarczyk

**2.00 pm LUNCH**

**POSTER SESSION II WEDNESDAY, MAY 26<sup>th</sup>, 2010**

**CHAIRPERSONS:** D. Mangelings and L. Vanden Bossche

**3.30 – 5.30 pm (COFFEE BREAK)**

**6.00 pm DINNER**

**SESSION V THURSDAY, MAY 27<sup>th</sup>, 2010**

**CHAIRPERSONS:** D. Mangelings and Z. Tesić

**9.30 – 10.00 am**

17. A comparative study of lipid composition of the brain of chicken and rat during myelination; A chromatographic-densitometric analysis

F. Helmy, A. Morris

**10.00 – 10.30 am**

18. Two dimensional separation of amino acids with thin-layer chromatography and pressurized planar electrochromatography in normal and reversed phase systems

A. Chomiccki, K. Kloc, E. Materna-Witkowska, T. H. Dzido

**10.30 – 11.00 am**

19. The use of technology in-needle extraction in the determination of organic compounds

M. Pietrzyńska, K. Bielicka-Daszkiewicz, A. Voelkel

**11.00 – 11.30 am**

20. Chosen strategy of basic drugs analysis in RP-HPLC systems

J. Flieger

**11.30 – 12.00 am**

21. The LC/MS/MS analysis of the nucleation precursors in the formation of secondary organic aerosols (SOA)

B. Witkowski, T. Gierczak

**12.00 am            CLOSING REMARKS**

**12.00 -12.30 am   COFFEE BREAK**

**12.30 pm            LUNCH**



## POSTER SESSION I

1.

Effect of mobile phase composition on the overall elution process in thin-layer chromatography

W. Prus, K. Kaczmarzski

2.

Study of porous materials by IGC/flash thermodesorption

M. Kasperkowiak, B. Strzemiecka, A. Voelkel

3.

Increasing the reproducibility of GC retention indices by modifying their measurements

E.S. Ivleva, I.G. Zenkevich

4.

Hydrophilic interaction planar chromatography of geometrical isomers of some Co(III) complexes

K. Salem A. M. Shweshein, P. Ristivojević, A. Radoičić, F. Andrić, Ž. Tešić, D.M. Milojković-Opsenica

5.

Comparison of chromatographic retention parameters of several basic compounds obtained on different stationary phases, i.e. C18 and phenyl bonded silica and various ionic liquids added to organic aqueous eluent systems

J. Flieger, A. Kieszko

6.

On spontaneous oscillatory condensation of phenylacetic acids in aqueous ethanol

M. Sajewicz, M. Leda, M. Gontarska, D. Kronenbach, E. Berry, I.R. Epstein, T. Kowalska

7.

On spontaneous oscillatory condensation of *S*-(+)-ketoprofen in acetonitrile

M. Sajewicz, M. Leda, M. Gontarska, D. Kronenbach, E. Berry, I.R. Epstein, T. Kowalska

8.

Chromatographic behavior of chosen basic drugs on cyanopropyl bonded silica gel eluted with organic aqueous eluent systems modified with ionic liquids

J. Flieger, S. Sas

9.

Application of micellar TLC in studying lipophylic properties of organic compounds

K. Stępnik, M. Janicka

10.

Revealing the structures of isomeric alkyl arenes using additive evaluation of gas chromatographic retention indices

A.I. Ukolov, I.G. Zenkevich

11.

Automatic interpretation of GC-MS data in toxicological screening

A.I. Ukolov, S.V. Vasilevsky, A.I. Tiunov, N.S. Khlebnikova, A.S. Radilov,  
E.I. Savelieva

12.

Application of gas chromatography in a comparative study of biomass,  
lignite and hard coal steam gasification

A. Smoliński, N. Howaniec

13.

TLC densitometric investigation of the degradation of 4-chlorophenol using  
advanced oxidation processes (AOPs)

M. Natić, J. Veljkovic, D. Dabić, D. Milojković-Opsenica, B. Dojčinović, G.  
Roglić, D. Manojlović, Ž. Tešić

14.

Determination of ethyl 2-cyanoacrylate in workplace air

J. Kowalska

15.

GC/MS analysis of some dialkyl esters of 1,2-cyclohexanedicarboxylic acid

E. Dziwiński, A. Tasarska, J. Lach

16.

Determination of aziridine in workplace air by HPLC method

A. Jeżewska

17.

BioArena – non analytical application of planar chromatography

M. Janicka, E. Tyihák, B. Ościk-Mendyk, K. Stępnik

18.

A comparison of the essential oil fingerprints derived from selected sage (*Salvia*) species with use of thin-layer chromatography directly and indirectly coupled with mass spectrometry

M. Sajewicz, Ł. Wojtal<sup>1</sup>, M. Natić, M. Waksmundzka-Hajnos, T. Kowalska

19.

A comparison of the phenolic compound fingerprints derived from selected sage (*Salvia*) species with use of thin-layer chromatography directly and indirectly coupled with mass spectrometry

M. Sajewicz, D. Staszek, M. Natić, M. Waksmundzka-Hajnos, T. Kowalska

## POSTER SESSION II

1.

Role of biogenic amines in the formation of *N*-nitrosamines during meat processing

G. Drabik-Markiewicz, E. De Mey, S. Impens, W. Verschelde, T. Kowalska, Y. Vander Heyden, H. Paelinck

2.

Development of the method for determination of ziprasidone and its impurities

M. Pavlović, M. Malešević, K. Nikolić, D. Agbaba

3.

Spirocyclopropane-type sesquiterpene hydrocarbons from *Schinus terebinthifolius* Raddi

R. Richter, S. H. von Reuß

4.

Spectrophotometric determination of the sum of flavonoids contained in twenty different sage (*Salvia* L.) species and the analysis of the sage extracts by means of HPLC-DAD and HPLC-ELSD

M. Sajewicz, D. Staszek, M. Waksmundzka-Hajnos, T. Kowalska

5.

Chromatographic and spectroscopic analysis of essential oils from *Salvia lavandulifolia* L. and *Salvia triloba* L.

M. Sajewicz, M. Matlengiewicz, J. Rzepa, Ł. Wojtal, M. Hajnos, M. Waksmundzka-Hajnos, T. Kowalska

6.

Determination of lipophilicity by TLC revisited - a comparative study on several techniques with simple molecule model solute set

Ł. Komsta, R. Skibiński, A. Gumieniczek, A. Berecka, B. Radkiewicz, M. Radoń

7.

HPLC method determination of formaldehyde released from chosen root canal sealers

M. Kantor Boruta, A. Jończyk, M. Lisowska-Kuźmich, W. Bojar

8.

Selective extraction of polar organic compounds as the key stage of chemical warfare metabolites identification in biomedical samples

E.S Ivleva., N.L Koryagina., N.S Khlebnikova, E.I.Savelieva

9.

GC/MS analysis of the urine for metabonomic research of autistic children

J. Kałużna-Czaplińska, E. Socha, A. Sokołowska, J. Rynkowski

10.

Urinary dicarboxylic acids in autism

J. Kałużna-Czaplińska, E. Socha, A. Sokołowska, J. Rynkowski

11.

Determination of the level of *D*-arabinitol and *D-/L*-arabinitol ratio in urine of autistic children using gas chromatography/electrone capture detection

W. Gryś, J. Kałużna-Czaplińska, J. Rynkowski

12.

Determination of the level of fumaric acid in urine of autistic children using SPE-HPLC

W. Gryś, J. Kałużna-Czaplińska, J. Rynkowski

13.

Urinary level of cysteine and mercury in autistic children

J. Kałużna-Czaplińska, M. Michalska, J. Rynkowski

14.

Determination of the level of homocysteine in urine of autistic children before and after a diet using gas chromatography/mass spectrometry

J. Kałużna-Czaplińska, M. Michalska, J. Rynkowski

15.

Methods of determination selected priority substances in water samples

I. Fulara, J. Wypych

16.

Determination of nicotine in hair by GC-NPD

K. Tyrpień and C. Dobosz

17.

Determination of lipophilicity of tritolyldipyrromethane derivatives using TLC

G. Zięba, M. Rojkiewicz, K. Jarzembek, V. Kozik, A. Jarczyk, M. Łannik,

P. Kuś

18.

Lipophilicity of new potential photodynamic therapy agents

M. Rojkiewicz, G. Zięba, V. Kozik, K. Jarzembek, A. Jarczyk, P. Kuś

19.

Size exclusion chromatography in the study of anionic polymerization of selected oxirane and thirane monomers

A. S. Swinaew, J. Jurek, M. Szklarska, B. Nowakowski, P. Płatek, B.

Swinarew, A. Stolarzewicz



**SESSION I MAY 25<sup>th</sup>, 2010**

**CHAIRPERSONS:** F. Geiss and Y. Vander Heyden

**SESSION II MAY 25<sup>th</sup>, 2010**

**CHAIRPERSONS:** B. Chankvetadze and J. Polański

**SESSION III WEDNESDAY, MAY 26<sup>th</sup>, 2010**

**CHAIRPERSONS:** D. Agbaba and M. Waksmundzka-Hajnos

**SESSION IV WEDNESDAY, MAY 26<sup>th</sup>, 2010**

**CHAIRPERSONS:** I. Zenkevich and A. Voelkel

**SESSION V THURSDAY, MAY 27<sup>th</sup>, 2010**

**CHAIRPERSONS:** D. Mangelings and Z. Tešić

1.

## **Application of Cyclodextrins as Chiral Selectors in Capillary Electrophoresis: Recent Studies on Enantioseparation and Chiral Recognition Mechanisms**

B. Chankvetadze

*Institute of Physical and Analytical Chemistry and Molecular Recognition and Separation Science  
Laboratory, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 0179  
Tbilisi, Georgia  
bezhan\_chankvetadze@yahoo.com*

Cyclodextrines represent one of the most powerful group of chiral selectors for enantioseparations in capillary electrophoresis (CE) [1, 2]. The properties of cyclodextrines, such as their solubility in aqueous and some non-aqueous solvents, UV-transparency of native cyclodextrines, enantioselective complex-formation ability with many chiral compounds, multivariate possibility of chemical derivatization, etc. contributed significantly to the success of these macrocyclic molecules as chiral CE selectors. In addition to above mentioned, being the medium size molecules with more or less defined structure makes cyclodextrins as useful targets for studies aimed to better understanding of fine mechanisms of enantioselective noncovalent intermolecular interactions.

This presentation summarizes our recent studies on enantioseparation of chiral drugs in CE using various native, as well as neutral and charged derivatives of cyclodextrins. The major emphasis is put on the relationships between separation and chiral recognition of cyclodextrins in aqueous and non-aqueous medium [2]. The application of other techniques, such as various nuclear magnetic resonance (NMR) spectroscopy methodologies and mass spectrometry, will be shown for better understanding of enantioselective effects in analyte-cyclodextrin interactions.

The examples of analyte involved in these studies include chiral drugs such as ketoconazole and terconazole [3], propranolol [4, 5], dimethindene, ephedrine, norephedrine, tetrahydroziline and others.

1. Chankvetadze, B.: Enantioseparations by using capillary electrophoretic techniques: The story of 20 and a few more years. *J. Chromatogr. A* **1168**, 45-70 (2007).
2. Chankvetadze, B.: Combined approach using capillary electrophoresis and NMR spectroscopy for an understanding of enantioselective recognition mechanisms by cyclodextrins. *Rev. Chem. Soc.* **33**, 337-347 (2004).
3. Lomsadze, K., Martinez-Giron, A. B., Castro-Puyana, M., Chankvetadze, L., Crego, A. L., Salgado, A., Marina, M. L., Chankvetadze, B.: About the role of enantioselective selector-selectand interactions and the mobilities of temporary diastereomeric associates in enantiomer separations using capillary electrophoresis, *Electrophoresis* **30**, 2803-2811 (2009).
4. Servais, A.-C., Rousseau, A., Fillet, M., Lomsadze, K., Salgado, A., Crommen, J., Chankvetadze, B.: Separation of propranolol enantiomers by capillary electrophoresis using sulfated  $\beta$ -cyclodextrin derivatives in aqueous and nonaqueous electrolytes: Comparative CE and NMR study. *Electrophoresis* 2010, in press.
5. Servais, A.-C., Rousseau, A., Fillet, M., Lomsadze, K., Salgado, A., Crommen, J., Chankvetadze, B.: Separation of propranolol enantiomers using capillary electrophoresis with various cyclodextrins: Comparative CE and NMR studies. *J. Sep. Sci.* 2010, in press.

2.

### **Use of chlorinated polysaccharide-based chiral stationary phases to update generic separation strategies for capillary electrochromatography.**

Ans Hendrickx<sup>1</sup>, Debby Mangelings<sup>1</sup>, Bezhan Chankvetadze<sup>2</sup>, Yvan Vander Heyden<sup>1\*</sup>

<sup>1</sup>*Department of Analytical Chemistry and Pharmaceutical Technology, Center for Pharmaceutical Research*

*(CePhaR), Vrije Universiteit Brussel-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium*

<sup>2</sup>*Department of Physical and Analytical Chemistry and Molecular Recognition and Separation Science Laboratory, School of Exact and Natural Sciences, Tbilisi State University, 380028 Tbilisi, Georgia*

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The separation of chiral molecules is an extensively studied field in pharmaceutical analysis because enantiomers display different pharmacodynamic and pharmacokinetic profiles in living systems [1]. Regulatory instances therefore demand the development of separation methods to determine the enantiomeric purity and stability of chiral drugs. Because the separation of these drug molecules often requires extensive method development, generic separation strategies, applicable on large sets of structurally diverse compounds, can be very useful.

Capillary electrochromatography (CEC) combines the properties of both high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), and is characterized by its miniaturized character, a high sample loading capacity, high efficiencies and fast separations [2]. It was earlier found suitable as separation technique to define a chiral separation strategy using polysaccharide-based chiral stationary phases [3].

However, new types of chiral stationary phases were commercialized in the meanwhile. Therefore, it is currently investigated whether these new types of CSP display a higher enantioselectivity than the older ones. Then they can replace an older CSP in the strategy, resulting in a higher success rate.

The screening conditions of the existing chiral strategy were tested for their applicability on four chlorine-containing polysaccharide-based stationary phases [4]. A test set of 48 structurally diverse drug compounds was analyzed using the screening conditions of the strategy. The enantioselectivity of these phases was compared with those of the four phases used in the existing strategy. The results led to different possibilities to upgrade the current screening strategy.

#### **References**

[1] A. Van Eeckhaut, Y. Michotte, "Pharmacological importance of chiral separations" in A. Van Eeckhaut, Y.

Michotte (Eds.) "Chiral separations by capillary electrophoresis", Taylor and Francis, Florida, 2010, pp. 1-22

[2] I.S. Krull, R.L. Stevenson, K. Mistry, M.E. Swartz, Capillary electrochromatography and pressurized flow

capillary electrochromatography: An introduction, HNB Publishing, New York, 2001

[3] D. Mangelings, M. Maftouh, D.L. Massart, Y. Vander Heyden, LC-GC Europe 19 (2006) 40-47

[4] S. Fanali, G. D'Orazio, K. Lomsadze, B. Chankvetadze, J. Chromatogr. B 875 (2008) 296-303

3.

**Comparison of different chromatographic supports for liquid chromatography applied to the separation of josamycin and erythromycin**

L. Van den Bossche, A. Van Schepdael, J. Hoogmartens, E. Adams

*K. U. Leuven, Laboratory for Pharmaceutical Analysis, O&N 2, Herestraat 49, PB 923, B-3000 Leuven (Belgium)*

Chromatographic supports for liquid chromatography (LC) should give, fast and highly efficient separations at low cost.

For this purpose, several strategies were developed, such as the use of silica based monolithic columns, sub-2  $\mu\text{m}$  porous particles and fused core technology particles. However, most of the analytical methods reported in the European Pharmacopoeia (Ph. Eur.) use conventional porous silica particle columns, which are characterized by relatively long analysis times and high solvent costs. Therefore, this study investigates whether the conventional silica columns can be replaced easily by monolithic supports or newer types of particle columns, in order to achieve faster analyses with similar/better resolution power. Hence, two Ph. Eur. methods for the separation of the macrolide antibiotics, josamycin and erythromycin, were examined. Potential benefits and problems for the transfer of a method from a conventional porous silica particle column to monolithic columns or small particle columns will be commented in terms of stability, sensitivity, efficiency, resolving power and analysis time.

For the monolithic supports, easy method transfer with minor adaptations of chromatographic parameters was observed. In addition, the monoliths showed good peak capacity, low backpressure and short analysis time. The use of small particle columns requires special LC instrumentation to cope with the high backpressures (up to 1000 bar). Also, the method transfer involves altering of several chromatographic parameters, such as injection volume, flow rate... Furthermore, this research demonstrates that with small particle columns the best analytical results were obtained when the same brand of stationary phase was used as in the conventional silica column on which the method was developed.

4.

**Separation of high polar glycosidic compounds from *Verbascum* sp. flower extracts by means of hydrophilic interaction thin-layer chromatography**

<sup>1</sup>Łukasz Cieśla, <sup>2</sup>Michał Ł. Hajnos and <sup>1</sup>Monika Waksmundzka-Hajnos

<sup>1</sup>*Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6 St, 20-081 Lublin, Poland.*

<sup>2</sup>*Department of Pharmacognosy, Medical University of Lublin, Chodźki 1 St, 20-093 Lublin, Poland.*

*Verbascum* is a genus of the *Scrophulariaceae* family, including approximately 250 species, native to Europe and Asia. Various mullein species have been commonly used as emollients, expectorants, and for treating infections of the respiratory system. According to previous investigations iridoid glycosides as well as oleanane type triterpenes are widespread in the family *Scrophulariaceae* and constitute a group of compounds with chemotaxonomic significance for the *Verbascum* genus. Unfortunately detection and identification of compounds of these groups encounter certain difficulties. Iridoids are nonvolatile compounds what hinders their analysis by gas chromatography. The application of HPLC with UV detection is also difficult due to the fact this compounds possess rather weak chromophores. What also makes the chromatographic analysis difficult, is iridoid glycosides' strongly hydrophilic character. For the sake of example aucubin and catalpol are characterized by the following logP values: -3.0 and -3.2, respectively. The analysis of triterpene saponins is also challenging, as triterpenoids occur in plant material as multicomponent mixtures of glycosidic forms. A need to develop a new simple method for the resolution and detection of these compounds, in complex mixtures, is steadily growing. However the resolution of high polar compounds, both in normal and reversed-phase systems, is somewhat difficult. In such case hydrophilic interaction chromatography (HILIC), known also as aqueous normal phase chromatography, may be a method of choice. It is believed that in HILIC the mobile phase forms a water rich layer on the surface of polar stationary phase, creating a liquid/liquid extraction system.

In this presentation a concept of using two perpendicular HILIC TLC systems for the resolution of high polar glycosidic compounds, from the flower extracts of selected *Verbascum* species, is presented. The obtained results are further used for the comparative studies of the investigated species.

5.

## **Drug impurity profiling. A methodology for method development**

M. Dumarey, Y. Vander Heyden

*Department of Analytical Chemistry and Pharmaceutical Technology*

*Center for Pharmaceutical Research (CePhaR)*

*Vrije Universiteit Brussel – VUB*

*Laarbeeklaan 103, 1090 Brussels, Belgium*

In drug impurity profiling an HPLC method is optimized that allows separating a drug compound from its impurities. At the moment the method is developed neither the number of compounds is known nor their structures. When method development is performed by means of an experimental design approach then first a screening is performed followed by a response surface design. However, here in HPLC method development we can skip the screening step, because we know for the different factors which are affecting the selectivity of the method most. It are in order of importance, the stationary phase manufacturer and the mobile phase pH, the organic modifier composition and to a lesser extent the slope profile and -time and the temperature. A sequential methodology will be presented to optimize drug impurity profiles. It contains three steps: first a stationary phase selection combined with pH optimization; secondly the organic modifier composition optimization, and finally an optimization of the gradient profile and the temperature. The stationary phase is selected among dissimilar phases and the separation modelled as a function of the pH. Organic modifier is optimized based on Snyder's solvents triangle, which in fact is a mixture design, and finally the slope and temperature are optimized using a factorial design.

6.

## **Thin-layer chromatography for fingerprinting and screening the biological activity of plant extracts**

Monika Waksmundzka-Hajnos and Łukasz Cieśla

*Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6 St, 20-081  
Lublin, Poland.*

Chromatographic fingerprint profiling is a very convenient and effective method for quality assessment of herbal materials. Several chromatographic techniques have been extensively applied for constructing chromatographic profiles, e.g.: HPLC, GC, HPTLC or HSCCC. HPTLC offers a number of unique features that can outperform the other separation techniques used for the fingerprinting. Great advantage of HPTLC is the speed of method development and also its flexibility. What is more, TLC is often a method of choice for the screening of plant extracts for the presence of biologically active compounds. It is particularly well suited for the direct biological detection, since the separation result is immobilized prior to the detection and moreover, the open solid bed layer allows direct access to the sample. In this presentation application of different thin-layer chromatographic techniques are presented for the fingerprinting of the variety of plant samples. The use of special modes of chromatogram development, applied for the fingerprinting, are described and their application for the purposes of the plant chemotaxonomy, are discussed. The quality control of pharmaceutical preparations, by means of TLC, are also presented. Advantages and disadvantages of TLC are widely discussed. The application of TLC for screening the biological activity of plant extracts is also addressed. The main focus is on the use of planar chromatography for detection and identification of free radical scavengers in plant extracts and botanical preparations. The possibilities of future studies and perspectives of method development are also outlined.

7.

## **On alternative fingerprinting solutions in phytochemical research**

T. Kowalska<sup>1</sup>, M. Waksmundzka-Hajnos<sup>2</sup>, M. Sajewicz<sup>1</sup>

<sup>1</sup> *Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland*

<sup>2</sup> *Department of Inorganic Chemistry, Medical University of Lublin, 6 Staszica Street, 20-081  
Lublin, Poland*

Kingdom of plants is an inexhaustible reservoir of compounds which have not yet been detected, isolated, and their respective structures properly identified, and moreover, many of those have not yet been synthesized either. Over the centuries, kingdom of plants has also proved a practically inexhaustible reservoir of curative compounds and pharmaceutical concepts, although until now, far from being sufficiently explored.

Presently, several critical issues in phytochemical research are on top of the agenda and the crucial one is application of flexible and efficient separation, isolation and identification tools, adequate to a given research task. The second crucial issue is availability of phytochemical standards, generally characterizing with relatively high costs.

This paper focuses on a discussion of selected novel fingerprinting strategies. First part of this presentation is devoted to the fingerprinting potential and flexibility of the TLC-MS interface device, able to transform thin-layer chromatography into an essential part of a multidimensional fingerprinting system. Second part of this presentation is devoted to the multidimensional fingerprinting strategy with use of the HPLC separation unit and a selection of the employed detecting systems in a parallel or consecutive set-up. Attention of the audience will be drawn to the MS, DAD, ELSD, and polarimetric detectors as those truly important for an efficient multidimensional fingerprinting of the natural products. Last not least, videoscanning of planar chromatograms as an additive means to generate digital fingerprints will also be mentioned.



## Approaching the Quality Assurance of Artesunate

Angelika Koch ,

*Frohme-Apotheke, Frohmestr. 14, D-22457 Hamburg*

Artemisinin (**2**) is a sesquiterpene lactone isolated from *Artemisia annua*, a herb that has traditionally been used in China for the treatment of malaria. Due to its insolubility in water Artemisinin is not recommended for intravenously administration. Therefore chemically modified derivatives with appropriate properties have been developed (fig.1).

Artesunate (**1**) is a slightly water-soluble hemisuccinate derivative of artemisinin (**2**) but it is unstable in neutral solutions and might be degraded to dihydroartemisinin/artenimol (**3**) or artemisinic acid (**4**).

Artesunate and its active metabolite dihydroartemisinin (**3**) reveal remarkable activity against otherwise multidrug-resistant *Plasmodium falciparum* and *P. vivax* malaria.

The functional group responsible for antimalarial activity of artesunate is the endoperoxide bond. Orphan designation of intravenously administered artesunate had been granted in the United States for immediate treatment of malaria. In addition to this application

Artesunate has now been analyzed for its anti-cancer activity against leukemia and colon cancer cell lines. Hence it makes a market in non-malaria countries, too.

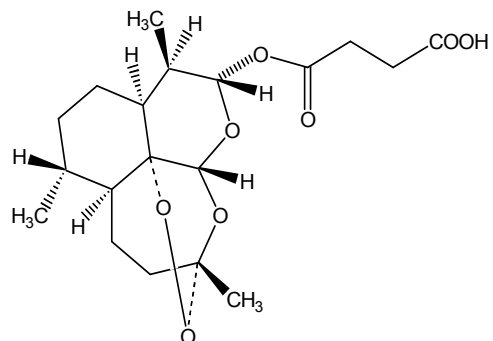
The demand and supply are increasing but there is a lack of defined specifications and purity test methods.

The International Pharmacopoeia offers drafts of the appropriate monographs. IR-spectrometry is used for identity tests and TLC and HPLC for purity tests.

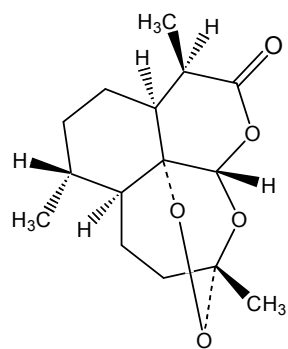
The TLC test uses ethyl acetate and toluene (5+95) as the mobile phase with a very low separation power It is proposed that the TLC method be omitted, whereas we think it would make sense to change the polarity of the mobile phase. We demonstrate the usefulness of an appropriate TLC method which is suitable to determine the identity of the raw substances and the related substances of artemisinin derivatives.

Furthermore we checked FTIR- spectra (regarding the striking differences across the range of interest (e.g. stretching of –OH, =O and –O) for suitability of a rapid purity test method (fig.2).

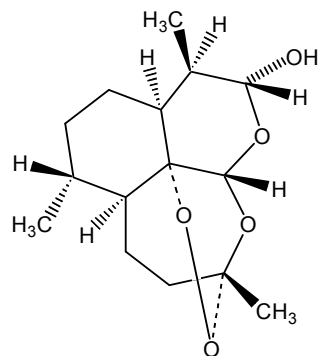
**Figure 1.** Chemical structures of artesunate and some of its derivatives



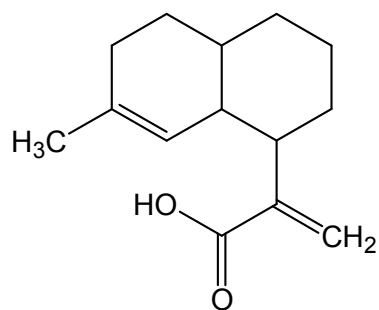
1: Artesunate



2: Artemisinin

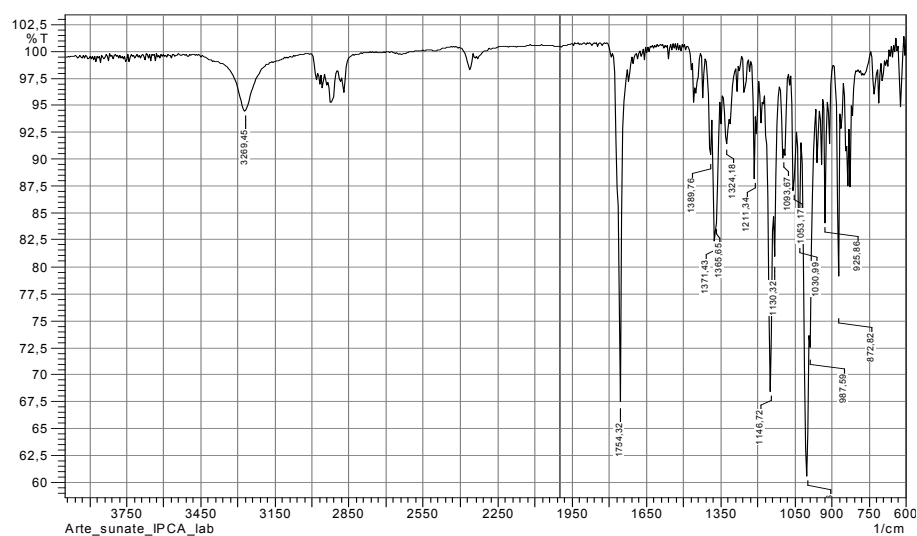


3: Dihydroartemisinin, Artemimol

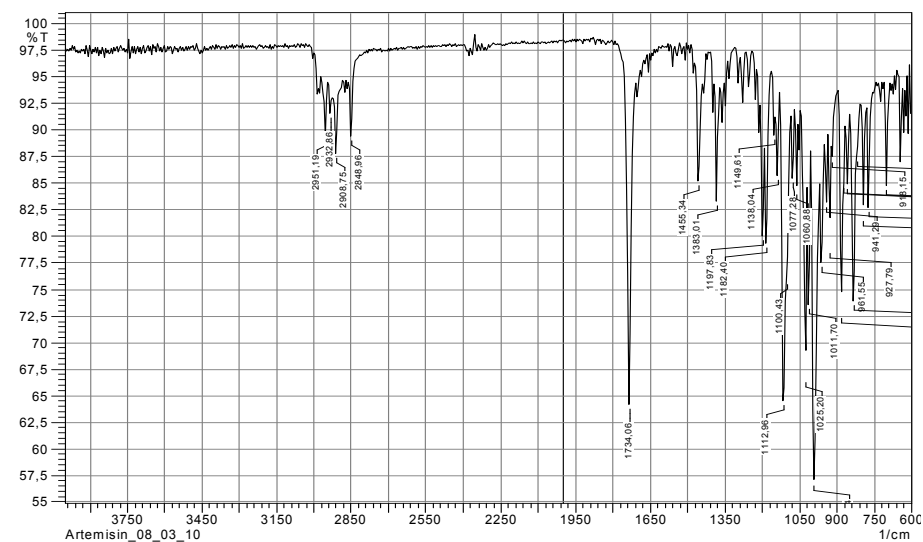


4: Artemisinic acid

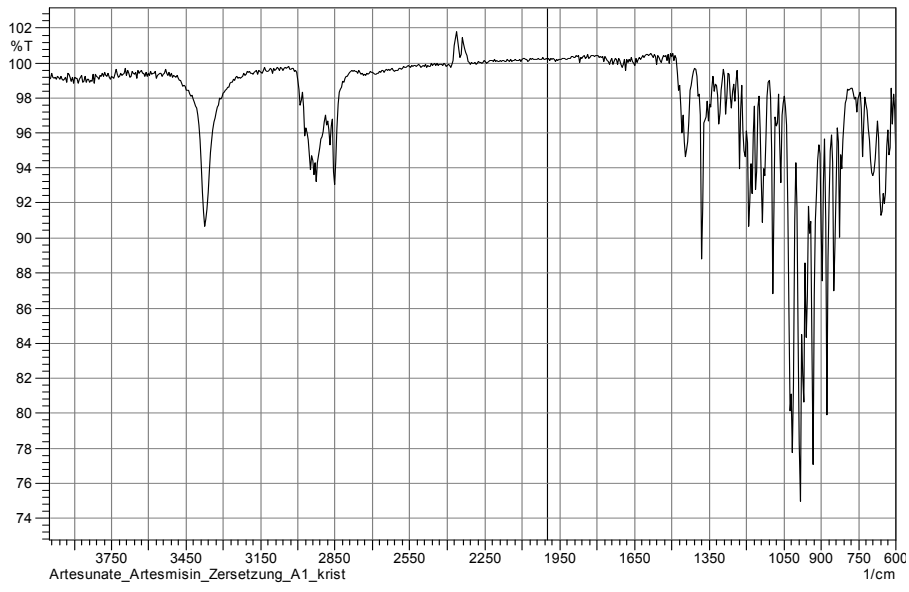
**Figure 2:** FTIR-spectra of Artesunate, Artemisinin and Dihydroartemisinin/Artemimol



**Artesunate (1)**



**Artemisinin (2)**



Dihydroartemisinin, Artenimol (3)

9.

## Teaching computers chemistry: on-line technologies in chemoinformatics

Jarosław Polański

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A first concept of social interaction by computer networking has been suggested in sixties and a first cable connection has been constructed in 1969 in the USA. Nobody at that time could have predicted the importance of this idea and its impact on the economy and science. Currently computers are more and more dependent on the web technologies. Accordingly, a number of chemical resources available in the web steadily increases. A term chemoinformatics has been coined recently to describe a discipline organizing and coordinating the increasing application of computers in chemistry, in particular also by networking chemical *in silico technologies*.

With the greater and greater potential of informatics *in silico chemistry* has significantly increased the scope of interest and the available field of investigations. Chemoinformatics focuses on drug design, molecular engineering and organic chemistry. The application of computer assisted methods for molecular manipulation and prediction, synthesis design and property oriented synthesis are illustrative examples. On-line chemical technologies collect the sites offering an access to chemical data, educational resources, free or commercial software, e-commerce, on-line chemistry journals and many others. In this lecture we will focus on the interactive molecular data resources which recently have significantly increase in usability and functionality. We will show illustrative examples in various of chemical branches. The advantages and problems will be discussed, including the data exchange standards, molecular codes and editors.

10.

## The renaissance of additive schemes for evaluation of retention indices in gas chromatography

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The evaluation of GC retention indices (RI) using “classical” additive schemes (2) is perceived sometimes like obsolete and primitive procedure comparing with contemporary QSPR approaches based on multi-parameter linear regressions (1):

$RI \approx \sum k_i A_i \quad (1)$ <p>where <math>A_i</math> are the descriptors of different origin, <math>k_i</math> – coefficients.</p>	$RI \approx RI_0 + \sum \Delta RI_i \quad (2)$ <p>where <math>RI_0</math> – the RI value for basic structure containing no substituents characterized by increments <math>\Delta RI_i</math>.</p>
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The case  $RI_0 = 0$  (only atomic increments are involved) is known [1]. However, precalculation and following summarizing of increments is resulted in a large uncertainty of results.

The alternative approach includes no RI increments at all, because it is based on the direct superposition of chemical structures. If we characterize the target molecule **ABCD**, we can combine it from the simpler precursors **ABC** and **BCD** subtracting the duplicated fragment **BC** by the following way:



The arithmetical operations with reference RI values directly correspond to this equality:

$$RI(ABCD) \approx RI(ABC) + RI(BCD) - RI(BC)$$

This kind of additive scheme was shown to be effective in precalculation of RIs of 839 polychlorinated hydroxybiphenyls [2], 211 structural isomers of 4-nonylphenols [3], products of free-radical chlorination of cyclohexane [4], ionic chlorination of aliphatic carbonyl compounds [5], bromosubstituted anilines [6], etc. Its features and not numerous restrictions are discussed.

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## Hansen Solubility Parameters – Their Determination by IGC and Applications

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The solubility parameter concept found an application in many industries for explanation different properties of the components forming a formulation. Knowledge of the solubility parameter data for different components is important to predict the magnitude of interaction between the components of formulation (miscibility, compatibility or adsorption) and further stability of the product.

Solubility parameter called *Hildebrand solubility parameter* or *Hildebrand parameter* is applied only for regular solution. So-called *Hansen solubility parameter* (HSP) is extension of the Hildebrand solubility parameter to polar and hydrogen bonding systems. Hansen assumed, that cohesive energy can be considered as a sum of contributions from dispersive ( $E_d$ ), polar ( $E_p$ ) and hydrogen bonding ( $E_h$ ) interactions:

$$-E_{coh} = -E_d - E_p - E_h \quad (1)$$

and the total solubility parameter ( $\delta_T$ ) is expressed as

$$\delta_T^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (2)$$

where:  $\delta_p$ ,  $\delta_p$ ,  $\delta_h$  denote dispersive, polar and hydrogen bonding contribution, respectively.

The estimation of HSP for the group of nanomaterials and modified nanomaterials from Inverse Gas Chromatographic (IGC) data is presented and discussed.

12.

## **Modelling of the chromatographic retention from organic modifier content by different equations- theoretical comparison and practical aspects**

Lukasz Komsta

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Binary mixtures of weak diluent and strong modifier are commonly used mobile phases in HPLC and TLC, giving a possibility to control the retention in wide range. An estimation of retention from the content of modifier is widely elaborated topic and many theoretical and empirical equations were proposed till now [1]:

1. Equations modelling  $\ln k$ : semilogarithmic model of Soczewiński-Wachtmeister, log-log model of Snyder-Soczewiński, quadratic and square-root models of Schoemakers, two models of Nikitas, a variable-power model of Zapała, recursive model of Zenkevich and recent Box-Cox transform model by Komsta
2. Equations modelling  $k$  or  $1/k$  proposed by: Row, McCann, Kaczmarski and Zapała
3. Two equations modelling  $R_F$  proposed by Kowalska.

After fitting the data to an equation for particular component and chromatographic system, its retention can be then interpolated (to find modifier concentration bringing best separation) or extrapolated to pure diluent (concentration equal to zero). The extrapolation in reversed chromatography is an important approach and common method for determination of solute lipophilicity.

The purpose of the presentation is to collect all existing retention equations and compare them theoretically (against their mathematical properties) and practically (on two TLC retention datasets) according to both interpolation and extrapolation abilities.

A particular attention will be pointed at uncertainty (confidence of prediction) during extrapolation and the resulting usefulness in lipophilicity estimation [2].

[1] Komsta, L. Acta Chromatogr. 2/2010 (in press)

[2] Unpublished results.



## Mapping Drug Architecture by MoStBioDat - Rapid Screening of Catechol Motifs

Andrzej Bak, Tomasz Magdziarz, Agata Kurczyk, Jaroslaw Polanki

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Computer-assisted simulations are the most progressive component of the present day chemical investigations, producing enormous amount of data. The constraint of processing and sharing such data is thought as a major impediment in the drug discovery process. Furthermore, among the steepest barrier to overcome in the high-throughput screening (HTS) studies is the restricted amount of a reliable, publicly available repositories combining the detailed drug data with the comprehensive drug target information. Only the proper dataset aggregation and unified standards of data organization enable massive *in silico* knowledge mining. By offering a uniform data storage and retrieval mechanism various data might be compared and exchanged easily.

Structure-based database screening is a rapidly growing and an efficient technique in the early stages of the drug development process, gaining considerably from the current progress in the computer technology. Particularly, the subsequent sampling of a virtually infinite chemical space (VCS) in order to optimize the ligand diversity of chemical libraries (VLS) with appropriate binding affinity places emphasis more on the probability field with accidentally developed drugs than on traditional principles of the rational drug discovery. In consequence, the tools and techniques for organizing and intelligently mining this information are highly desirable.

In an effort to make the virtual screening more accessible the Molecular and Structural Bioinformatics Database (MoStBioDat) project has been established as a management platform for an efficient storage, access and exchange of the biomolecular data with an extensive array of software tools for the structural similarity measures and pattern matching. It could potentially serve as a dual purpose storage environment integrated with database management system (DBMS) to explore 3D drug-target interactions or compare and measure the structural similarities between chemical structures.

In the current studies we have investigated the application of MoStBioDat software platform for the massive analysis of the spatial arrangements and conformational examinations of hydroxyl groups in the catechol-containing compounds, widely regarded as the main substructure block in many antiviral inhibitors. The geometrical orientation of the hydroxyl groups seems to determinate the ability of catechol derivatives to recognize the surrounding environment by forming the inter- and intra-molecular hydrogen bonds. The detailed analysis of the torsion angles, taking into account the spatial coordinates of the hydroxyl groups and the adjacent aromatic carbon atoms has been conducted using 3D structures taken from the freely accessible repositories.

## Drug metabolism development concepts and its bioanalytical technics

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Drug-metabolism research relies on multidisciplinary approaches comprising receptor biology, enzymology, recombinant DNA technology, biochemical toxicology, and drug disposition into study design and conduct balanced *in vitro* and *in vivo* experiments.

Successful drug-metabolism research must integrate to allow a full understanding of the mechanisms of individual variability in drug therapy and drug safety.

The present lecture intends to give an overview of the process and up-to-date bioanalytical tools of the *in vitro* and *in vivo* drug metabolism. Several examples illustrate the possibilities of the quick fingerprint radio-bioanalytical examination of drug molecule in the comparison of species.

For *in vitro* or *in vivo* biotransformation investigations drugs labeled radioactively with  $^3\text{H}$ - and/or  $^{14}\text{C}$ - isotopes provide the possibility to track and quantitatively analyze the metabolites in complex biological matrices using separation techniques coupled to radioactivity detection methods. Nowadays the radiochemical detection of different on-line hyphenated techniques (GC-RD, HPLC-RD, OPLC-RD) are of great impact in the complex study of the pharmacokinetics and metabolite kinetics of the parent compound and its metabolites, while the hyphenated off-line techniques (OPLC-DAR/PIT – Digital Autoradiography; Phosphor Imaging Technology) are essential in the metabolite isolation and purification. The different types of radioactive detection enables high selectivity (only the  $^3\text{H}$ -,  $^{14}\text{C}$ - labeled compound and its metabolites are detectable) with extremely good sensitivity.

*In vitro* metabolism studies bring important decisional elements for the selection of the best candidate(s) entering clinical development and represent valuable tools to optimize future clinical studies. Pharmacokinetic and metabolism information of different species, contributing to registration, are also summarized.

The application of the new, flexible and rapid high-performance complex solution and their possible combinations, including single- and multi-step separation and isolation in metabolism research will also be presented.

### **Evaluation of methacrylate-based monolithic stationary phases for the analysis of drug molecules in the capillary format.**

Indiana Tanret, Debby Mangelings, Yvan Vander Heyden \*

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The separation of pharmaceutical samples with efficient methods is relevant in several fields, like drug development, drug safety assessment, quality control and toxicity studies. Monolithic stationary phases offer many advantages, of which the most important are easy column preparation and the possibility to tailor their morphology. They are prepared via polymerization of a so-called polymerization mixture that consists of monomers, poreforming solvents and an initiator of the polymerization reaction. The shape and functionalities of monolithic columns can easily be adapted by varying the composition of the polymerization mixture [1]. In our study, the potential use of methacrylate-based monoliths for the analysis of small molecules with basic or acidic properties, i.e. drug molecules, was evaluated. In a first stage, central-composite design-based experiments were performed to find a polymerization mixture from which columns with good chromatographic properties could be synthesized [2,3]. This polymerization mixture was then used for the analysis of drug molecules in capillary electrochromatography (CEC) [4] and pressure-assisted CEC. CEC combines the properties of capillary electrophoresis (CE) and liquid chromatography (LC). As in CE, the mobile phase is driven by the electro-osmotic flow (EOF), while the presence of a stationary phase reminds of liquid chromatography (LC). Pressurized CEC (pCEC) is based on CEC, but the flow is controlled both electrophoretically and by pressure [5].

Finally, the synthesized monolithic capillary columns were used within a so-called lab-on-a-chip set-up, where the injection, separation and detection all happen on one small surface. The separation on a chip can be performed in either electrophoretic, electrochromatographic or pressurized mode, depending on the needs of the analysis. The chips used in our experimental work were prepared in-house by etching into a polydimethylsiloxane layer.

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### **Multidimensional SHIMADZU chromatography**

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Chromatographic analysis has become the fundamental technique for the exceptional and ultimate identification of a sample of interest. The presentation will enlight different techniques developed by Shimadzu .

The technical design of the newest instruments and outstanding GC×GC, GC×GC-MS, LC×LC, LC×LC-MS and LC×GC-MS, techniques will be presented. These new state of art chromatographic techniques are called 2D and comprehensive solutions.

The benefits and advantages of these techniques over standard techniques will be discussed. The basis of these techniques are multi column chromatographic analysis which lead to the perfect separation of the studied sample, and straight identification of analytes not detected or separated with the standard GC and LC techniques. The key role of MS detection will be presented.

A wide range of application of these techniques, in different analytical fields will be presented.

**A comparative study of lipid composition of the brain of chicken and rat during myelination; A chromatographic-densitometric analysis.**

F. Helmy, Morris

*Delaware State University, Dover, DE*

The lipid profile of brain from 10 day and 18 day old chick embryo, one day old chick and adult chicken (10 weeks old), as well as full-term fetus rat, 21 day old male rat, young adult male rat, and pregnant female rat brain; were analyzed by thin layer chromatography and densitometry. The emphasis was on the major glycolipids of brain during myelination (i.e. galactocyl diglyceride (GDG), normal fatty acid and hydroxyl fatty acid ceramide monohexosides (n-CMH and h-CMH respectively), and ceramide monohexide sulfatides (CMS) as well as the choline lipids sphingomyelin (SM), and phosphatidyl choline (PC), and the species of phosphatidyl ethanolamine plasmalogen (PE<sub>1</sub> and PE<sub>2</sub>).

10-day old chick embryo brain revealed a low concentration of GDG, n-CMH, h-CMH, and CMH-S. The concentration of these glycolipids increased gradually as the chick embryo advanced in development, indicating age relatedness, and reached the highest level in the brain of adult chicken. Rat brain did not begin to show the presence of these lipids until at least 21 days old, possibly indicating that the nervous system of the bird model develops faster than that of mammals. In addition, phosphatidyl ethanolamine plasmalogen (PE), was shown to be the only alkenyl phospholipid in all samples analyzed. Two molecular species have been identified (PE<sub>1</sub> and PE<sub>2</sub>), and PE<sub>2</sub> has been shown to correlate with the myelination process. PE<sub>2</sub> is not seen until after hatching in chick brain, while it is seen at the 21 day old stage rat brain. Phosphatidyl choline (PC), and sphingomyelin (SM), were also present. SM concentration increased gradually during development, and reached its highest level in adult chicken brain, as in rat brain.

The correlation of myelination, brain development and the level of these glycolipids, indicate the important role of these glycolipids in both brain maturation and function.

18.

**Two dimensional separation of amino acids with thin-layer chromatography and pressurized planar electrochromatography in normal and reversed phase systems**

Adam Chomicki, Kinga Kloc, Ewa Materna-Witkowska, Tadeusz H. Dzido

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Combination of different chromatographic modes is very attractive tool for separation of complicated sample mixtures, especially of biological origin such as peptides or amino acids. High efficiency of two dimensional separation (2-D) is achieved with respect to different selectivity of both modes involved in this process.

Thin layer chromatography (TLC) is very popular method in biomedical, pharmaceutical and environmental protection analyses. It has advantages such as low analysis cost, simple sample preparation, various methods of detection, chromatographic plates with sample bands on it can be stored after separation process and many other.

Pressurized planar electrochromatography (PPEC) is a relatively new separation mode in which the mobile phase is driven into movement by electric field (electroosmotic effect). The mode is characterized by few advantages in comparison to thin-layer chromatography such as high performance, short time of separation process and different selectivity. The last attribute is concerned with electrophoretic effect, which is involved in separation process when solute molecules undergo dissociation.

The attributes of TLC and PPEC mentioned above are very advantageous for combination of both methods into two dimensional separation process (2D TLC/PPEC). Such combination leads to considerable increase of separation efficiency. This feature of 2D TLC/PPEC mode will be demonstrated in our presentation for separation of some dye and amino acid mixtures applying normal and reversed phase systems.

## **The Use of Technology in-needle Extraction in the Determination of Organic Compounds**

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A new method of in-needle samples preparation has been used for the determination of samples containing organic compounds in water and gaseous samples. Specially designed needle is packed with sorbent on which the analytes are retained.

In-needle method combines the advantages of solid phase extraction (SPE) and stationary phase microextraction (SPME) despite their drawbacks, such as labor expense in the case of the SPE and necessity of careful handling of expensive fiber for SPME. Conventional sample preparation methods still requires large amount of organic solvents. Classic liquid–liquid extraction (LLE) or solid-phase extraction (SPE) are relatively complicated and time-consuming procedures. In-needle extraction compared with these methods is much more economical. The amount of solvent can be reduced to less than one milliliter. In-needle extraction device is cheaper than SPE or SPME. Another advantage is its mobility. In-needle extraction device can be taken to the place where samples will be collected. Analytes can be retained in needles and transported into laboratory.

The extraction was made by pumping the aqueous/gaseous 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.

Several solutions has been checked to find the best in-needle extraction device. The most important parameters are: needle size, sorbent size, pore diameter. The needle filling should assure satisfactory flow rate with good recovery. Flow velocity is higher for bigger size of grains. Fine-grained fill provides a short path of diffusion of a substance inside the grains. Kinetic properties will be better for the sorbent having thinner active layer.

## **Chosen strategy of basic drugs analysis in RP-HPLC systems**

Jolanta Flieger

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Basic compounds constitute quite a big group of separable analytes by reversed-phase high performance chromatography (RP-HPLC). This separation method covers the compounds which are important due to their biomedical applications. The most common strategy of controlling the retention of these compounds in RP systems is special modification of the mobile phase.

In case of weak bases required retention and efficiency of chromatographic system can be achieved by the use of addition of buffer components enabling suppression of analyte ionization. For the stronger bases creation of ion-pairs is required. Due to the presence of hydrophobic chains either cationic or anionic additives tend to be strongly adsorbed by hydrophobic stationary phase and the initial properties of a column are hard to recover again.

Attractive alternative, in context to previously used methods of basic compounds analysis, is application of chaotropic effect in RP-HPLC. It appears that salts possessing anions with chaotropic properties provide not only retention increase of protonated basic molecules in agreement with their order in liotropic Hofmeister series but they also improve efficiency of chromatographic system, peak symmetry and additionally radically perfect separation selectivity. The interest in this chromatographic technique constantly increases because of its application simplicity connected with method of system modification and fast recovery of the column initial properties. Mechanism of retention in this technique is still the subject of speculations. Participation of dynamic ion exchange mechanism as well as creation of ion-pair in the mobile phase is mentioned in context of chaotropic effect.

Recently also short chain perfluorinated acids may replace the need for addition of hydrophobic "ion-pairing" reagents, chaotropic salts or ionic liquids which have to be applied together with additional components of buffering systems. Although in the past, higher concentrations of such acidic modifiers were broadly avoided because silica based stationary phases could undergo degradation under highly acidic environment. Now approach to mobile phase modifiers can be revised owing to the advancement of silica based packings and availability of RP columns with excellent chemical stability.



## **The LC/MS/MS analysis of the nucleation precursors in the formation of secondary organic aerosols (SOA)**

Bartłomiej Witkowski\*, Tomasz Gierczak\*

*\*Department of Chemistry, Warsaw University, al. Żwirki i Wigury 101, 02-089 Warsaw,  
Poland*

Aerosols are very important constituents of the atmosphere. They can act as the cloud-condensation nuclei (CCN), contribute to the light-scattering effect and could be hazardous to humane health. Gas-phase ozonolysis of alkenes is known to produce aerosols. Since aerosols may affect environment it is important to understand the process of particle formation of SOA produced from ozonolysis of alkenes (for instance terpenes; vast amount of  $\alpha$ -pinene is introduced every year into the atmosphere from the biogenic sources). Many theories concerning nucleation precursors of aerosols produced from gas-phase ozonolysis of alkenes were developed. It was proposed that the second-generation dicarboxylic acids are responsible for self-nucleation and particle growth. After extensive studies diacids were excluded as nucleation precursors, because they are produced too slowly and they are too volatile to induce self-nucleation. Recently, the theory taking into account the formation of dimers was suggested. Ozone addition to the carbon-carbon double bond initially produces the high-energy primary ozonide. This ozonide rapidly decomposes, producing species called excited Criegee intermediate (ECI). ECI can decompose, producing wide variety of products with various oxygen-containing functional groups, or it can be collisionally stabilized with  $N_2$  or  $O_2$  to become thermally stabilized Criegee intermediate (SCI). Studies show that SCI plays an important role in the particle formation process during gas-phase ozonolysis of alkenes. Scavenging SCI with low molecular weight compounds significantly decreased particle number in comparison with experiments where no scavenger was used. Literature sources report that SCI have strong reactivity towards oxygen-containing functional groups. Reaction with carbonyl-containing compounds and carboxylic acids produces secondary ozonide and hydroperoxide. It was proposed, since SCI is the most reactive with carboxyl containing compounds, that nucleation precursors may be the products of gas-phase reaction between SCI and carbonyl-containing acids.

The primary aim of this study is to prepare the analytical method for the analysis of hydroperoxides formed in the reaction of SCI with carbonyl-containing acids. In our experiments cyclohexene was used as a model compound. Because standards for cyclohexene oxidation products are not available, the hydroperoxides were generated using liquid-phase ozonolysis of cyclohexene in the presence of investigated scavenger. The scavengers used included carbonyl-containing acid (first-generation products) and diacids (second-generation products). Synthesized hydroperoxides were analyzed by high performance liquid chromatography (HPLC) coupled with triple quadrupole mass spectrometer with electrospray ion source (ESI). Tandem mass spectra ( $MS^2$ ) for structural studies of synthesized compounds were obtained.

## **POSTER SESSION I**

**MAY 25<sup>th</sup>, 2010**

**CHAIRPERSONS:** W. Verschelde and Ł. Komsta

1.

## **Effect of mobile phase composition on the overall elution process in thin-layer chromatography**

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The solute retention and its band profile in the TLC system are strictly dependent upon the mobile phase composition. Determination of this dependence seems to be especially complex in the case of that kind of adsorbents that possess variety of active sites on their surface. Such heterogenic surface usually provides adsorption sites of different ability to interact both with molecules of solute and with more polar component of the mobile phase. The example of such popular adsorbent having at least two different active sites on its surface is alumina. This stationary phase is very common in numerous TLC applications. We have found that for binary mobile phase, there is a range of stronger component concentration that gives both peaks of Gaussian shape and triangle-like shape as well. Mobile phase composition determines the scope of interaction between analyte (solute) and active sites of adsorbent and therefore affects observed isotherm of adsorption. Profound insight in modeling of this process should reflect the fact of competitive adsorption of solute and components of mobile phase. This phenomenon is not observed in the TLC as frequently as in the LC mode of chromatography because concentrations applied here are usually in low level. Nevertheless we observed non-Gaussian peaks in our TLC study of phenylacetone on alumina chromatographed with mixture of toluene and 1,4-dioxane with relatively low concentration of the last one. We also suggested an appropriate model describing observed retention process.

2.

## **Study of Porous Materials by IGC/flash Thermodesorption**

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Inverse Gas Chromatography (IGC) became very popular method for the measurement of physico-chemical properties of solid materials. In contrast to analytical chromatography the stationary phase is the sample under investigation, while a substance in the mobile phase acts as a probe molecule. This means the roles of the phases are inverted.

The combination of IGC and thermal desorption method allows to determine the type of solid structure and estimate the contributions of micropores, mesopores and outer surface area to the adsorption process. Adsorption mechanism in micropores is completely different in comparison to behavior of the adsorbate in the mesopores and at the outer surface area. The adsorption in the smallest pores can be described by so-called “theory of volume filling of micropores” (TVFM), while in the mesopores and the outer surface prevails mono-/multilayer sorption mechanism (BET equation).

In combined IGC/flash thermodesorption experiment different zeolites were investigated. The obtained chromatograms show two peaks: the first corresponds to the elution, while the second – to the thermodesorption. The first peak, representing the mesopore and outer surface adsorption, is associated with desorption of only physisorbed probe molecules. Some probe molecules, due to their increased adsorption potential, are retained in the micropores. Therefore, after the increase of temperature the thermodesorption peak (micropore sorption) is observed.

The combination of IGC with flash thermodesorption proved to be a very good method for the characterization of porous materials and separation micropore and mesopore/outer surface adsorption due to different adsorption mechanisms.

## Increasing the reproducibility of gc retention indices by modifying their measurements

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Calculating the gas chromatographic retention indices (**RI**) usually implies the registration of the maxima of chromatographic peaks. The column overloading or non-linearity of sorption isotherms cause a distortion of peak shapes and shift the positions of their maxima, however the profiles of fronts of chromatographic peaks often remain undistorted.

We recommend to reconsider the idea of RI calculations using retention times measured not at the maxima of chromatographic peaks, but at the points on their fronts corresponded to the same levels of analytical signals, which was proposed first by A.I.M. Keulemans at the end of 1950s. It permits us to compensate RI variations caused by different relative quantities of target analytes and reference compounds. It can be illustrated by data for nitrobenzene, analyzed on the packed column with standard non-polar phase (see Table), where  $\gamma$  [1] is the following ratio:

$$\gamma = \frac{S_x}{S_n + S_{n+1}}$$

where  $S_x$  is peak area of the target component,  $S_n$  and  $S_{n+1}$  are peak areas of reference alkanes with  $n$  and  $n+1$  carbon atoms in the molecules.

**Table.** The reproducibility of **RI**s of nitrobenzene calculated in the maxima of peaks ( $RI_{\max}$ ) and at the different levels of the signals at their fronts.

$RI_{\max}$	RI (8 mV)	RI (4 mV)	RI (2 mV)	$\gamma$
1052	-	1081	1076	0.04
1084	1086	1079	1075	0.27
1096	1093	1082	1078	0.43
1111	1090	1082	1077	0.95
1110	-	1083	1077	1.20
1118	1085	1078	1074	1.75
1126	-	1082	1077	2.20
1143	-	1080	1077	3.10
<b>Average values</b>				
<b>1108 ± 28</b>	<b>1088 ± 4</b>	<b>1081 ± 2</b>	<b>1077 ± 1</b>	

As we can see, the fewer level of registration of signals leads to decreasing of absolute **RI** values and increasing of their reproducibility ( $S_{RI}$  values decrease from 28 to 1 i.u.). The values RI (2 or 4 mV) indicate no dependence on  $\gamma$  at all. The proposed method of **RI** calculation permits us to compensate partially both the influence of overloading of GC systems and non-linearity of sorption isotherms.

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### Hydrophilic Interaction Planar Chromatography of Geometrical Isomers of Some Co(III) Complexes

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Hydrophilic interaction liquid chromatography (HILIC) has recently been introduced as a highly efficient chromatographic technique for the separation of a wide range of polar solutes [1]. In the HILIC mode, an aqueous-organic mobile phase combined with a polar stationary phase was used to provide normal-phase retention behavior. HILIC is often considered as a normal-phase separation in a reversed-phase fashion mode. Studies on the separation mechanisms in different chromatographic systems were the topic of our long-term investigations and the results were reported in numerous papers. Among these studies, examinations of chromatographic behavior of different classes of metal complexes are of a special significance because their environmental and biomedical interest [2]. Continuing these investigations in this work the applicability of hydrophilic interaction chromatography to the analysis of geometrical isomers of some Co(III) complexes was explored.

The chromatographic behavior of series of cobalt(III) complexes of the anionic, cationic and neutral type were investigated under HILIC conditions on thin-layer of silica-gel. The chromatography was carried out with solvent systems: water (0-100%)/organic solvents and water (0-100%)/organic solvents/various amounts of electrolytes.

On the basis of the results obtained, possible retention mechanisms were considered.

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5.

**Comparison of chromatographic retention parameters of several basic compounds obtained on different stationary phases i.e. C18 and Phenyl bonded silica and various ionic liquids added to organic aqueous eluent systems**

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Ionic liquids (ILs) were firstly applied in liquid chromatography by Poole et al. in 1986. They are most commonly applied as additives in low concentration (smaller than 60 mM) in organic aqueous solutions in reversed-phase mode. It is obvious that the chromatographic results are affected by either cations or anions of a modifier. Activity of anions could be explained using the theory of chaotropicity, while action of cations is connected with suppression of silanophilic interactions. In this work the measurements of chromatographic parameters of several basic compounds revealing retention and efficiency were performed on different hydrophobic stationary phases i.e. C18 and Phenyl bonded silica gel whereas the mobile phase containing organic solvent/water/ionic liquid remained constant. Obtained results were compared and discussed according to retention mechanism appearing at applied conditions. Blocking of silanophilic interactions was also taken into account in conclusions.

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## On Spontaneous Oscillatory Condensation of Phenylacetic Acids in Aqueous Ethanol

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It is generally believed that condensation of amino acids and hydroxy acids (resulting in peptides and poly(hydroxy acids), respectively) is rather difficult because energetically unfavourable, as it needs a considerable energetic input in order to split one water molecule from each pair of binding compounds [1]. This conviction affects many present-day presumptions regarding, e.g., prebiotic condensation of amino acids resulting in formation of peptides coupled through peptide bonds (NH-C=O). Hence, the experiments are still devised which involve ion irradiation of amino acid solutions to imitate the presumable prebiotic conditions of peptide formation [2]. Moreover, computational simulations are carried out to prove that energetically, polycondensation of amino acids (and hydroxy acids) would be more favourable, if carbon, oxygen, and/or nitrogen atoms in amino acid and hydroxy acid molecules were replaced by their respective analogues, i.e., silicon, sulphur, and phosphorus atoms [3].

In this study, an experimental evidence is provided to prove that condensation with certain low-molecular-weight amino acids and hydroxy acids carried out at ambient temperature can be effortless, if it is carried out in 70% aqueous ethanol. We present the results of the investigation carried out with use of the non-chiral high-performance liquid chromatography with diode array detector (HPLC-DAD) and mass spectrometry (LC-MS) on the dynamics of condensation of *S*-, *R*-, *rac*-phenylglycine, and *S*-, *R*-, *rac*-mandelic acid dissolved in 70% aqueous ethanol and stored for the longer periods of time.

It seems that energetically effortless condensation of amino acids and hydroxy acids is inseparably linked with an ability of these acids to undergo a spontaneous oscillatory chiral conversion, first described in papers [4-6]. With the obtained experimental results, we managed to convincingly demonstrate that condensation of phenylglycine and mandelic acid is the oscillatory process.

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## On Spontaneous Oscillatory Condensation of *S*-(+)-Ketoprofen in Acetonitrile

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This study is continuation of our earlier investigations of chemical stability of the ketoprofen, when dissolved in the low-molecular-weight solvents and stored for the longer periods of time in a solution (1-5). In those earlier experiments, we have discovered an ability of this profen to undergo a spontaneous oscillatory *in vitro* chiral inversion and also to undergo condensation. The kinetic-diffusive model was proposed to illustrate formation of the oscillatory time and space waves of the locally changing concentration of the respective antimer pairs.

In this study, experimental evidence is provided to prove that condensation with ketoprofen carried out at ambient temperature can be effortless, if this profen is dissolved in acetonitrile. We present the results of the investigation carried out with use of the non-chiral high-performance liquid chromatography with diode array detector (HPLC-DAD) and mass spectrometry (LC-MS) on the dynamics of condensation of *S*-(+)-ketoprofen dissolved in acetonitrile and stored for certain period of time.

It seems that energetically effortless condensation of ketoprofen is inseparably linked with its ability to undergo a spontaneous oscillatory chiral conversion. With the obtained experimental results, we managed to convincingly demonstrate that condensation of ketoprofen is the oscillatory process.

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8.

## **Chromatographic behavior of chosen basic drugs on cyanopropyl bonded silica gel eluted with organic aqueous eluent systems modified with ionic liquids**

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Recent years have been characterized by the increase of the application of the so called "ionic liquids" (ILs). Ionic liquids have very unique properties of a new class of solvents that make them fashion in organic synthesis, catalysis, electrochemistry or green chemistry. However, when used diluted in a chromatographic mobile phase, ILs lose all their fashion properties and become just salts. The ions, being the components of salts may incite in the chromatographic system both a synergistic effect and an antagonistic effect, providing improvement of efficiency and separation selectivity. Not meaningless, as for the benefit of the achieved effects, is the possibility of suppressing of silanol interactions.

The aim of the following study is the use of the chosen ionic liquids (1-ethyl-3-methyl-imidazolium hexafluorophosphate (EMIM PF<sub>6</sub>), 1-butyl-3-methyl-imidazolium hexafluorophosphate (BMIM PF<sub>6</sub>) and 1-butyl-3-methyl-imidazolium chloride (BMIM Cl) as the additives for the mobile phase in RP-HPLC of ionogenic basic compounds. By the application of HPLC technique and Cyanopropyl bonded silica gel, chromatographic parameters expressing efficiency of the system were established.

In this work, the retention mechanisms (solute interactions in ionic liquids modified systems) of solutes of different nature was demonstrated.

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## Application of micellar TLC in studying lipophylic properties of organic compounds

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Hydrophobic character of organic substance is important physico-chemical property in relation to its biological activity. The most widely accepted hydrophobicity index is octanol-water distribution constant,  $\log P$ . Because this value is extremely difficult to measure experimentally due to problematic and tedious procedure, different computational methods are applied. Moreover, reversed-phase liquid chromatography (column and planar) is used for estimation of chromatographic hydrophobicity indices. In isocratic RPLC extrapolated retention to pure water,  $\log k_w$ , or  $\varphi_o$  parameters are proposed for the purpose. Although RPLC is a simple technique and permits to obtain reproducible data, using it in hydrophobicity measurements is not perfect. The value of extrapolated  $\log k_w$  parameter strongly depends on organic modifier and residual silanols of alkyl-bonded stationary phases. To overcome these difficulties new stationary phases imitating biosystem are proposed for studying hydrophobicity - immobilized artificial membranes IAMs, immobilized proteins, ceramides, keratin or cholesterol. Moreover alternative techniques such as counter-current chromatography (CCC) or micellar liquid chromatography (MLC) are applied in such investigations. Micellar liquid chromatography is a mode of conventional RPLC using surfactant solution above critical micellization concentration (cmc) as the mobile phase. The retention of solute in this technique depends on the type of interactions with the micelles and the surfactant modified stationary phase. In MLC two values, i.e., retention parameter  $k$  and  $k_m$  (the retention parameter at zero micellar concentration), are proposed as hydrophobicity indices and correlated to  $\log P$  values.

In our investigations micellar TLC was proposed to study hydrophobic properties of newly synthesized organic substances. Two surfactants, Brij35 and SDS, were applied as mobile phase components and different organic solvents, i.e., methanol, acetonitrile, acetone, dioxane and tetrahydrofuran, were used as the mobile phase modifiers. As stationary phase RP-CN HPTLC plates were utilized. Very interesting correlations between chromatographic parameters and  $\log P$  values calculated from molecular structures of test solutes confirm the importance and usefulness of micellar TLC in studying hydrophobicity of new substances.

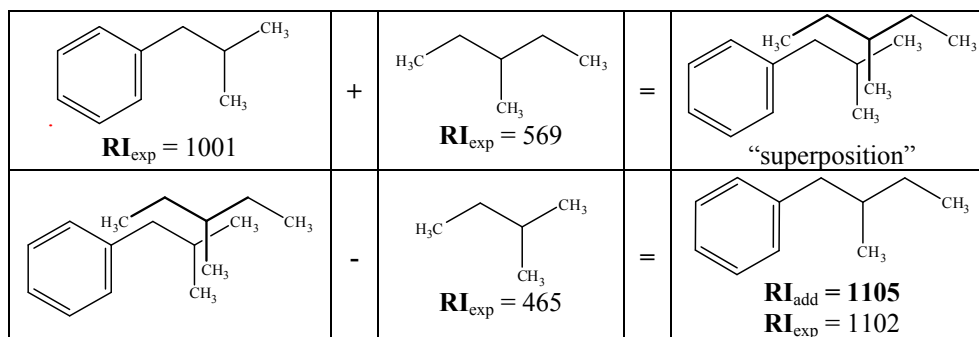
## Revealing the structures of isomeric alkyl arenes using additive evaluation of gas chromatographic retention indices

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There are many cases in analytical practice when we have to identify compounds with similar mass spectra in complex mixtures. Particularly, revealing the structures of isomeric products of Friedel-Crafts reaction, namely the alkylation of arenes, cannot be based on their mass spectra solely. Isolation and purification of each product of alkylation for its identification seems to be highly time- and money-consuming work. So far as the differences between mass spectra of isomeric alkyl arenes can be negligible, interpretation of data should be started with GC retention indices (**RI**) of products. If these data are unknown for estimated reaction products, evaluated values can be used instead of experimental.

This method of RI prediction is the simplest one; we can consider it with the example of RI calculation for (2-methylbutyl)benzene. We should assemble the “superposition” (see Scheme) of required structures (2-methylpropylbenzene and 3-methylhexane) and then subtract the molecule including the “duplicated” fragments (2-methylbutane). The simple arithmetic calculations provide to estimated **RI** value ( $1001 + 569 - 465 = 1105$ ). The experimental RI value for this compound is 1102.



**RI<sub>add</sub>** – the additively evaluated value

**RI<sub>exp</sub>** – one of the values from NIST/EPA/NIH (2005) database.

Formally schemes of such design don't imply pre-calculation of any increments. The average deviation of calculated and experimental RI's values within the series of alkyl arenes is 11 i.u., which is comparable with interlaboratory **RI** reproducibility. It shows that additive RI estimation is efficient in evaluation of structures of products of organic reactions, when both substrates and reagents are known.

The application of this approach for products of alkylation of alkyl arenes by alcohols is considered.

### **Automatic interpretation of gc-ms data in toxicological screening**

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One of the main problems in analytical toxicology is the identification of components of multicomponent mixtures of volatile organic compounds (VOC). For this purpose GC-MS is an indispensable technique. The chromatograms of biological samples contain a lot of peaks of background compounds which are often hardly distinguishable from the peaks of target compounds. The list of probable contaminants includes hundreds of compounds, which makes manual identification time- and labor-consuming. Automated GCMS data interpretation allows higher sample throughput. The software for automated interpretation of GC-MS data (AMDIS) was developed by the US National Institute of Standards and Technologies. It allows identification of components of complex mixtures even in the case of incomplete chromatographic resolution. However, the AMDIS with on-line large-volume nonspecific MS databases is unsuitable for toxicological screening. We performed a series of experiments and obtained evidence showing that the AMDIS in an open version (with on-line NIST 05 MS library) fails to identify components of model VOC mixtures, which could be readily identified by manual interpretation. This problem raised the necessity of creating domestic MS libraries compatible with the AMDIS software. However, the use in analysis of samples from complex matrices (like a corps material) was observed false-positive identification of light alcohols, ketones and aldehydes. False answers were rejected by help of gas chromatographic retention indices interpretation. So it became evident that concurrent interpretation of GC retention and MS data is necessary for unambiguous identification. Reference values for VOC retention indices and mass spectra were obtained experimentally.

With the using of references and own experimental data we create the list of 2252 compounds, including industrial and domestic poisons, toxic admixtures of alcohol, drugs, stimulants, products of toxic compounds decomposition, background components of urine, saliva and blood. A mixture of 180 components of various chemical origin was analyzed with GC-MS, and mass spectra and retention indices obtained were assembled in database. It was shown experimentally that automated identification with domestic database was done without false negative and positive results. Automated GC-MS data interpretation in analysis of natural (including high salted) water, synthetic urine and other samples spiked with VOCs was more efficient in comparison with manual data interpretation.

12.

**Application of gas chromatography in a comparative study of biomass, lignite and hard coal steam gasification**

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An increasing world's energy demand and environmental concerns related to GHG emissions as well as depleting fossil fuel resources and unstable prices of crude oil and natural gas cause a renewed interest in utilization of renewable energy sources, in particularly biomass.

In the paper a comparative study of steam gasification of biomass, lignite and hard coal, in a laboratory scale fixed bed reactor at the temperature of 700<sup>0</sup>C are presented. The effectiveness of steam gasification of biomass, lignite and hard coal samples, in terms of flows and composition of the main gaseous products and carbon conversion were studied. The procedure and results of fuels' chars reactivity testing in the process with a use of gas chromatography as well as the results of experimental data analysis with chemometric methods. The highest reactivity  $R_{50}$  and  $R_{max}$  were observed for biomass samples. A negative correlation between chars reactivities and heat of combustion, calorific value, carbon, nitrogen and fixed carbon content in a sample, total synthesis gas yield and CO content in synthesis gas were also observed. As it was expected synthesis gas produced in the process of biomass steam gasification was characterized by relatively lower calorific values when compared to gas produced through steam gasification of lignite and hard coal. It suggests that biomass gasification should be treated not as an alternative but as a complement for lignite and hard coal gasification.

**TLC densitometric investigation of the degradation of 4-chlorophenol using advanced oxidation processes (AOPs)**

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Chlorophenoles are contaminants with toxicity for humans and animals. They are non biodegradable and their removal is difficult. Advanced oxidation processes (AOPs) are one of alternatives for water treatment standard techniques. These processes completely degrade chlorophenoles to carbon (IV) - oxide or to biodegradable and less toxic intermediers.

In this work degradation was performed with AOPs using non-thermal plasma reactor based on coaxial dielectric barrier discharge. Three different sets of conditions for degradation of 4-chlorophenol was examined using falling film dielectric barrier discharge (DBD) reactor: DBD, DBD/H<sub>2</sub>O<sub>2</sub> and DBD/Fe<sup>2+</sup>. Degradation of 4-chlorophenol was monitored using thin-layer chromatographic method with densitometric detection (Camag TLC Scanner 3). RP18 silica plates were used with acetone-water-triethylamine 60:38:2 (v/v) as mobile phase. Scanning and densitometric analysis was performed at 240 nm.

## **Determination of ethyl 2-cyanoacrylate in workplace air\***

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Ethyl 2-cyanoacrylate (ECA,  $C_6H_7NO_2$ , CAS No. 7085-85-0) is a clear, colourless liquid with a strong, acrid odour. It reacts readily with water to form a solid polymer. It is soluble in methyl ethyl ketone, toluene, acetone, *N,N*-dimethylformamide, and nitromethane. Contact with alcohols, amines, or water may cause polymerization.

Various homologues of cyanoacrylate (CA) adhesive have been studied and used, including methyl- (MCA), ethyl- (ECA), isobutyl-, isohexyl-, and octyl-CA. The cyanoacrylates are used as adhesives both domestically and in a wide range of industrial environments — e.g., the manufacture of lampshades, plastics, electronics, scientific instruments, shoes and jewellery. The other known application of MCA and ECA is also visualization of fingerprints for criminal investigations.

The main health effects that have been observed to date in relation to occupational exposure to ECA are eye and respiratory tract irritation. Based on the harmfulness of ethyl 2-cyanoacrylate the exposure limit values: NDS-MAC (TWA) of  $1 \text{ mg/m}^3$  were proposed by the Interdepartmental Commission for Maximum Admissible Concentrations Intensities.

The determination method is based on the adsorption of ethyl 2-cyanoacrylate vapours on phosphoric acid-treated XAD-7 sampling tubes (80/40 mg sections), desorption with 2 ml of 0,2% (v/v) phosphoric acid in acetonitrile and high performance liquid chromatographic (HPLC/UV) analysis of the resulting solution. This method makes it possible to separate the ethyl 2-cyanoacrylate in the presence of methyl 2-cyanoacrylate. Calibration was carried out with standard solutions of ethyl 2-cyanoacrylate, using the following conditions: measurement range from 0,1 to  $2,5 \text{ mg m}^{-3}$ , for determination of 12 l air volume, 20  $\mu\text{l}$  injected sample. This method was fully validated.

### **Acknowledgement**

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**GC/MS analysis of some dialkyl esters of 1,2-cyklohexanedicarboxylic acid**E. Dziwiński<sup>1</sup>, A. Tasarska<sup>2</sup>, J. Lach<sup>1</sup><sup>1</sup>*Institute of Heavy Organic Synthesis „Blachownia”, Kędzierzyn-Koźle, Poland,*<sup>2</sup>*Institute of Chemistry, Opole University, Opole, Poland*

Dialkyl phthalates are used very often as the plasticizers of the different polymers. Because this type of compounds is recognized as the potential cancerogenes, they should be replaced by other types of compounds such as dialkyl esters of 1,2-cyclohexanedicarboxylic acid. The synthesis of these new plasticizers is based on the high pressure hydrogenation reaction of the appropriate dialkyl phthalates in the presence of catalyst.

The aim of our study was the application of gas chromatography – mass spectrometry (GC/MS) method to the identification of the hydrogenation products of di-n- and iso- butyl and octyl phthalates. The identification of these products was done in two ways:

- by exact measurement of retention times or relative retention times of the compounds analyzed and determination for them chromatographic retention arithmetic or Kovat's indices. The determined values of indices are very helpful, especially in the GC analyses of their isomers.

and/or

- by registration during GC/MS analysis and detailed interpretation the mass spectra of the individual esters. The general feature of mass fragmentation of dialkyl esters of 1,2-cyclohexanedicarboxylic acid is the presence in their mass spectra the most intense and characteristic peak at  $m/z$  155 corresponding to the ion with protonated 1,2-cyclohexanedicarboxylic acid anhydride structure, similarly as in the case of mass spectra of dialkyl phthalic acid esters where peak at  $m/z$  149 corresponds to the ion with protonated phthalic acid anhydride is present.

The application of the GC/MS method is very useful in qualitative and quantitative analyses both dialkyl esters of 1,2-cyclohexanedicarboxylic acid and dialkyl esters of phthalic acid occurring in the complex mixtures.

## Determination of aziridine in workplace air by hplc method

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Aziridine (ethyleneimine) is a colourless oily liquid with an intense odour of ammonia. Aziridine is an intermediate and monomer in the preparation of cationic polymers, such as polyaziridine. This polymer are used for improvement of wet strength of paper, in rocket and jet fuels, lubricants, as flocculating agents and in protective coatings, in textile finishing and for adhesives, polymer stabilizers, and surfactants. Occupational exposure to aziridine may occur in its production and in the preparation of polyaziridine polymers. The substance can be absorbed into the body by inhalation of its vapour, through the skin and by ingestion.

Aziridine is possibly carcinogenic and mutagenic to humans. It may cause heritable genetic damage to human germ cells.

The Interdepartmental Commission for Maximum Admissible Concentrations and Intensities in Poland established the NDS-MAC (TWA)<sup>1)</sup> value of 0,62 mg/m<sup>3</sup> for aziridine.

Determination of a worker's exposure to airborne aziridine is made using a XAD-2 resin tube (100/50 mg sections) coated with 1-naphthylisothiocyanate. The aziridine derivative formed was subsequently desorbed with acetonitrile and analysed by high performance liquid chromatography using a diode array detector. The working range for a 12-L air sample is 0,062 to 1,24 mg/m<sup>3</sup>.

Project carried out within the National Programme: "Improvement of Safety and Working Conditions" (2008 - 2010).

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<sup>1)</sup> Maximum Admissible Concentrations

**BioArena – non analytical application of planar chromatography**

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BioArena is a system, which integrates the modern technique and biological results of bioautography with layer liquid chromatography, suitable for investigating biochemical interactions. It has proved to be very effective way for examining the mechanism of toxicity of substances. Planar chromatography is a suitable technique for studying both the hydrophobicity and biological activity of new substances.

In our investigations a group of fourteen newly synthesized organic compounds has been investigated. The substances have potentially high bioactivity and they may be regarded as herbicides for use in agriculture. The lipophilicity of the test substances has been described by two indices – the retention in water,  $\log k_w$ , calculated from chromatographic data obtained by RP TLC on RP-18 as stationary phase with buffer–methanol mixtures as mobile phases, and  $\log P$  parameters calculated from molecular structures. The mechanism of action of the substances tested was examined in BioArena system. We reported practical determination of the crucial role of HCHO in the mechanism of action of new compounds. In the studies we observed a decrease of toxicity against *Pseudomonas savastanoi* pv. *phaseolicola* when HCHO molecules were eliminated by use of HCHO-capturing molecules, i.e. L-arginine and reduced glutathione and an increase in toxicity when  $\text{Cu}^{+2}$  ions, formaldehyde promoters, were added to the system.

The results obtained with BioArena as a complex separation and detection system support earlier observations that formaldehyde and its reaction products play a special and crucial role in the effects of antibiotic in general. Planar liquid chromatographic technique seems to be suitable for studying both the hydrophobicity and biological activity of new potential pesticides.

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**A comparison of the essential oil fingerprints derived from selected sage (*Salvia*) species with use of thin-layer chromatography directly and indirectly coupled with mass spectrometry**

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The low-temperature analysis of essential oils derived from plants by means of TLC is a relatively novel approach to investigating these volatile compounds [1-3], and it challenges the long established approaches of the first choice, most of them based on the use of gas chromatography.

The low-temperature TLC analysis and fingerprinting of essential oils derived from various different sage (*Salvia*) species has already been presented in our papers [4,5].

In this study, we compare the performance of the TLC-MS system described in paper [5] (and used to obtain TLC/mass spectrometric fingerprints of essential oils derived from the different sage species) with performance of the new TLC-LC-MS separation/fingerprinting strategy. With use of this novel approach, preliminary low-temperature fractionation of the essential oils derived from the different sage species is followed by the high-performance liquid chromatographic separation and fingerprinting of individual fractions with use of the LC-MS system. Abundant chromatographic evidence is provided and relevant practical conclusions are drawn.

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**A comparison of the phenolic compound fingerprints derived from selected sage (*Salvia*) species with use of thin-layer chromatography directly and indirectly coupled with mass spectrometry**

M. Sajewicz<sup>1</sup>, D. Staszek<sup>1</sup>, M. Natić<sup>1,2</sup>, M. Waksmundzka-Hajnos<sup>3</sup>, T. Kowalska<sup>1</sup>

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In our earlier studies [1-4], we presented the results of investigations focused on a comparison of phenolic compounds and essential oils originating from twenty different sage (*Salvia*) species by means of fingerprints obtained by TLC/densitometry, HPLC/DAD, and HPLC/ELSD. In paper [4], a comparative analysis of chromatographic fingerprints recorded for the different sage (*Salvia*) species with use of chemometrics was also given.

In this study, we compare fingerprinting performance of the TLC-MS system earlier described in paper [5] with the performance of the new TLC-LC-MS separation combined with fingerprinting. With use of this new approach, the preliminary TLC fractionation of phenolic acids and flavonoids derived from the several sage species is followed by the high-performance liquid chromatographic separation and fingerprinting of individual fractions with use of the LC-MS system. In that way, multidimensional fingerprints are obtained, with an enhanced content of analytical information. Abundant chromatographic evidence is provided and relevant conclusions are drawn.

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**POSTER SESSION II WEDNESDAY, MAY 26<sup>th</sup>, 2010**

**CHAIRPERSONS: D. Mangelings and L. Vanden Bossche**

1.

## **Role of biogenic amines in the formation of *n*-nitrosamines during meat processing**

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Y. Vander Heyden<sup>3</sup> and H. Paelinck<sup>1</sup>

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The formation of *N*-nitrosamines in a wide range of foods, especially in meat and meat products, can be bait by different factors like the presence of sodium nitrite, conditions of heating, storage time, bacteriological status or pH. Their formation in the presence of biogenic amines (BA) creates an additional toxicological risk. Raw meat contains certain amounts of biogenic amines e.g., spermidine, spermine, cadaverine and putrescine. The concentration of the latest ones can increase due to bacterial proliferation under inappropriate storage conditions. Presence of biogenic amines in meat is monitored for quality reasons, because BAs can be used as chemical indicators for bacterial spoilage of the final products [1-3].

The aim of this study was to determine the role of biogenic amines i.e., putrescine, cadaverine, spermidine or spermine on the formation of *N*-nitrosamines in cured meat products. Such products were processed with different amount of sodium nitrites ( 0 mg kg<sup>-1</sup>, 120 mg kg<sup>-1</sup>, 480 mg kg<sup>-1</sup>), 0 or 1000 mg kg<sup>-1</sup> of the stated biogenic amines, and heated at 85°C, 120°C, 160°C and 220°C. Experimental evidence was produced using gas chromatography in combination with Thermal Energy Analyzer (GC-TEA). The obtained analytical results were statistically evaluated by means of the Univariate Analysis of Variance (ANOVA) approach.

From the obtained data could be concluded that higher processing temperatures and higher added amounts of sodium nitrite increase the yields of *N*-nitrosodimethylamine (NDMA). Addition of cadaverine and spermidine caused a significant increase on the *N*-nitrosopiperidine (NPIP). The other added amines, i.e. putrescine and spermine, did not had a measurable influence on the nitrosamines concentrations. Beside *N*-nitrosopyrrolidine (NPYR) in some rare cases, no other volatile *N*-nitrosamines are detected.

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2.

## **Development of the method for determination of ziprasidone and its impurities**

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Ziprasidone is an atypical antipsychotic drug, belongs to second generation, which possesses high affinity for adrenergic ( $\alpha_1$ ), histamine ( $H_1$ ), and serotonin ( $5-HT_2$ ) receptors as well as dopamine ( $D_2$ ) receptors. It is used for the treatment of schizophrenia and in acute manic or mixed episodes associated with bipolar disorder. For acute agitation in patients with schizophrenia, ziprasidone may be given as the mesilate by intramuscular injection.

As it is not official active pharmaceutical ingredient in European Pharmacopoeia, there are not so many data available on simultaneous quantification of ziprasidone and its impurities in bulk powder and in dosage forms. Therefore, the purpose of this investigation was to develop and validate a selective RP-HPLC method for analysis of ziprasidone and its five impurities which differ in polarity and pKa<sub>s</sub>. During the preliminary study some important data about chromatographic behavior of substances were found. Relationship between chemical structure and chromatographic elution was established.

Satisfactory chromatographic separation - good resolution, peak symmetry, retention and selectivity factor, was achieved using combination of gradient and isocratic elution. The most important factors in the separation were column type and the profile of chromatographic elution. Separation were performed on the Hewlett Packard 1100 Series chromatographic system (Agilent Technologies, Germany) with column Waters Spherisorb<sup>®</sup> ODS 1, (4.6mm×250 mm, 5.0  $\mu$ m). Injection volume was 40  $\mu$ l. All analyses were performed at 25 °C and the UV detection was performed at 250 nm using diode array detector. Mobile phase consists of water phase - 1% TEA in 0.05M potassium dihydrogen phosphate solution whose pH was adjusted to 2.5 by orthophosphoric acid, and organic phase – acetonitrile. The flow rate was 1.5 ml/min and run time was 20 minutes.

Relationship between peak areas and the amounts of the active pharmaceutical ingredient and all of five impurities was proven by validation parameters - linearity, recovery, precision and quantitation limit, which found to be satisfactory. Finally, method is convenient for the purity control of ziprasidone and assay, both in raw materials and dosage forms.



3.

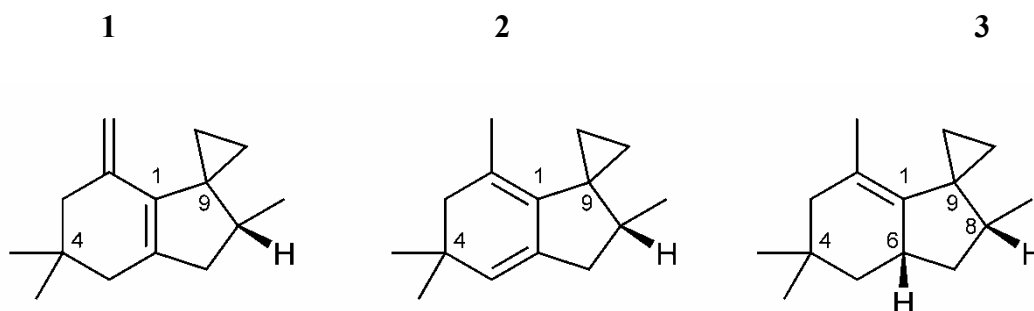
### Spirocyclopropane-type Sesquiterpene hydrocarbons from *Schinus terebinthifolius* RADDI

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*Schinus terebinthifolius* RADDI is an evergreen shrub or tree of the Anacardiaceae, native to South and Central America. The leaves and reddish fruits are rich in essential oil. Earlier investigations reported high concentration of monoterpenes (Stahl, 1983; Malik, 1994) along with some sesquiterpene hydrocarbons (Singh et al., 1998). In order to obtain additional data, dried fruits of *S. terebinthifolius* were hydrodistilled (Sprecher, 1963) to afford the essential oil, which was investigated by GC and GC-MS (GC-column: CP-Sil-5). A combination of column chromatography on silicagel 60, preparative GC on polysiloxane SE-52, and repeated semipreparative GC using megabore thickfilm capillary columns coated with DB-1 and DB-1701, three unknown sesquiterpene hydrocarbons (1, 2, 3) were selected. Structure assignments were carried out by using NMR spectroscopy\*.



These new natural products are 9-spiro(cyclopropano)-4,4,8-trimethyl-2-methylenbicyclo[4.3]non-1(6)-ene (terebanene, **1**), 9-spiro(cyclopropano)-2,4,4,8-tetramethylbicyclo[4.3]nona-1,5-diene (teredenene, **2**), and (6*R*\*,8*R*\*)-9-spiro(cyclopropano)-2,4,4,8-tetramethylbicyclo[4.3]non-1-ene (terebinthene, **3**).

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\* In press

4.

**Spectrophotometric determination of the sum of phenolic acids and flavonoids contained in twenty different sage (*Salvia* L.) species and the analysis of the sage extracts by means of HPLC-DAD and HPLC-ELSD**

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Phenolic acids and flavonoids are the two groups of chemical compounds widespread in the plant kingdom that exert a positive influence on human health. Their particularly important biological role consists in an antioxidative and radical-scavenging activity. The group of phenolic acids embraces the highest number of active compounds which play a significant role in various different defensive mechanisms in the plant organisms (e.g., protecting them against infections, an excessive exposure to sun, or injuries [1]). The main biological role of flavonoids in humans relates to the blood vessels and to the blood circulatory system, although certain flavonoids exert even a more complex impact on human health.

The objective of this study was spectrophotometric determination of the sum of phenolic acids and flavonoids contained in the extracts originating from twenty different sage (*Salvia* L.) species, and the chromatographic fingerprint analysis of the respective extracts by means of high-performance liquid chromatography with the diode array and the evaporative light scattering detectors (HPLC-DAD and HPLC-ELSD).

Botanical material originated from the Pharmacognosy Garden of Medical University, Lublin, Poland, harvested in the three consecutive vegetation seasons (i.e., in 2007, 2008, and 2009). This material has been extracted in the two different ways proposed in the literature [2], one targeting phenolic acids and the other flavonoids.

The main aim of spectrophotometric and chromatographic analysis was to expose seasonal and inter-species composition differences in the contents of phenolic compounds. By means of the HPLC analysis, the DAD and ELSD fingerprints of the scrutinized extracts were collected. Moreover, the chromatographic analysis served an identification purpose also, and it was carried out against the acquired phytochemical standards.

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The work of one author (D.S.) was partially supported by PhD scholarships granted to her in 2009 within the framework of the ‘University as a Partner of the Economy Based on Science’ (UPGOW) project, subsidized by the European Social Fund (EFS) of the European Union.

## Chromatographic and spectroscopic analysis of essential oils from *Salvia lavandulifolia* L. and *Salvia triloba* L.

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Mixtures of volatile compounds which can have a diverse chemical nature and intense olfactory characteristics are known as essential oils. Chemically, essential oils can be terpenes, esters, alcohols, aldehydes, ketones, phenols, ethers, and hydrocarbons. Contributions from each individual class of compounds and quantitative proportions among these compounds determine therapeutic properties of a given essential oil [1].

Currently, a fast developing hybrid field formed at an intersection of natural medicine and the knowledge of medicinal plants is known as aromatherapy. Within its framework, the patient is treated with biologically active olfactory agents derived from herbal essential oils which penetrate his organism through the respiratory system and skin. Certain plants produce up to several hundred volatile compounds and many of those have never been properly identified yet [2].

This paper presents consecutive results derived within the framework of a complex research project on composition of essential oils originating from the different sage (*Salvia* L.) species. The analyses performed so far have enabled identification of 24 volatile compounds belonging to the class of terpenes [3, 4]. Presently, we introduce a combined evidence on chemical composition of essential oils obtained from the two different and essential-oil-rich sage species (i.e., from *Salvia lavandulifolia* L. and *Salvia triloba* L.) by means of <sup>13</sup>C NMR spectroscopy and the *headspace*-GC-MS. This attempt is basically meant to gain an additional and a more in-depth perspective on composition of essential oils from the *Salvia* L. genus, owing to the complementary identification results derived by means of <sup>13</sup>C NMR spectroscopy that accompany those obtained by means of the *headspace*-GC-MS technique.

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The work of one author (Ł.W.) was partially supported by PhD scholarships granted to him in 2009 within the framework of the ‘University as a Partner of the Economy Based on Science’ (UPGOW) project, subsidized by the European Social Fund (EFS) of the European Union.

6.

**Determination of lipophilicity by TLC revisited - a comparative study on several techniques with simple molecule model solute set**

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

The purpose of the study was to compare several approaches of TLC lipophilicity determination: a single TLC run, extrapolation of a retention, principal component analysis of a retention matrix, PARAFAC on a three-way array and a PLS regression.

All techniques were applied to 35 model solutes with simple molecules, using RP18 thin layer plates and nine concentrations of six modifiers: acetonitrile, acetone, dioxane, propan-2-ol, methanol and tetrahydrofuran.

Comparative analysis formed several general recommendations:

1. Methanol and dioxane were the best modifiers, while acetonitrile gave the worst and unacceptable correlation of retention with lipophilicity.
2. Surprisingly good correlations were obtained for single TLC runs and this method is underestimated in the literature.
3. Advanced chemometric processing proposed recently, such as PCA, PARAFAC and PLS did not show a visible advantage comparing to classical methods.
4. A need to use of robust regression and robust correlation measures, due to presence

7.

## **HPLC method determination of formaldehyde released from chosen root canal sealers**

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*National Medicines Institute, Warsaw, Poland*

The present investigation was concerned with determination of formaldehyde released from root canal filling and sealing materials. For the analysis four root canal sealers were chosen: EZ-Fill Endodontic Filling Cement, Endomethasone N Root Canal Sealer, AH Plus Jet Root Canal Sealing Material and Endodontic Cement N2. The cements samples (homogenous pastes) were prepared according to manufacturers' instructions.

Formaldehyde released from pastes was analyzed by RP - HPLC after derivatization with 2,4-dinitrophenylhydrazine and extraction with dichloromethane and acetonitrile. Analysis was performed by using a C18 chromatographic column, a mixture of acetonitrile and water (70:30) as a mobile phase, and UV/VIS detection (360 nm).

The standard curve was linear across the range 0,05 – 16 ppm of formaldehyde with a correlation coefficient 0,9996; the limit of detection was 0,01ppm. Precision (RSD) of the assay was 5,3% for pastes containing about 2000 ppm of formaldehyde and 6,9% for lower contents (about 2 ppm). Recovery studies were performed by fortifying each paste with an amount of formaldehyde equal to that which was determined. The results were in the range 89% for pastes containing about 2000 ppm of formaldehyde and 76 – 86% for those containing about 2 ppm.

The results allow for application of this HPLC method for the analysis of formaldehyde released from the root canal sealers.

8.

### **Selective extraction of polar organic compounds as the key stage of chemical warfare metabolites identification in biomedical samples**

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Biomatrices are the most challenging objects for investigation because of their complex and unpredictable qualitative and quantitative compositions. Biomatrices actively metabolize absorbed compounds even after their excretion from the body. Chemical warfare (CW) biotransformation products are usually polar and hydrophilic, which entails their high affinity to biomatrices and low recovery in standard extraction procedures.

Direct analysis of polar CW metabolites can be achieved by means of HPLC and CE techniques, but their sensitivity and selectivity are lower compared to GC. As participants of the OPCW Confidence Building Exercise on Biomedical Sample Analysis we had to develop optimal procedures for sample preparation for electron impact and chemical ionization GCMS analysis of *O*-alkylmethylphosphonic acids, thiodiglycol, and  $\beta$ -lyase metabolites of sulphur mustard. These procedures include extraction of target compounds from synthetic urine, clean-up and derivatization. Each sample should be tested for the presence of each analyte with minimum sample volume and time consumption and with a maximum reliability of identification. Sample preparation appeared to be a bottleneck.

Attempted SPE application was unsuccessful, probably due to a high concentration of artificially spiked masking agents. We have developed a universal procedure, involving purification of the synthetic urine by successive treatment with benzene and hexane in neutral and acid media. The purified synthetic urine was mixed with equal volume of acetonitrile, sodium chloride was added to the mixture, and the separated acetonitrile layer containing CW metabolites was separated (salt-assisted dispersive liquid-liquid extraction). The residual aqueous layer was extracted with acetonitrile and diethyl ether, the organic extracts were combined, concentrated and derivatized for subsequent GCMS analysis.

Thus we report here a procedure involving clean-up of synthetic urine by successive treatment with organic solvents, followed by salt-assisted dispersive acetonitrile extraction as a simple and reliable method for selective extraction of polar organic compounds from complex matrices.

## GC/MS analysis of the urine for metabonomic research of autistic children

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In combination with genomics, transcriptomics and proteomics, metabonomic analysis is being increasingly used in the clinical chemistry. Metabonomics is defined as “the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification”. Application of metabonomics in clinical investigations is supported by a large number of publications. One of the aims of such research is discovering biomarkers, candidates specific for the condition, for assessing disease progression, determining drug efficacy, and potentially allowing selection of a patient. Urine, which is readily obtained, represents one of the most common sample types for this work but analysis can also be performed on other biofluids and secretions as well as whole tissues and tissue extracts. Combined techniques such as e.g. GC-MS, HPLC-MS, GC-TOF-MS are widely used in metabolic studies [1-4]. The use of GC-MS-based methods for the study of metabolite urinary profiles in research on autism can allow obtaining information on possible metabolic disorders in people affected by this disease. Autism is a developmental disorder, defined on the basis of behavioural characteristics which result from impairments in social communication and reciprocal social interaction, repetitive and restrictive behaviours, and imaginary thought [5].

The aim of this study was to perform metabolic profiling using GC-MS. The urine specimens were collected from 10 autistic children who underwent rehabilitation at the Navicula (Research Centre in Mental Retardation “Navicula” Centrum in Lodz, Poland) and 5 healthy children. The method involves extraction of analytes from urinary samples and derivatization with MSTFA.

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## Urinary dicarboxylic acids in autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder diagnosed in early childhood. All children with ASD demonstrate deficits in social interaction, verbal and nonverbal communication, and repetitive behaviors or interests [1]. Several metabolic defects have been associated with autistic symptoms, these include phenylketonuria, histidinemia, adenylosuccinate lyase deficiency, dihydropyrimidine dehydrogenase deficiency, 5'-nucleotidase superactivity, and phosphoribosylpyrophosphate synthetase deficiency, mitochondrial dysfunction. Early diagnosis of the metabolic disorders and proper therapeutic interventions in some patients may significantly improve both cognitive abilities and behavioral deficiencies [2,3]. A very important tool used in the diagnosis of several metabolic disorders is the analysis of urinary dicarboxylic acids [4]. The chronic urinary excretion of dicarboxylic acid may be due to: 2-ketoglutarate degradation, ketosis, tissue ischemia (in case of succinate), ketosis, lactic acidosis; hypoglycemia, beta-oxidation defects HMG-CoA lyase deficiency, systemic carnitine deficiency, succinic semialdehyde DH deficiency, CPT II deficiency (in case of adipate, suberate, sebacate) uremia and peroxisomal diseases (in case of odd dicarboxylic acids) [5].

The aim of the study was to determine the level of succinic, adipic, suberic and azelaic acids in urine of autistic and healthy children. Urine samples were taken from 30 autistic children who underwent rehabilitation at the Navicula (Research Centre in Mental Retardation "Navicula" Centrum in Lodz, Poland) and from 10 healthy children. Before GC-MS analysis dicarboxylic acids were extracted from urine and derivatised with BSTFA.

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11.

**Determination of the level of D-arabinitol and D-/L-arabinitol ratio in urine of autistic children using gas chromatography/electrone capture detection**

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D-arabinitol is 5-carbon sugar alcohol (pentiol) is produced by many pathogenic yeast species. These include *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida kefyr*, *Candida lusitanae* and *Candida guilliermondii*. Human mammalian cells are capable of producing both D- and L-arabinitol. D-arabinitol is a well known marker for invasive candidiasis. Clinical signs of invasive candidiasis can be unspecific, and diagnosis is still mostly based on blood cultures; however, blood cultures have been assumed to be positive for *Candida* in only 24 to 60 % of cases. Both D-arabinitol and L-arabinitol are normally present in serum and urine, and the DA/LA ratio can be determined with coupled chromatography techniques. An elevated urine D-arabinitol/L-arabinitol (DA/LA) ratio is a sensitive sign of invasive candidiasis.

Autism belongs to a group of disorders whose process can be modified by mycosis. Many symptoms of autism can be the result of pathological *Candida*'s increase. Treating with antifungal drugs and application of gluten-free and casein-free diets can significantly improve clinical state of autistic subjects.

The aim of this work is to determine D-arabinitol and DA/LA ratio in urine of autistic children in comparison with healthy individuals using gas chromatography/electron capture detection (GC/ECD).

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**Determination of the level of fumaric acid in urine of autistic children using SPE-HPLC**

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Fumaric acid is the trans-isomer of malic acid that enters the citric acid cycle. It is necessary to generate cellular energy for tissue fuel. It is formed by oxidation of succinate by enzyme succinate dehydrogenase. Fumarate is then converted by the enzyme fumarase to malate. Fumaric acid is also a byproduct at certain stages in the arginine-urea cycle and purine biosynthesis. In healthy individuals, fumaric acid is formed in the skin from the exposure to sunlight. A deficiency in one or more Krebs' cycle intermediates and an inhibition of normal energy production may cause a wide range of metabolic disturbances and symptoms. A deficiency of fumaric acid is linked to chronic fatigue, psoriasis and fumarate hydratase deficiency, which is an autosomal recessive disorder of the Krebs cycle with variable presentation, usually involving developmental delay with neurological features such as epilepsy, hypotonia and encephalopathy. The commercial demand for fumaric acid has been increasing because of its extensive applications in the food industry. It is used in beverages and baking powders, as a substitute for tartaric acid and in place of citric acid.

There is evidence that some cases of autism have been associated with several different organic conditions, including bioenergetic metabolism deficiency. Cases of mitochondrial respiratory chain disorders have also been described as being associated with autism.

The aim of this work is to measure the level of fumaric acid in urine of autistic children in comparison with healthy individuals. Investigations were carried out with an application of high pressure liquid chromatography technique/ultraviolet detection (HPLC-UV) based on solid phase extraction method (SPE).

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## Urinary level of cysteine and mercury in autistic children

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Autism is heterogeneous neurodevelopmental disorders behaviorally defined by significant deficits in social interaction and communication. There are numerous theories concerning the specific causes of autism. One of these theories is exposure to toxic metals. In literature one can find suggestions that there are strong links between childhood autism and mercury toxicity (1-3).

The function of glutathione in human organism is connected with toxic metals and their removal. The cysteine thiol (-SH) group of glutathione binds mercury and protects essential proteins from functional inactivation. Mercury binds to cysteine thiol groups on intracellular proteins and inactivates their functions. A studies revealed the importance of transsulfuration metabolic imbalance present in many autistic children, characterized by significant reductions of cysteine in organism and reduced glutathione levels relative to controls (4,5).

The aim of the present study was to find out whether there are some tangible differences and correlation between the level of cysteine and mercury in urine of autistic children. Urine samples were collected from 30 autistic children (4-11 years) who underwent rehabilitation at the Navicula ( Research Centre in Mental Retardation “Navicula” Centrum in Lodz).

Cysteine in urine was determined by gas chromatography/mass spectrometry (GC/MS). The total mercury content was measured with Mercury Analyser MERCURY SP-3D, Nippon Instrument Corporation.

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## **Determination of the level of homocysteine in urine of autistic children before and after a diet using gas chromatography/mass spectrometry**

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Nutrition plays an important role in the development and behavior of autistic children. Autistic people have a high prevalence of gastrointestinal disease and dysbiosis. Improper diets and bad conditions of alimentary system can have a strong influence on the intensification of autism symptoms (1).

The level of homocysteine, which is eliminated with urine, provides essential data about diets and functioning of the alimentary system. Homocysteine is metabolised on two pathways: remethylation to methionine or transsulfuration to cysteine. A defect in either of these pathways leads to an accumulation of higher concentration of homocysteine in organism. Remethylation is a process which involves the presence of folic acid and vitamin B<sub>12</sub>. Transsulfuration is a process which involves the presence of vitamin B<sub>6</sub>. Vitamins B<sub>6</sub>, B<sub>12</sub> and folic acid are necessary for lowering the level of homocysteine. Improper dietary intakes of these nutrients can lead to vitamins deficiencies, which can produce elevations in homocysteine levels (2-4).

In our studies, we present the results of the level of homocysteine in the urine of autistic children before and after the diet enriched with vitamins from group B. Urine samples were collected from a group of autistic children (4-11 years) who underwent rehabilitation at the Navicula ( Research Centre in Mental Retardation “Navicula” Centrum in Lodz). Gas chromatography/mass spectrometry (GC/MS) was used to determine the levels of homocysteine in urine.

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## **Methods of determination selected priority substances in water samples**

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

The aim of this work was to devise methods of determination selected priority substances, which were partitioned for three groups. Group I involving : di(2-ethylhexyl)phthalate,  $\alpha,\beta$ -endosulfan, alachlor, chlorpyrifos, octylphenols and trifluralin was analyzed by GC/MS. For compounds: simazine, atrazine, diuron, isoproturon, chlorfenvinphos – group II, HPLC/DAD was chosen as a final method of determination.. Group III, represented by volatile organic compounds: trichlorobenzenes, trichloromethane, benzene, 1,2-dichloroethane, dichloromethane was analyzed by SPME-GC-MS.

As a extraction technique of water samples for compounds from groups I and II Solid Phase Extraction (SPE) was applied comparing the two kinds of sorbents, C<sub>18</sub> phase and copolymer SDB. For volatile organic compounds - group III, Head Space-Solid Phase Microextraction (HS-SPME) was used.

The usefulness of the devised methods was estimated by comparing obtained limits of quantification of examined substances with limit values according to ordinance of the Ministry of Environment 20 August 2008 regarding the way of classification of surface water state (DZ. U. Nr 162, poz. 1008)

The devised methods were tested on natural environmental samples collected from areas with different contamination levels. Our researches have proved occurrence of priority substances in water which are situated in near neighborhood of industrial plants.

## Determination of nicotine in hair by GC-NPD

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It has been proved, that variety of xenobiotic can accumulate in hair during its growth. In addition, hair analysis belongs to noninvasive methods of assessment of exposure to tobacco smoke. Hair concentrations of nicotine was worked out by gas chromatography with nitrogen detector preceded by solvent extraction procedure.

Sixty milligrams of each hair portion were transferred to a polypropylene centrifuge tube for washing. Samples were washed using 6 mL of dichloromethane by sonication for 30 minutes to remove nicotine adhering to the surface of the hair. When the hair strands were dry after the washing step, 3mL of 1mol/L NaOH were added and the samples were incubated at 50°C for 24h, then cooled to room temperature. After incubation, 7 mL dichloromethane was added to the tubes. The tubes were finally shaken using centrifuged at 2000g for 10 minutes. The organic phase in each tube was then transferred to a clean polypropylene evaporation tube. To prevent volatilization of nicotine, 35  $\mu$ L n-octanol was added to each tube. The extraction process was repeated and the organic layers from each extractions were evaporated together in a water bath at 50°C for approximately 20 minutes. Finally, methanol was added to the remaining of octanol and the final solution was transferred to an insert mounted in an autosampler vial and analysed by GC-NPD (Shimadzu, Japan) with ZB-5 (30m x 0,25mm x 0,50 $\mu$ m) column. Temperature programme: 50°C (for 1min); 50°C to 215°C (10°C/min for 1 min); 215°C to 275°C (20°C/min) for 2 min) was used. Detector and injector temperatures were 300°C and 250°C, respectively. Helium and flow rate of 2 cm<sup>3</sup>/min was applied as carrier gas.

The main nicotine metabolites - cotinine can be determined in much more concentrations, but 3-trans'-hydroxycotinine should be derivatised before gas chromatographic determination. Therefore we were only interested in the measurement of nicotine with detection limit of 0.15 ng/ $\mu$ L, determined by GC-NPD.

Moreover, n- octanol addition does not improve nicotine recovery from hair. The worked out procedure can be also apply in nicotine replacement therapy(NRT).

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**Determination of lipophilicity of tritolyldiporphyrin derivatives using TLC**

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The worldwide laboratories are working to develop a new, effective methods for combat diseases, particularly cancer. One of the developing methods of the fight against cancer is Photodynamic Therapy, which is based on the use of photosensitizers, among other for this reason can be used the porphyrin rings.

One of the parameters, which allows to determine the biological activity of some compound is its lipophilicity. Lipophilicity can also be determined by TLC (*thin-layer chromatography*) on reversed phase, in addition to the traditional method of determining this parameter (which is the *shake-flask method*). In presented study, lipophilicity was determined for carboxyalkyl derivatives of tritolyldiporphyrin, which can potentially be used as the photosensitizers in Photodynamic Therapy.

### **Lipophilicity of new potential photodynamic therapy agents**

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Photodynamic therapy (PDT) is quickly developing method for the treatment of different kinds of cancer diseases based on possibility of light interaction with chemical compounds. However every medical application of new chemical compounds is faced with delivery problems into the organism. Very important feature of photosensitizer good for photodynamic therapy is its selectivity to pathological tissue. Attractive method for delivery and increasing selectivity seems to be the possibility of placing the photosensitizer into liposoms.

Lipophilicity is an important parameter which allows foreseeing of biological activity or the accumulation of drugs in the organism, especially their penetration into cell membranes. Thin layer chromatography as an alternative to the traditional *shake-flask* method is a quick and convenient way for determination of lipophilicity. In this work we assigned lipophilicity for series of new derivatives of tetra(hydroxyphenyl) porphyrins as potential agents in PDT with use of reversed phase thin layer chromatography.