INSTITUTE OF CHEMISTRY, UNIVERSITY OF SILESIA,
KATOWICE, POLAND

THE 39th SYMPOSIUM

CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS

JUNE 1st -3rd, 2016

KATOWICE – SZCZYRK

POLAND

PROGRAM

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TUESDAY, MAY 31st, 2016

1.00 pm LUNCH

2.30 – 4.30 pm WORKSHOP PART 1

Electrochemical simulation of selegiline metabolism

Appointed instructor: Przemysław Mielczarek

Department of Biochemistry and Neurobiology at the AGH University of Science and Technology in Krakow, Poland

1. Selegiline extraction from Segan medicine
2. Selection of basic electrolyte for metabolism simulation
3. Electrochemical oxidation of selegiline on glassy carbon electrode by means of ROXY™ system (Antec, The Netherlands)
4. Selection of optimal parameters for electrochemical oxidation
5. Sample preparation for *on-line* EC-MS and *off-line* EC/LC-MS/MS analyses

Presentation proposed by Shim-Pol - Paweł Stalica

4.30 -5.00 pm COFFEE BREAK

5.00 – 7.00 pm WORKSHOP PART II

UHPLC-MS/MS analysis of selegiline metabolites

Appointed instructor: Marek Smoluch
Department of Biochemistry and Neurobiology at the AGH University of Science and Technology in Krakow, Poland

1. Preparation of sample obtained in training 1 for LC-MS analysis
2. Reversed phase chromatography (UHPLC Nexera, Shimadzu) coupled to mass spectrometry detection (LCMS 8050, Shimadzu)
3. Discussion on possible modes of MS analyses (Full Scan, SIM, MRM, MS^n)
4. Obtained results analysis
5. Demonstration of a new, compact LC System from Shimadzu

7.15 pm DINNER

SESSION I WEDNESDAY, JUNE 1st, 2016

CHAIRPERSONS: Irena Vovk and Danilo Corradini

8.55 – 9.00 am OPENING ADDRESS

9.00 – 9.30 am

1. HPTLC and HPLC analysis of phytonutrients in food samples
Irena Vovk

9.30 – 10.00 am

2. Reversed phase HPLC of bioactive compounds in food matrices of plant origin: fundamental and practical aspects
Danilo Corradini

10.00 -10.30 am

3. Liquid chromatographic tandem mass spectrometric analysis of polyphenolic compounds in Italian spontaneous and cultivated berries: target and non-target approaches for their comparison and valorisation
Massimo Del Bubba

10.30 – 11.00 am COFFEE BREAK

11.00-11.30 am

4. The polyphenol-related EFSA health claim:
A meaningful parameter for olive oil's health-promoting potential?
Thomas Jakschitz

11.30-12.00 am

5. Planar chromatography and chemometrics in determination of food authenticity
Jelena Trifković

12.00-12.30 am

6. Effect of Liquid-Solid Extraction techniques on the yield of secondary metabolites from plant material
Monika Waksmundzka-Hajnos

1.00 pm LUNCH

SESSION II WEDNESDAY, JUNE 1st, 2016

CHAIRPERSONS: Danica Agbaba and Andjelija Malenović

2.30 – 3.00 pm

7. Investigation into the phenomena affecting the retention behavior of basic analytes in chaotropic chromatography
Andjelija Malenović

3.00 – 3.30 pm

8. Chemometric approaches for the analysis of genotoxic impurities in bulk drugs via LC-MS/MS
Yannis Dotsikas

3.30 – 4.00 pm

9. Quantitative Structure – Retention Relationship modeling in green liquid chromatographic separation of selected drugs
Ana Protić

4.00 – 4.30 pm

10. Chromatographic assessment of pharmaceutical dissolution profiles
Łukasz Komsta

POSTER SESSION I WEDNESDAY, JUNE 1st, 2016

CHAIRPERSONS: Anna Bodzoń-Kułakowska and Ana Protić

4.00 – 6.00 pm (COFFEE BREAK)

6.30 pm BONFIRE

SESSION III THURSDAY, JUNE 2nd, 2016

CHAIRPERSONS: Piotr Suder

9.00 – 9.40 am

11. Serum high-end biomarker analysis – lipid and peptide detection in Multiple Sclerosis and Complex Regional Pain Syndrome

Simone König

9.40 – 10.05 am

12. Designer drugs as the bane of modern times - non-targeted and targeted analysis of psychoactive substances in biological material

Agata Kot-Wasik

10.05 – 10.30 am

13. A metabolite profiling approach driven by automatic compound identification - Identification of detoxification mechanisms of plant secondary metabolites in insects

Aiko Barsch

10.30-11.00 am Coffee break

11.00 – 11.30 am

14. Analysis of chosen bioactive secondary metabolites synthesized by cyanobacteria and lichens

Beata Bober

11.30 – 12.00 am

15. An untargeted data analysis of multi capillary column - ion mobility spectrometry (MCC-IMS) dataset from a breathomics study

Ewa Szymańska

12.00 - 12.30 am

16. Mass spectrometry imaging (MSI) combined with thin-layer chromatography (TLC) as a technique for small molecules analysis

Anna Bodzon-Kułakowska

1.00 pm LUNCH

SESSION IV THURSDAY, JUNE 2nd, 2016

CHAIRPERSONS: Jerzy Silberring and Zbigniew Szewczuk

2.30 - 3.15 pm

17. Peptides labeled with cyclic quaternary ammonium salts for sensitive sequencing by electrospray tandem mass spectrometry
Zbigniew Szewczuk

3.15 – 3.35 pm

18. NGC Chromatography System – comprehensive solution for protein purification
Jerzy Jankowski - Bio-Rad

3.35 – 4.05 pm

19. Fingerprints to study indole alkaloids from *Psychotria nemorosa*: in extraction and fractionation optimization and indicating iteresting compounds
Yvan Vadner Hayden

4.05 – 4.35 pm

20. Determination of ethanol as a residual organic solvent in pharmaceutical preparation of human albumin using head space-

gas chromatography-flame ionization detection (HS-GC-FID) in drug quality control laboratories
Moustafa Khalifa

4.35 – 5.05 pm

21. Enantioseparations using immobilized polysaccharide-based chiral stationary phases in supercritical fluid chromatography
Debby Mangelings

5.05 – 5.25 pm

22. A novel label-free and universal detector for liquid chromatography systems using millimeter-wave technology
Yuchen Zhang

POSTER SESSION II THURSDAY, JUNE 2nd, 2016

CHAIRPERSONS: Jelena Trifković and Agata Kot-Wasik

4.30 – 6.30 pm (COFFEE BREAK)

7.00 pm DINNER

SESSION V FRIDAY, JUNE 3rd, 2016

CHAIRPERSONS: Łukasz Komsta and Robert Skibiński

9.00 – 9.30 am

23. Hyphenation of liquid chromatography and bioautography methods for analysis of antibacterial compounds in plants

Irena Choma

9.30 – 10.00 am

24. Open screening method for analysis of biological material on the presence of medicinal drugs that have severe influence on fitness to drive

Bogdan Tokarczyk

10.00 – 10.30 am

25. The chromatographic analysis of head and neck cartilage biocompatibility

Andrzej Swinarew

10.30-11.00 am

26. Hybrid approach combining chemometrics and likelihood ratio for evaluation of chromatograms for forensic purposes

Agnieszka Martyna

11.00-11.20 am

27. New psychoactive substances (NPS) contained in „designer drugs" – chromatographic and spectroscopic analysis
Milena Majchrzak

11.20-11.40 am

28. Biopurification of air from VOCs mixture - optimization of biodegradation process
Paulina Dybał

11.40 – 12.00 am

29. Comparison of composition of the volatile fraction in commercial samples of *Cistus incanus* L
Dariusz Szeremeta

12.00 am CLOSING REMARKS

12.15 am LUNCH

POSTER SESSION I

1.

Pesticide residues in surface and drainage waters of agriculture intensive area at El-Behira province, Egypt

Moustafa A. Abbassy, Mamdouh A. Marzouk, Moustafa A. Khalifa, Omar. A. Omar, Eman. Noureldin

2.

Synthesis of deuterium labeled denatonium cation and its application in the quantitative analysis of Bitrex® by liquid chromatography-mass spectrometry

Remigiusz Bąchor, Alicja Kluczyk, Piotr Stefanowicz, Zbigniew Szewczuk

3.

Multi-dimensional (3D/4D-QSAR) probability-guided pharmacophore mapping: Investigation of activity profile for series of drug absorption promoters

Andrzej Bak, Violetta Kozik, Adam Smolinski, Joseph Jampilek

4.

Present state and future perspectives of using countercurrent chromatography (CCC) in phytochemical research

Ewa Długosz

5.

Application of TLC-densitometry in pharmaceutical analysis of finasteride

Małgorzata Dołowy, Alina Pyka-Pająk, Agata Piontek

6.

TLC-densitometric analysis of clobetasol propionate in pharmaceutical preparation

Małgorzata Dołowy, Alina Pyka-Pająk, Katarzyna Pala

7.

LC-MS, GC-MS and NMR based untargeted metabolomics in searching for urine biomarkers of bladder cancer

Julia Jacyna, Renata Bujak, Stéphane Balayssac, Aleksandra Sawicka, Małgorzata Patejko, Marcin Markuszewski, Myriam

Malet-Martino, Marcin Matuszewski, Roman Kaliszan, Michał J. Markuszewski

8.

The hydrogen-deuterium exchange in betaine moiety does not affect chromatographic behaviour of peptides labeled with quaternary ammonium derivatives

Alicja Kluczyk , Remigiusz Bąchor, Bartosz Setner, Piotr Stefanowicz, Zbigniew Szewczuk

9.

Untargeted metabolomics with the use of LC-TOF/MS and GC-MS for selection of tentative markers of renal cell carcinoma

Marta Kordalewska, Renata Bujak, Karolina Siedlecka, Wiktoria Struck-Lewicka, Arlette Yumba Mpanga, Marcin Markuszewski, Marcin Matuszewski, Roman Kaliszan, Michał J. Markuszewski

10.

Determination of antioxidant properties of selected cruciferous plants

Violetta Kozik, Andrzej Bąk, Krystyna Jarzembek, Marcin Rojkiewicz, Katarzyna Pytlakowska, Aleksandra Łangiewka, Patrycja Glenc, Marta Skorek

11.

Corrosion in continuous bio-degradation of sulfur-containing, volatile organic compounds

Violetta Kozik, Andrzej Bąk, Paulina Dybał, Krystyna Jarzembek, Slawomir Kuś, Russell Kane, Damian Kasperczyk, Krzysztof Barbusinski

12.

Synthesis and physico-chemical properties of derivatives of graphene oxide as potential drug carriers

Violetta Kozik, Andrzej Bąk, Brygida Baboń, Katarzyna Pytlakowska, Barbara Hachuła, Marcin Rojkiewicz, Wojciech Pisarski

13.

Lipophilicity of isomeric N-methylquinolinesulfonamides

Maria Maślankiewic, Danuta Kwapulińska, K. Marciniec

14.

Application of UHPLC-PDA-ESI-MS^n for phytochemical profiling of antioxidant active extracts from selected *Sorbus* species

Aleksandra Owczarek, Magdalena Matczak, Monika Anna Olszewska

15.

Thin-layer chromatographic identification of phenolic acids in cosmetic raw materials

Marta Skorek, K. Jurczyk, Mieczysław Sajewicz, Teresa Kowalska

16.

Functuonalization of quinolone derivatives by Reimer-Tiemann reaction: the GC-MS and TLC investigation

Marcin Szala

17.

Chemometric analysis of some histamine H_1- and H_2-receptor antagonists on the basis of chromatographic data and molecular descriptors

Grażyna Żydek, Krzysztof Baj, Elżbieta Brzezińska

18.

Chemomeric analysis of some dopamine receptor agonists and antagonists on the basis of chromatographic data and molecular descriptors

Grażyna Żydek, Magdalena Strąkowska, Elżbieta Brzezińska

19.

Determination of acrylamide in real samples

Agnieszka Psiuk, Michał Daszykowski

POSTER SESSION II

1.

Speciation and determination of trace and ultratrace amount of arsenic and selenium on the graphene oxide decorated with cerium oxide

Anna Baranik, Beata Zawisza, Anna Gągor, Rafał Sitko

2.

Novel insights into pH-dependent retention behavior of analytes in chaotropic chromatography

Jelena Čolović, Marko Kalinić, Ana Vemić, Slavica Erić, Anđelija Malenović

3.

A comparative study of chromatographic behaviour and lipophilicity of selected imidazoline derivatives

Slavica Filipic, Aleksandra Antic, Milena Vujovic, Katarina Nikolic, Danica Agbaba

4.

Determination of microbiological activity of hop extracts from different varieties of *Humulus lupulus* by TLC + direct bioautobiographic method

Grzegorz Jóźwiak, Barbara Majer-Dziedzic, Justyna Kwiecińska, Monika Waksmundzka-Hajnos

5.

Application of TLC, HPLC and GLC in the Analysis of Metronidazole and Secnidazole

Lina Yu Klimenko, Galyna L. Shkarlat, Oksana V. Shovkova, Zoia V. Shovkova

6.

Studying the changes of excise duty components in diesel oil samples under influence of a reducing agent using gas chromatography with nitrogen chemiluminescence detector

Beata Krakowska, Joanna Orzeł, Ivana Stanimirova, M. Sznajder, M. Zaleszczyk, I. Grabowski, Michał Daszykowski

7.

Investigation of micro- and nanostructures spontaneously formed in monocomponent proteinogenic amino acid solutions

Anna Maciejowska, Agnieszka Godziek, Ewa Talik, Mieczysław Sajewicz, Teresa Kowalska

8.

The ways of detecting counterfeit plant protection products

Patrycja Marczewska

9.

Fanal poisoning of 3-MMC

Joanna Margasińska, D. Wiechuła, Milena Majchrzak, Marcin Rojkiewicz, Jadwiga Nędza, Rafał Celiński

10.

Determination of caffeine content in fat-burning supplements

Jadwiga Nędza, Renata Polaniak, Joanna Margasińska, Rafał Celiński, Elżbieta Grochowska-Niedworok

11.

Determination of ziprasidone and its impurities by thin-layer chromatography

Darija Obradović, Slavica Filipic, Katarina Nikolic, Marija Čarapić, Danica Agbaba

12.

Chromatographic methods for qualitative evaluation of oxidative stress markers in urine samples

Joanna Orzel, Michal Daszykowski

13.

Thermal stability of silica based stationary phases for liquid chromatography by vibrational spectroscopy techniques

Wojciech Prus, Mateusz Dulski, Dorota Biniaś, Roman Wrzalik

14.

RP-18 thin layer chromatographic study of selected biological and physicochemical properties of substances

Anna W. Sobańska, Monika Korzela, Elżbieta Brzezińska

15.

RP-18 thin layer chromatographic investigations of lipophilicity of selected steroid hormones

Anna W. Sobańska, Elżbieta Brzezińska

16.

Chromatographic investigations of plasma protein binding of selected drugs

Anna W. Sobańska , Elżbieta Brzezińska

17.

The development of the conditions for thin-layer chromatographic separation and the detection of bioactive flavonols and depsides in plants

Helena D. Smolarz, Katarzyna Szewczyk, Magdalena Czechowicz

18.

Finding a set of orthogonal solvents for effective plant extraction by chemometric analysis of chromatograms

Katarzyna Szewczyk, Łukasz Komsta, Agnieszka Skalska-Kamińska, Anna Gumieniczek, Ryszard Kocjan

19.

Photocatalytic degradation study of tiapride by ESI-LC-MS method

Jakub Trawiński, Robert Skibiński

SESSION I

WEDNESDAY, JUNE 1st, 2016

CHAIRPERSONS: Irena Vovk

and Danilo Corradini

1.

HPTLC and HPLC analysis of phytonutrients in food samples

Irena Vovk, Vesna Glavnik, Alen Albreht, Breda Simonovska, Eva Kranjc, Katerina Naumoska

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

Major groups of phytonutrients are polyphenols (e.g. flavonoids, phenolic acids), isothiocyanates, carotenoids, triterpenoids, phytosterols etc. This is a big group of compounds with different bioactivities, like antioxidant activity, enhancement of immune response or cell-to-cell communication, lowering blood pressure and/or cholesterol level, causing death of cancer cells, etc. Some phytonutrients are investigated because of their interaction with medicines. However, many phytonutrients are still not known to be present even in some of the most popular unprocessed food (e.g. fruits or vegetables), which we consume on a daily basis. Therefore, new analytical methods are needed to control food quality and safety and to provide more information about food composition, as well as to gain knowledge about new possible ingredients for functional food and food supplement products [1, 2]. Chromatographic techniques and especially their combined use and hyphenation to mass spectrometry (MS) and UV/vis spectrophotometry are indispensable tool in analysis and discovery of new of phytonutrients. Development of the methods for screening, qualitative and quantitative analysis of phytonutrients in different matrices is a base for the investigation of the composition of different foods, especially regarding the minor constituents. We will present the potential of the methods based on high performance thin-layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) in qualitative and quantitative analysis of selected phytonutrients (e.g. flavonoids, carotenoids, triterpenoids, phytosterols) in food samples including food supplements. The examples will show targeted and non-targeted analyses using different stationary phases (silica gel, RP C18, diol, cellulose) and detection techniques (HPTLC-image analysis, HPTLC-densitometry HPTLC-MS, HPLC-UV/Vis, LC-MS, LC-MS^n).

References:

[1] I. Vovk, V. Glavnik. Analysis of dietary supplements. In: C.F. Poole (Ed.): Instrumental thin-layer chromatography. Elsevier, Amsterdam, 2015, pp. 589-635.

[2] I. Vovk, A. Albreht. TLC-MS analysis of carotenoids, triterpenoids, and flavanols in plant extracts and dietary supplements. In: T. Kowalska, M. Sajewicz, J. Sherma, Joseph (Eds.): Planar chromatography - mass spectrometry, (Chromatographic science series, vol. 110). Boca Raton: CRC, Tayor & Francis Group, 2016, pp. 305-325.

2.

Reversed phase HPLC of bioactive compounds in food matrices of plant origin: fundamental and practical aspects

Danilo Corradini[1], Laura De Gara[2], Isabella Nicoletti[1], Francesca Orsini[2]

[1]National Research Council, Institute of Chemical Methodologies, Area della Ricerca di Roma 1, I-00015 Montelibretti, Rome, Italy; [2]University Campus Bio-Medico of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy; danilo.corradini@cnr.it

Reversed phase high performance liquid chromatography (RP-HPLC) is widely employed for the identification and quantification of biomolecules in edible plants and food matrices of plant origin. Most of these compounds are secondary metabolites produced within the plants besides the primary biosynthetic and metabolic routes. They hold various types of important functions in plant tissues, such as protection, attraction or signalling, and most of them, occurring as "non-nutritive" compounds in plant food, have found to play important roles in disease prevention and health-promoting effects. This communication discusses the results of our recent studies carried out to investigate a variety of factors that influence the chromatographic behavior of plant secondary metabolites, mainly phenolic compounds, with the purpose of developing novel RP-HPLC methods for the identification and quantification of bioactive compounds in plant extracts and foodstuff. We have investigated the dependence of retention behavior of a variety of biomolecules in RP-HPLC on the experimental parameters, such as flow rate, column length and internal diameter, dwell volume, temperature, isocratic and gradient elution mode, variation of the organic solvent concentration in gradient elution mode (gradient shape and duration). The influence of the considered parameters on the chromatographic behavior of the selected compounds has been evaluated in the framework of solvophobic theory [1], using a chromatographic modeling software that allows the development of RP-HPLC methods according to a Quality by Design (QbD) criteria, with the result of decreasing the number of experiments requested for method development and increasing flexibility in routine operations. Practical applications of the investigated approaches to the analysis of biomolecules in samples extracted from edible plants and processed food are then discussed.

References:

[1] Cs. Horvàth, W. Melander, I. Molnar, J. Chromatogr. *125* (1976) 129 - 156.

3.

Liquid chromatographic tandem mass spectrometric analysis of polyphenolic compounds in Italian spontaneous and cultivated berries: target and non-target approaches for their comparison and valorisation

Massimo Del Bubba, Claudia Ancillotti, Lorenzo Ciofi, Sandra Furlanetto
Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy; delbubba@unifi.it

Berries of different *Vaccinium* species are widely considered important sources of polyphenolic compounds, especially anthocyanidin, in the human diet, thus providing interesting health-protecting attributes [1]. In fact, these compounds are well-known for their anti-inflammatory, antihypertensive, anti-microbial and anti-cancer properties [2].

Among *Vaccinium* berries, *V. myrtillus* (i.e the bilberry) is a wild species native to mountain areas of Northern and Central Europe, widely diffused also in Italian Alps and Apennines. In these zones the increasing presence of a different *Vaccinium* species, namely *V. uliginosum* subsp. *gaultherioides* (locally named "false bilberry") has been recently observed. The cultivation of *V. corymbosum* berries (i.e. the blueberry) is also widespread in the same area. Many studies focusing on the determination of selected anthocyanins and less frequently of other phenolic compounds, were carried out on bilberries from different European areas, as well as on various blueberry cultivars [3,4]. Nevertheless, no comprehensive investigation of the polyphenolic profiles of these *Vaccinium* species has been published to date, whereas for "false bilberry" the first information concerning its polyphenolic composition has been recently obtained by our team [5]. In this lecture the results of an in-depth comparison of the polyphenolic metabolomes of the three aforementioned *Vaccinium* species are presented. Data were obtained by coupling liquid chromatography with advanced quadrupole-linear trap-quadrupole, triple quadrupole and quadrupole-time of flight mass analysers, and were used in a Principal Component Analysis, achieving the clear separation of object scores in the principal component cartesian plane. Among the most interesting results, a general prevalence of anthocyanins in bilberry than in "false bilberry" and blueberry was highlighted, with the exceptions of (i) malvidin-3-glucoside and xyloside derivatives, and (ii) acylated anthocyanins, respectively.

References:

[1] O. Paredes-López, M. L. Cervantes-Ceja., M. Vigna-Pérez, and T. Hernández-Pérez Plant Food Hum. Nutr. 65 (2010) 299-308.
[2] M. Daglia Curr. Opin. Biotech. 23 (2012) 174-181
[3] S. Može, T. Polak, L. Gašperlin, D. Koron, A. Vanzo, N. P. Ulrih, V. Abram J. Agric. Food Chem. 59 (2011) 6998-7004
[4] L. Zoratti, L. Jaakola, H. Haggman, L. Giongo J. Agric. Food Chem. 63 (2015) 8641-8650.
[5] C. Ancillotti, L. Ciofi, D. Pucci, E. Sagona, E. Giordani, S. Biricolti, M. Gori, W.A. Petrucci, F. Giardi, R. Bartoletti, U. Chiuminatto, S. Orlandini, S. Mosti, M. Del Bubba Food Chem. 204 (2016) 176–184.

4.

The polyphenol-related EFSA health claim: A meaningful parameter for olive oil's health-promoting potential?

T.A.E. Jakschitz[1], M. Fischnaller[1], O.M.D. Lutz [1], G.K. Bonn[1,2], L. Skaltsounis[3], D. Corradini[4]

[1]*ADSI - Austrian Drug Screening Institute, Innrain 66a, 6020 Innsbruck, Austria*
[2]*Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria*
[3]*Laboratory of Pharmacognosy and Natural Products Chemistry, University of Athens, 15771 Athens, Greece*
[4]*Institute of Chemical Methodologies, National Research Council, Area della Ricerca di Roma 1, I-00015 Montelibretti, Rome, Italy*
e-mail: Thomas.Jakschitz@adsi.ac.at

In 2012, the European Food Safety Authority (EFSA) approved the health claim 432/2012, attesting olive oil polyphenols a protective effect on blood lipids from oxidative stress. A beneficial effect is obtained upon a daily intake of 20 g of olive oil, containing at least 5 mg of "hydroxytyrosol and its derivatives". However, considering the structural diversity and the encompassing mass range of hydroxytyrosol's derivatives (i.e. oleocanthal, oleuropein, ligstroside, etc.), EFSA's health claim is valid for the majority of extra virgin olive oils.

In order to prove the content of hydroxytyrosol and its derivatives in olive oil a methodology for extraction, hydrolysis and quantitative determination by means of LC-UV has been developed, optimized and tested in different laboratories.

Many polyphenols are esters that are easily hydrolyzed in the human's stomach and duodenum, yielding either hydroxytyrosol or tyrosol, which are resorbed *via* the gastro-intenstinal tract's (GIT) wall. Utilizing a parallel artificial membrane permeability assay (PAMPA), we estimated the pH-dependent GIT permeability of such health benefiting substances. Comparison is drawn between commercially available standard substances (*e.g.* tyrosol and hydroxytyrosol) and polyphenols extracted from olive oils hydrolyzed *in vitro* by gastric acid and digestion enzymes.

The results of these experiments should give a hint whether the health-promoting potential of an olive oil is best represented by a sum parameter or by direct quantification of the two most basic structural compounds tyrosol and hydroxytyrosol.

5.

Planar chromatography and chemometrics in determination of food authenticity

Jelena Trifković[1], Irena Vovk[2], Dušanka Milojković-Opsenica[1], Živoslav Tešić[1]

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[2]*Laboratory for Food Chemistry, National Institute of Chemistry, Hajdrihova 19, SI-1000, Ljubljana, Slovenia*
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Natural products extracts are complex mixtures that contain vast number of compounds. The chromatographic profile is usually taken into consideration for an assay of authenticity and quality of food. Fingerprint analysis of food samples could be defined as a set of characteristic chromatographic signals, which comparison leads to sample recognition. The entire chromatogram is treated as unique multivariate fingerprint, i.e., multidimensional vector, without special identification of single peaks. Recently, high-performance thin-layer chromatography (HPTLC) become a method of choice for such kind of studies due to the development of novel microstructured and nano monolithic stationary phases and powerful scanning and image capturing and processing devices and algorithms. It is often used as an alternative to high-performance liquid chromatography (HPLC).

Development of efficient and reliable fingerprint HPTLC method requires chemometric approach at several levels starting with application of experimental design and optimization techniques for the separation step, followed by data acquisition, and signal manipulation, and finally solving classification and modeling problem. Based on the similarity/dissimilarity analysis or correlation matrix, a number of unsupervised and supervised chemometric methods could be performed. Selection of particular chemometric technique depends on its features and the nature of a problem to be solved.

However, application of multivariate image analysis and chemometric tools for image processing and classification in thin-layer chromatography is still very poor, and in most instances, fingerprint analysis is conducted in subjective manner based on manually noted peak differences.

6.

Effect of Liquid-Solid Extraction techniques on the yield of secondary metabolites from plant material

Monika Waksmundzka-Hajnos and Anna Oniszczuk

Department of Inorganic Chemistry, Medical University of Lublin, 20-093 Lublin, Poland

The extraction of phenolic compounds from plant materials compounds may be performed by traditional or by modern methods (such as: accelerated solvent extraction (ASE), ultrasound assisted extraction (USAE), microwave assisted solvent extraction (MASE) etc. The novel methods require shorter extraction time, use of low amount of solvents, allow for simultaneous parallel processing of several samples, and are automatic, but are more expensive.

The objective of this work was comparison of different extraction techniques developed for isolation of selected polyphenols from various plant materials such as: *Sambucus nigra* L. inflorescence, *Polygonum avicularae herb*, *Equisetum arvense* L. herb, *Tilia cordata* inflorescence etc.

The highest extraction yields of phenolic compounds from *Sambucus nigra* inflorescence were obtained by use of Soxhlet extraction. Ultrasonification, microwave-assisted solvent extraction, and accelerated solvent extraction result in similar extraction yield of phenolic acids. The highest extraction yields of the compounds from *Polygonum aviculare* herb were obtained by use of microwave-assisted extraction in a closed system. For extraction of ferulic acid from *P. aviculare* herb the highest yield was obtained by use of USAE, while highest extraction yields of rutin and isoquercetrin were obtained by use of ASE.

One can attempt to explain the different yields of the methods used for extraction of phenolic acids from the different plant materials. The differences might be caused by the distinct cellular structures of flowers (for *S. nigra*) and foliage (stem and leaves, for *P. aviculare*) and/or the different dimensions of the cells. Stems are largely composed of hard, mechanically resistant tissue, for example colenchyma, sclerenchyma, and elements of wood whereas leaves and flowers comprise mainly delicate parenchyma cells with large intercellular spaces. It is probable that destruction of the compact, hard structures of *Polygonum aviculare* stems and diffusion of solvent into this material requires more drastic extraction conditions. Phenolic compounds can, moreover, form strong bonds with lignin, a component of the cell walls of stems and leaves. Such lignin complexes are difficult to break down, and then classic extraction methods are less efficient. In such circumstances techniques which destroy the cell structure – USAE, ASE and MASE – result in higher yields of the phenolic compounds.

SESSION II

WEDNESDAY, JUNE 1st, 2016

CHAIRPERSONS: Danica Agbaba and Andjelija Malenović

7.

Investigation into the phenomena affecting the retention behavior of basic analytes in chaotropic chromatography

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Due to the complex mechanism underlying analytes' retention in ion-interaction chromatography with chaotropic additives, a lot of effort has been put into understanding of those systems from the various aspects. To that aim, many authors were studying and describing the influence of chaotropic ions' and organic modifiers' type and concentration, mobile phase ionic strength and stationary phase hydrophobicity on analytes' chromatographic behavior. Nevertheless, the effect of the mobile phase pH had been overlooked and studied only as factor affecting analyte's ionization. Consequently, we have studied the phenomena affecting the retention behavior of 34 structurally diverse basic drugs by systematic variation of aqueous phase pH value, sodium hexafluorophosphate concentration, and acetonitrile content in the mobile phase. Increasing pH from 2 to 4 led to longer retention times, even with analytes which remain completely protonated. An explanation for this phenomenon was sought by studying the adsorption behavior of acetonitrile and chaotropic additive onto stationary phase. It was shown that the pH value variation in the studied range led to larger surface excess of acetonitrile and consequentially enhanced adsorption of chaotropic agent, which significantly contribute to magnitude of surface potential and further longer retention times of oppositely charged analytes.

To study how analytes' structural properties influence their retention, quantitative structure-retention modelling was performed next. Chromatographic parameters and values of analytes' structural descriptors were correlated to retention data using a "mixed modelling" approach and support vector regression (SVM). The established model exhibited good predictive capabilities, incorporating three mobile phase and four molecular descriptors. While the ETA_EtaP_B_RC and XlogP can be considered as molecular descriptors which describe factors affecting retention in any RP-HPLC system, TDB9p and RDF45p are molecular descriptors which account for spatial arrangement of polarizable atoms and they can clearly relate to analytes' behavior on the stationary phase surface, where the electrostatic potential develops. Analysis of molecular descriptors forming the model provides further support to our earlier observations that the complementarity between the electronic structure of the analyte and the electric double layer developed on the stationary phase surface – represents a key structural determinant of retention in chaotropic chromatographic system.

Furthermore, we thought this issue should also be addressed with regard to the extended thermodynamic retention model that provides the most extensive consideration of the mechanisms underlying the separation in ion-interaction chromatography − contribution of the double layer formation and its electrostatic influence on the analytes retention, as well as the ion-pair formation between the chaotropic agent and the analyte. Therefore we have

developed comprehensive and readily applicable empirical retention model that includes the magnitude of surface potential changing with mobile phase pH, and the most important molecular descriptors affecting the interaction with electric double layer, as well as the ion-pair formation both in the stationary and mobile phase.

8.

Chemometric approaches for the analysis of genotoxic impurities in bulk drugs via LC-MS/MS

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Trace level genotoxic impurities (GTIs) in pharmaceutical products or bulk drugs require sensitive analytical methodologies for their analysis. The need to control most genotoxic impurities to the low ppm level presents significant analytical challenges. Such low levels usually require application of mass spectrometer (MS) detectors, which are characterized by their inherent sensitivity and remarkable selectivity. Therefore, chromatographic separation, unless needed, is not generally a requirement. However, two major conditions should be fulfilled when utilizing LC-MS methods: compatible mobile phases and avoidance of injecting extremely high concentrations of analytes. The latter constitutes a serious issue for the case of GTIs, since very high levels of the active pharmaceutical ingredient (API) should be utilized in order to obtain detectable levels of GTIs. Such condition renders efficient chromatographic separation between API and GTIs so that to direct API to waste and GTIs to MS detector via a switch valve. To this purpose application of experimental design combined with grid-point search optimization or experimental design - quality by design (QbD) approach may be used to provide suitable chromatographic conditions that can assure GTIs analysis along with MS protection. The aforementioned principles were applied to the detection of GTIs in the case of bulk meropenem and rabeprazole. Initial experiments with UV detectors were conducted helping reach efficient chromatographic separation between meropenem and its GTIs (S5, M9, 318-BP) and rabeprazole and its GTIs (CPAR, FBCI), respectively, utilizing compatible-to-MS LC conditions. Optimal conditions obtained via experimental design were transferred to LC-MS instrumentation for the validation of the final LC-MS/MS methods according to international guidelines.

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9.

Quantitative Structure – Retention Relationship modeling in green liquid chromatographic separation of selected drugs

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Liquid chromatography is generally considered not a green analytical technique as it requires organic toxic solvents for the separation. On the other side, this offers more possibilities for "greening". One of the strategies for greening liquid chromatography is the search for "green" components of the mobile phase. Acetonitrile and methanol are the most popular solvents employed in analytical HPLC, but they suffer from a number of drawbacks from the environmental point of view. The use of different types of cyclodextrin (CD) as mobile phase additives allowed us to increase the proportion of water in the mobile phases without loss in the resolution or efficiency of the separations, leading initially to a considerable reduction of the proportion of methanol and acetonitrile in the mobile phase.

A detailed knowledge of the stability and structure of the inclusion complexes between CDs and drug molecule is necessary to obtain a more detailed picture of its influence on the chromatographic process. Usually, the more lipophilic part of the molecule, which is the one responsible for the interaction with the stationary phases, is inside the CD cavity. Therefore, diminished retention times can be expected in the presence of CDs, even in mobile phases containing high proportions of water. The association constants determine drug-CD complex stability and along with concentration of CDs and other chromatographic parameters, influence retention behavior of the drugs.

This presentation is intended to give proposal how to build a *Quantitative Structure – Retention Relationship* **(**QSRR) models with a good predictive ability using molecular descriptors, drug-CD complex association constants and chromatographic parameters. The structural descriptors are usually derived by computational chemistry methods for the energy-optimized conformations and association constants could be derived from *Docking* studies. Only uncorrelated descriptors are selected to produce a QSRR equation. When CD-s is used as mobile phase additive, the driven force for separative displacement of analytes is the equilibrium between the stationary phase, the mobile phase and CD-s. As this is the new field of interest, some questions are to be answered in order to define the best choice of parameters to describe the drug-CD complex retention mechanisms in chromatographic system.

10.

Chromatographic assessment of pharmaceutical dissolution profiles

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The liberation process of a drug is the first step of its action after administration and it is (together with adsorption) the main factor responsible for bioavailability of the drug formulation. The liberation depends on the solubility of the active pharmaceutical substance and on the formulation itself (excipients, drug carriers, controlled release techniques etc.).

Analysis of this process requires registering concentration of liberated drug as a function of the time under specific laboratory conditions. The data obtained in this way is called a dissolution profile and the analysis is most frequently done chromatographically.

The equipment used to test dissolution is designed to simulate conditions inside the human stomach and it is currently normalized worldwide (the requirements of most pharmacopoeias are almost the same).

The apparatus can be based on basket, rotating paddle, reciprocating cylinder and flow-through cell, however paddle equipment is used most frequently. There is also a visible trend to normalize other requirements and considerations of testing procedure.

The obtained dissolution profile can be compared with a reference profile by various methods. A simple statistical analysis can be done with t-test and ANOVA test. More advanced model-independent methods are similarity coefficients and indices.

Finally, the profiles can be fitted to some model explaining the release process (zero order, first order, Hixson-Crowell, Higuchi, Baker-Londsale etc.) and the comparison can be done between the obtained parameters and their confidence intervals.

The presentation is intended to give a concise summary of current requirements and trends in dissolution profile testing for pharmacists and chromatoghraphers who are not familiar with pharmaceutical analysis.

SESSION III

THURSDAY, JUNE 2nd, 2016

CHAIRPERSONS: Piotr Suder

11.

Serum high-end biomarker analysis –
lipid and peptide detection in Multiple Sclerosis and Complex Regional Pain Syndrome

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Complex Regional Pain Syndrome (CRPS) is a severe and often disabling syndrome, which develops after trauma in ~5% of all cases; most often after distal radius fractures. CRPS is characterized by several clinical features including spontaneous pain and hyperalgesia. Increased neuropeptide release from peripheral nociceptors has been suggested as a possible pathophysiological mechanism triggering the symptoms. Resolvins, on the other hand, are thought to have a potent anti-hyperalgesic effect in inflammatory pain. These lipids are endogenous anti-inflammatory mediators generated during the resolution phase of acute inflammation. Another fatty acid, conjugated linoleic acid (CLA) and/or its metabolites, ameliorated autoimmune inflammation in several models of autoimmunity. In Multiple Sclerosis, immune alterations are believed to reflect an intrinsic immune dysbalance due to impaired immune-regulatory functions on the one hand and augmented proinflammatory effector cell responses on the other hand. We have employed liquid chromatography (LC)-tandem mass spectrometry methods using ion trap and Q-TOF technology to analyze both peptides and lipids in this context. In particular Ionkey (chip-based LC)-MS/MS on Synapt G2 Si proved to be very useful for the purpose.

12.

Designer drugs as the bane of modern times - non-targeted and targeted analysis of psychoactive substances in biological material

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Boosters are designed drugs of hallucinogenic, narcotic or psychoactive action. Although they appeared in the European market in the 90s, in Poland they are still gaining popularity. The emergence and presence on the market of a large number of new drugs means that analytical laboratories are working on the development of qualitative and quantitative methods applied for their analysis. Case studies will be described based on the analysis of biological materials (blood, urine, gastric content) taken from patients after ingestion of a substance from the group "NBOMe".

Identification of boosters in the material was done using high-resolution MS. Selection of precursor ion and fitting by the elemental composition (molecular formula) to a specific monoisotopic mass with an accuracy of 5 ppm has been done; usage of database to predict the likely structure of the compounds was performed. Due to the fact that by searching of compounds in selected databases (eg. ChemSpider, PubChem, Metlin) during assignment of possible structures fitted to the measured accurate mass many results (sometimes several thousands!) could be obtained, the fragmentation of ions in the auto MS/MS mode and targeted MS/MS mode was also performed. This approach resulted in limitation of the number of hits up to several times. Modification of the sample preparation stage, reduction of the MS signal suppression and the use of quantitative analysis based on weighted calibration curve with deuterated internal standard were finally evaluated. The resulting booster levels in serum varied at several tens ng/ml.

The following chemical and toxicological measurements for the routine analysis of biological material were done: immunochemistry, liquid-liquid extraction, gas chromatography with flame ionization detection (HS-GC-FID) and mass spectrometry (GC-EI-MS). Ethanol traces in urine and the presence of 11-nor-Δ9-tetrahydrokannabinolo-9-carboxylic acid metabolite in blood and urine samples were observed. The chromatographic analyses revealed the presence of new psychoactive substances in blood samples: 25B-NBOMe at the level of several tens ng/mL and 4-CMC of a few ng/mL. The high level of 25B-NBOMe in the stomach and the presence of metabolites of THC-COOH in the blood and urine samples confirmed poisoning by new boosters. Risk of acute poisoning 25C-NBOME is explained by the possibility of an overdose of very low active dose (on the order of several hundred micrograms).

13.

A metabolite profiling approach driven by automatic compound identification - Identification of detoxification mechanisms of plant secondary metabolites in insects

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Link to Homepage: https://www.bruker.com/applications/life-sciences/metabolomics.html

Exploring the diversity of natural products in plants requires efficient methods to gain sufficient structural information to rapidly discriminate known compounds from novel or closely related ones. This process can be rendered extremely challenging when analyzing profiles of genetically-manipulated plants in which natural product biosynthetic pathways are manipulated. Especially on further trophic levels, like herbivores feeding on those plants, the consequences of these manipulations are difficult to grasp. Efficient workflows which combine statistical data mining and automatic compound identification routines are therefore needed. Here, we present a software solution for a quick and efficient metabolite profile screening to unravel the function of wild tobacco plants in which the expression of several glycosyltransferase genes has been manipulated. These genes are part of the biosynthetic pathway leading to defensive 17-hydroxygeranyllinallool diterpene glycosides (HGL-DTGs). Furthermore we analyze the next trophic level, by profiling the metabolites and their changes within the tobacco hornworm *Manduca sexta*. We illustrate the power of this software based workflow for the discovery of gene-mediated glycosylations in the HGL-DTG pathway in tobacco and their consequences in the herbivore *M. sexta*.

14.

Analysis of chosen bioactive secondary metabolites synthesized by cyanobacteria and lichens

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www.wbbib.uj.edu.pl/zaklad-fizjologii-i-biologii-rozwoju-roslin1

Cyanobacteria and lichens occurring worldwide are well known to produce a wide range of secondary metabolites with a high structural diversity. Cyanobacteria produce and may release into the aqueous environment compounds affecting taste and odour of water (e.g. geosmin), toxins (e.g. neurotoxic or cytotoxic alkaloids, hepatotoxic cyclic peptides, lipopolysaccharides) as well as linear, cyclic or multi-cyclic oligopeptides (e.g. microginins, cyanopeptolins). Although the main efforts are concentrated on monitoring of the toxins appearance, numerous cyanobacterial compounds exhibit a specific enzyme inhibition, antimicrobial, immunosuppressive and even antitumor activity and are considered as a promising source for pharmacology and industry. Great potential of lichens as producers of many bioactive metabolites such as UV-absorbing mycosporine-like amino acids is still poorly investigated. Despite the numerous analytical methods have been developed for characterization of cyanobacterial and lichens secondary metabolites profiles, the main problems are the complex matrices and low availability of standards. The aim of the presentation is to provide a brief overview of analytical procedures as well as a summarization of possibilities and limitations of instrumental analytical techniques typically employed for determination of these compounds. The examples will include analysis of cyanobacterial alkaloids, linear and cyclic oligopeptides as well as lichen mycosporine-like amino acids. The high biological activity of these secondary metabolites opens up new perspectives of their practical application.

15.

An untargeted data analysis of multi capillary column - ion mobility spectrometry (MCC-IMS) dataset from a breathomics study

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Ion mobility spectrometry combined with multicapillary column separation (MCC-IMS) is a well-known technology for detecting volatile organic compounds (VOCs) in gaseous samples. Recently, a new untargeted data size reduction strategy for analysis of MCC-IMS data was developed [1]. It comprises of wavelet transform, mask construction and a sparse discriminant analysis. It allows to reduce MCC-IMS data size from 500 000 to 50 variables relevant to the goal of analysis.

In this study, the untargeted data size reduction strategy is applied to a large breathomics dataset. The goal of the study is evaluate the effects of various respiratory diseases on breath metabolic profile. Breathomics dataset includes MCC-IMS spectra of 193 breath samples from 107 patients with various respiratory diseases (including asthma, lung cancer, COPD) and 86 controls, collected at four different sites e.g. hospitals.

A significant effect of respiratory diseases on breath profiles is revealed at two stages of an untargeted data analysis strategy: in masks of different sample classes and in results of sparse-PLS-DA models [2]. Nevertheless, a significant effect from sample location is also found. Finally, different chemometric approaches are considered to remove the influence of sample collection location on discrimination of controls and respiratory disease patients.

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16.

Mass spectrometry imaging (MSI) combined with thin layer chromatography (TLC) as a technique for small molecules analysis

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Imaging mass spectrometry is an established and well-recognized technique for surface analysis. During this process, certain molecules located at a precise position on the surface, are desorbed. If only they are able to ionize under defined conditions, their *m/z* values might be detected by a mass spectrometer. It means, that the substances from the surface could be identified according to their molecular weight and verified by fragmentation. Thus, at the end, we may obtain the information about molecular composition of the analyzed surface.

Thin layer chromatography (TLC) allows for separation of complex mixtures of various substances. In the classic version of this technique, identification of the separated compounds is based on their retention factors. To estimate this value, spots characteristic for particular components are visualized using either UV - light or chemical reaction. The simplicity, cost-effectiveness and speed of TLC analysis, are the key reasons of its popularity. Nevertheless, visualization of the obtained chromatograms might sometimes be the drawback of this technique, especially when spots representing different components overlap.

Combination of simplicity and robustness of TLC analysis, with sensitivity and specificity of DESI-MS, introduces a new quality in this kind of research. It offers the possibility of unambiguous identification of almost every substance on the TLC plate, even if they are located in overlapping spots. Moreover, previous knowledge about separated sample is not necessary, since we are able to identify unknown substances present in separated sample.

Up to date, TLC/DESI-MS technique has been used for analysis of botanical samples, quality control of dietary supplements, separation of products of electrochemical reactions, and as a simple and rapid method for screening plant material for illicit substances. It could also be used as a promising strategy to obtain qualitative data on lipidome. Further progress in this technique certainly proves its usefulness for the analysis of complex mixtures.

SESSION IV

THURSDAY, JUNE 2nd, 2016

CHAIRPERSONS: Jerzy Silberring and Zbigniew Szewczuk

17.

Peptides labeled with cyclic quaternary ammonium salts for sensitive sequencing by electrospray tandem mass spectrometry

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The *de novo* peptide sequencing utilizing electrospray tandem mass spectrometry was successfully used in discovery of new peptide biomodulators and biomarkers. However, the insufficient ionization efficiency of some peptides and the resulting limited sensitivity is one of the main problems during analysis of trace amount of peptides by mass spectrometry. Therefore, the development of sensitive detection techniques for the efficient analysis of such samples by increasing the ionization efficiency of peptides is of utmost importance.

Recently, we developed an efficient method of synthesis of peptide conjugates containing various *N,N,N*-trialkylglycine moieties and applied as ionization enhancers for analysis of peptides at the attomole level using nano-LC-ESI-MRM technique.[1] Although the procedure was useful in combinatorial chemistry[2], its application in peptide sequencing is limited due to Hofmann elimination during fragmentation experiments (MS/MS)[3]. To overcome the possibility of this unwanted fragmentation we developed cyclic quaternary ammonium ionization tags, where all bonds susceptible to cleavage are protected in the form of 5- or 6-membered ring heterocycles (Fig. 1).

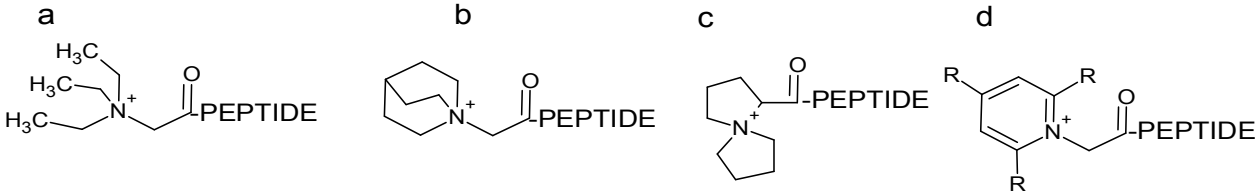

Fig. 1. Peptide conjugates containing linear (a) or cyclic ionization tags: 1-azoniabicyclic (b)[4], 5-azoniaspiro (c)[5] or 2,4,6-trisubstituted pyridinium (d) scaffolds

We also designed new derivatization reagents to introduce the ionization tags to amino or sulfhydryl groups of peptides in solution and found a method of tag conversion into their isotopologues for the quantitative research. [6] These ionization tags lower the detection limit of the analyzed peptides 10-1000 times. We believe that the application of such labeling may revolutionize comparative proteomics, leading to the development of new biomarkers based on proteins of low abundance.

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18.

NGC Chromatography System – Comprehensive Solution for Protein Purification

Jerzy Jankowski

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NGC Medium-Pressure Chromatography Systems offer a single preparative chromatography solution that scales to fit user applications and throughput requirements. The system major application is biomolecules purification.

The modular design of the NGC System provides multiple system configurations, each addressing different requirements. Each system is composed of interchangeable modules that confer different system capabilities.

Preconfigured 10 and 100 ml NGC™ Systems are available and could be easily upgradable as throughput and automation needs change — by simply add new modules.

By use the website configurator, it is possible to build own system configuration to meet specific workflow and throughput needs.

ChromLab Software is the integrated software package for the NGC™ Chromatography System. It controls all functions for laboratory-scale protein purification including instrument setup and calibration, method development, real-time monitoring and system control, chromatogram comparison, and peak analysis.

The NGC Chromatography Systems offer multiple system configurations, optional upgrades, automated multidimensional (Multi-D) chromatography workflow setup, and a common software platform for lab-scale separations. Suitable for analytical and preparative chromatography and protein purification scale-up.

19.

Fingerprints to study indole alkaloids from *psychotria nemorosa*: in extraction and fractionation optimization and in indicating interesting compounds

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Some *Psychotria* plants are used by Amazon Indians to prepare Ayahuasca, a hallucinogenic beverage. In addition, some tribes from Middle America use *Psychotria* species to treat dementia. In fact, the modulatory action of *Psychotria* alkaloid fractions and isolated compounds on enzymes related to neurodegenerative disorders was already demonstrated. One species is *Psychotria nemorosa*, which displays prominent inhibitory activity on butyrylcholinesterase (BChE) and monoamine oxidase-A (MAO-A). The extraction is a difficulty to overcome to access the plant metabolome. Several approaches have been described, mainly maximizing the number of chemical features as optimization goal. However, they may lack reliable data-handling methods. Thus, this study aims optimizing the extraction and fractionation to access the alkaloids metabolite profile of *P. nemorosa*, a source of multifunctional indole alkaloids (MIAs). Based on earlier results, ultrasound assisted extraction (UAE) was selected as extraction method. The alkaloid fraction was obtained by standardized liquid-liquid extraction (LLE), and analyzed by means of UPLC-DAD. In a first part, the extraction procedure was optimized, using a fractional factorial screening design (SD) to evaluate the influence of five factors. Two were further optimized via a central composite response surface design (RSD). Effect fingerprints were calculated and drawn for the SD. Heights of important peaks, indicated by SD, were modeled as responses. In a second part, as alternative to LLE, solid phase extraction was applied, and the fractionation was optimized in a Box-Behnken RSD, using sum of peak areas as response.

As a strategy to indicate potential MIAs, a chemometric approach was used. Forty three samples of *P. nemorosa* leaves were submitted to the optimized UAE and fractionation. The fractions were analyzed with UPLC-DAD and assayed for their BChE and MAO-A inhibition. The chromatographic fingerprints were first aligned using Correlation Optimized Warping. Principal Component Analysis was applied to explore the data structure. Linear multivariate calibration techniques, Partial Least Squares (PLS) and Orthogonal Projections to Latent Structure (O-PLS), were used to model the activities as a function of the fingerprints. The PLS regression coefficients were noisy, making the interpretation of their plot and the indication of potentially active peaks difficult. However, the O-PLS model had a lower error and an improved interpretability of the coefficients. Plotting these regression coefficients relative to the fingerprints, four peaks were indicated as multifunctional compounds, with the capacity to impair both BChE and MAO-A activities. To confirm these results, a semi-prepHPLC technique was used and a fraction containing the four peaks was purified and its activity evaluated *in vitro*. The results reinforce the prediction obtained by O-PLS modelling, confirming these four compounds as multifunctional indole alkaloids.

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20.

Determination of ethanol as a residual organic solvent in pharmaceutical preperation of human albumin using head space-gas chromatography-flame ionization detection (hs – gc -fid) in drug quality control laboratories

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Organic solvents play a key role in the production of pharmaceutical products during the manufacturing processes of drug substances, drug products and excipients. (synthesis of active substances ,separation, purification drying and in product formulations) . Many of them have toxic effects on humans or environmentally have hazardous properties and cannot be completely removed . Therefore, they should be avoided unless their use can be justified on the basis of risk-benefit assessmen . In order to control concentration levels of residual solvents in drug substances, products and excipients, national and international guidelines were introduced. In 1997, the International Committee for Harmonization (ICH) in their guideline Q3C [1], classified the ,commonly used organic solvents into three classes in terms of their level of hazard to humans and the environment and to regulate the concentration level of each solvent. Determination of residual organic solvents in pharmaceutical preparations has many analytical problems. The main source of problems is their high volatility and hydrophobic properties, which is directly related to the difficulty in sampling and their preparation for analysis. Moreover, the determination of polar residual solvents in pharmaceutical preparations continues to present an analytical challenge mainly because these compounds are difficult to remove from water or other polar solvents. It is a drug manufacturer and governmental quality control laboratories responsibilities to ensure that any residual solvents present in the final pharmaceutical preparation are not harmful to humans and that products do not contain levels of residual solvent higher than the recommended safety limits . Here our research is related to determination of ethanol as a residual solvent in pharmaceutical preparation of human albumin . For that purpose analytical procedure based on HS- GC – FID as analytical tool has been developed and validated . Ethanol was extracted from samples using headspace technique with extraction recoveries ranged 94 – 104 %. . Separation of ethanol was achieved on GC capillary column . For quantification analysis ,FID was used as a detector and the calibration curve was constructed and was linear with $R^2 \geq 0.9988$. The obtained validation data showed that the method appears sensitive , accurate , precise ,specific and relatively simple in both sample preparation and equipment .The procedure provides a very useful tool for routine analysis of ethanol as a residual solvent in pharmaceutical preparation of human albumin in drug quality control laboratories . In our drug quality control laboratories , human albumin samples (500 samples of pharmaceutical preparations) were tested using this method and the results showed that99.5% of the investigated samples was full fill the requirements of manufacturer and USP [2] specifications .

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Keywords : Residual Solvent in Pharmaceuticals , HS-GC- FID

21.

Enantioseparations using immobilized polysaccharide-based chiral stationary phases in supercritical fluid chromatography

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Since their introduction on the market, immobilized polysaccharide-based chiral stationary phases have been intensely investigated in high-performance liquid chromatography. These phases can be employed with a wide range of modifiers, potentially extending the application range of the polysaccharide-based stationary phases. Because an increasing number of stationary phases are being introduced in the field of chiral chromatography it is important to evaluate their enantioselectivity in different techniques.

In this study, three immobilized chiral polysaccharide-based stationary phases (Chiralpak IA, IB, and IC) were evaluated by means of supercritical fluid chromatography (SFC) with a test set of pharmaceutical racemates. First, the performance of the phases is evaluated using traditional modifiers, then with mixtures of atypical modifiers, and finally the separation results were compared with those on coated stationary phases with an equivalent chiral selector. Principal Component Analysis allowed to get a visual overview of the enantioselective patterns of the different chromatographic systems and allowed determining the (dis)similarity between individual systems. The three immobilized chiral stationary phases produced high cumulative success rates when they were screened with both traditional and atypical modifier mixtures.

22.

A novel label-free and universal detector for liquid chromatography systems using millimeter-wave technology

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Several detection techniques, such as UV-VIS spectroscopy, fluorescence spectroscopy and mass spectrometry have been employed in analytical liquid chromatography (LC) for many years. While UV and fluorescence detectors sometimes require labelling of compounds to enable their detection, MS instrumentation is universal but quite expensive. In recent years, the development of millimeter wave- and 3D-printing technology [1] has enabled the monitoring of interactions between electromagnetic waves and biological substances in micro-fluidic channels. Due to the dielectric difference between bio-molecules and mobile phases used in LC, it is possible to achieve universal detection without labelling work. In this study, a millimeter-wave sensor with operation frequency at 60 Giga-Hertz is developed and evaluated for its applicability as label-free and universal detector for a capillary liquid chromatographic system. 3D printing technology is used to fabricate the sensor structure to guarantee not only sensitivity but also to enable the microfluidic compatibility with the system as well. As a proof-of-concept demonstration, 2 UV absorbing substances (trans-stilbene oxide and praziquantel) and 1 non-UV absorbing compound (sorbitol) are injected into an open-tube setup of the LC system. The results of the millimeter wave detector are compared to that of an UV detector which is integrated in the LC instrument. The outcome shows potential of the millimeter wave sensor as an alternative label-free detector.

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SESSION V

FRIDAY, JUNE 3rd, 2016

CHAIRPERSONS: Łukasz Komsta and Robert Skibiński

23.

Hyphenation of liquid chromatography and bioautography methods for analysis of antibacterial compounds in plants

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Chromatography, mostly high-performance liquid chromatography (HPLC), is a powerful tool for analyzing plant constituents. However, it does not provide direct information on their biological properties. This can be solved by thin-layer chromatography – direct bioautography (TLC-DB), that is a hyphenation of TLC with biological detection, performed directly on a developed and dried TLC plate. TLC-DB belongs to effect directed analysis (EDA) methods and may be used for searching biologically active substances even in very complex matrices. Although TLC-DB can be based on any biological effect, e.g., antioxidant, enzymatic, antiestrogenic, antibacterial, or antifungal, this term is used predominantly, when antimicrobial properties are estimated [1,2].

TLC-DB against several bacterial strains, including pathogenic and luminescent bacteria, was used as a bio-guiding method to detect substances with antibacterial activity in *Matricaria recutita* L., *Achillea millefolium* L., *Salvia officinalis* L., *Thymus vulgaris* L., *Hypericum perforatum* L. and *Chelidonium majus* L. extracts [3-5] and in their pharmaceutical preparations [6]. The targeted substances, found by TLC and TLC-DB in the analytical scale, were isolated in larger amounts using semi-preparative TLC. The isolated fractions were subjected to LC-MS/MS analysis for their structural identification.

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24.

Open screening method for analysis of biological material on the presence of medicinal drugs that have severe influence on fitness to drive

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An increasing use of drugs and mind-altering substances is a substantial challenge to toxicology laboratories, as it becomes necessary to develop targeted analytical method for the ever growing number of xenobiotics. One alternative to such methods is non-targeted screening analysis, also known as systematic toxicological analysis, which relies on identification of xenobiotics from a database of, for example, mass spectra. Its main advantage is wide applicability and relatively low analysis cost, covering mainly the purchase of equipment with an appropriately extensive database. The purchase and use of standards is not necessary, contrary to targeted analyses. The method can be easily expanded by extending the database.

Driving under the influence of drugs (DUID) is serious problem in Europe. That is why 36 institutes from 18 European countries from 2006 to 2011, participated in the Integrated Project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) to find answers to questions concerning the use of drugs or medicines that affect people's ability to drive safely. One of the aims was to gain new insights to the real degree of impairment caused by psychoactive drugs and their actual impact on road safety and develop and agree on input for the establishment of a European categorisation system for medicines and driving. In order to categorise a medicine with regard to driving, several steps are identified using pharmacodynamic and pharmacokinetic as well as pharmaco-vigilance, experimental, epidemiological and additional data (e.g. from accidentology). The DRUID expert group established and agreed that, according to its influence on the ability to drive, a medicine could, regarding to driving, be categorized as followed: category 0 (no or negligible influence on fitness to drive), category I (minor influence on fitness to drive), category II (moderate influence on fitness to drive) and category III (severe influence on fitness to drive). In our studies we focused on drugs from category III.

The goal of this project was to develop a screening method for identification drugs affecting psychomotor skills in whole blood and urine.

The screening method proposed by the equipment manufacturer was modified to include drugs influencing psycho-physical abilities, listed in the III group of substances in the DRUID project report. The supplied library was supplemented with missing mass spectra obtained from the analysis of standards. The analytical material was blood from blood donation center as with as blood and urine routinely sent to the Institute of Forensic Research for analyses. The analytes were isolated by liquid-liquid extraction with ethyl acetate (pH 11) and diethyl ether (pH 3). Samples were analysed by liquid chromatography with mass spectrometry, LC-MS/MS with linear ion trap detection (QTRAP). Analyses were carried out using a SCIEX 3200 QTRAP LC-MS/MS System. Separation was performed on a Kinetex C18 (50x3 mm, 2.6 μm particle size) column using gradient elution of water and 1% (v/v) ammonium formate in methanol and acetonitrile 1:1 mixture.

The assay was found to be selective for all tested compounds. The LC-MS/MS method has proven to be appropriate for identification of the components in whole blood and urine. It was successfully applied in day routine toxicological analysis. The procedure can be easily extended.

Keywords: systematic toxicological analysis, drug screening, LC-MS/MS

25.

The chromatographic analysis of head and neck cartilage biocompatibility

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Laryngectomy is used in the treatment of larynx cancer, serious injury or necrosis caused by radiation therapy. Laryngeal resection often results in major aspiration problems, making larynx preservation during surgical removal of tumors. To increase the comfort of patients after surgery there are attempts to reconstruct the larynx using cartilage from other parts of the body. Extra tissue fills the space where the cancerous tissue has been removed. These tissues will help fill in the spaces missing after removing the cancer. Among the sources of cartilage for reconstruction are nose (nasal septum) and external ear. In our study we present the preliminary results of the comparative analyses of the nose, ear and larynx cartilage using chromatographic methods.

To analyze nose, ear and larynx cartilage we used UHPLC system equipped with Aeris Peptide XB-C18 (150 x 2.1 mm; particle size: 1.7 μm) column. The column contained uniform porous silica layers grown around a solid, spherical silica core. The following parameters were used temperature: 30 °C sample eluent: physiologic saline concentration from: 800 ppm each component injection volume: 10 μL, mobile phase A: water B: acetonitrile; flow rate: 0.2-0.9 mL/min. Detection of the separated peaks was realized by the multiple DAD and FLD detectors enabling detection of trace-level components. Both phases were mixed with advanced micro-mixer.

The method seems to be really useful in biological (medical) material estimation in head and neck surgical reconstruction.

26.

Hybrid approach combining chemometrics and likelihood ratio for evaluation of chromatograms for forensic purposes

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The rapid development of the instrumental analytical techniques triggers the new possibilities for examination of samples of the microtrace size, which is the issue in the forensic field [1]. The advancement of the analytical techniques enables recording thousands of parameters characterising samples in a single measurement. With growing complexity of data there is an increasing need for developing the tools assisting in their interpretation, especially concerning reduction of data dimensionality. Chemometric methods, which are efficient in data compression, cannot be directly applied for interpreting the evidence data due to ignoring some aspects such as the rarity of the observed features, which is of utmost importance when interpreting the data for forensic purposes. All relevant factors are accomplished in the likelihood ratio (LR) framework, which enables reporting the evidential value of the physicochemical data in a way appreciated by the forensic community. LR ($LR=f(E|H_1)/ f(E|H_2)$) interprets the data (E) in the light of two contrasting propositions: H_1 stating that compared recovered and control materials come from the same source (e.g. suspected car), and H_2 assuming that compared recovered and control materials do not come from the same source [1]. Its only drawback lies in the fact that LR easily copes with data with the number of variables far less than the number of samples they describe and is poorly adaptable for reverse cases for highly multidimensional data delivered by advanced analytical techniques such as chromatograms [2].

The aim of the presented research was to report the evidential value of highly multidimensional data by using a hybrid models combining chemometric techniques for data compression and adopting their outcome as the input for LR models [2].

The developed models were used to solve the comparison problem of chromatograms obtained for the samples of polypropylene and analysed with pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Pieces of polypropylene originating from the elements of vehicles body and plastic containers are likely to be found on the scene of car accident. They were pyrolysed at a temperature 750°C for 15 s in the stream of carrier gas (helium). Obtained volatile organic compounds were transported via heated interface to GC-MS module, where they were separated and detected. The proposed LR models engaged the dissimilarity representation [3] for generating the new variables further encompassed in LR approach. Their performance was controlled by estimating the levels of false positive and false negative responses and by inspecting the empirical cross entropy curves.

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27.

New psychpactive substances (NPS) contained in "designer drugs" – chromatographic and spectroscopic analysis

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Beginning from mid-2000, on the global drug market compounds known as new psychoactive substances (NPS) or colloquially, as designer drugs started very effectively appearing. „Designer drugs" are groups of substances which are from the structure and mechanism of action point of view similar to illegal psychotropic substances or narcotics like amphetamine, phencyclidine, cannabis. Currently, among these compounds the most numerous group constitute the derivatives of cathinone, a biologically active alkaloid derived from the plant known as khat (*Catha edulis*). Lots of these substances are prohibited but some of them still not. However, possibilities of synthetic modifications of the original cathinone and their earlier obtained derivatives are enormous, so that once a given substance is put on the list of the prohibited psychotropic agents the new modified compounds almost instantaneously sprout on the market.

Due to steadily growing problem of new psychoactive compounds appearing on the market, elaboration of improved and/or novel identification and quantification methods becomes an urgent and very challenging task. Implementing an existing database with novel information on physicochemical and pharmacological properties of these compounds can facilitate rapid identification thereof by analytical chemists and toxicologists.

In this study, among others we present chromatographic and spectroscopic identification psychoactive substances contained in "designer drugs".

28.

Biopurification of air from VOC's mixture - optimization of biodegradation process

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Volatile Organic Compounds (VOCs) and odorous substances pose a significant part of hazardous pollution. The biogenic sources of VOC are related with occurrence tropical and coniferous forests, the vegetation processes and a number of physical activities such as volcanic eruptions or fires as well. Notwithstanding, the major cause of the environmental air pollution is anthropogenic activities. VOCs are commonly produced by a variety of chemical and petrochemical industries. Some VOCs may be harmful to human health in the long – term exposure and provoke serious illnesses. For example, sore throats, feelings of tiredness and dizziness, asthma, immune and reproductive system problems, mutagenic or carcinogenic effects. For an environmental point of view, VOCs lead to increased amount of ozone at troposphere thereby contribute to photochemical smog and the greenhouse effect [1-3].

There are plenty of methods for polluted air treatment and removal. The biologically based methods raise interest due to exploitation of natural ability of microorganism to degrade pollutants. They are environmentally friendly, without providing post – processes waste. The VOC's biotreatment carried out in the Compact Trickle Bed Bioreactor (CTBB) based on Know How of Ekoinwentyka has become an attractive alternative for many physical and physicochemical methods of air purification. Basically, this method has many advantages. The main pros include low pressure and low temperature of the biodegradation process, friendliness to human beings and surrounding environment, lack of secondary waste and low operating costs [4].

The aim of this work was biopurification of air stream from styrene, ethyl alcohol and dimethyl sulfide mixture using a trickle bed bioreactors operating with continuous bed fid with a mineral salt solution. The primary object of the investigation was searching for the optimal operational conditions in terms of air pollutant concentration and nutrients addition for a best purification efficiency.

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29.

Comparison of composition of the volatile fraction in comercial samples of *Cistus incanus* L

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Herbal medicine or phytotherapy (which consists in practical use of knowledge on medicinal plants and treatments with herbal agents that can replace or support conventional treatment) is becoming very popular and increasingly common [1]. Current studies confirmed an effectiveness of herbs. Among the herbs, the *Cistus* species has gained great popularity. Preparations of this plant exhibit a wide spectrum of the activities including an antioxidant, antibacterial, antiviral and antifungal activity, gastroprotective properties, and inhibition of the prostate hypertrophy process [2].

The aim of this study is to compare the volatile fraction derived from herbal samples of one species, *Cistus incanus* L., originating from different manufacturers and geographic regions. Each plant sample was subjected to hydrodistillation according to the standard procedure [3] and essential oils obtained in that way were analyzed by means of gas chromatography coupled with the mass spectrometric detection (GC/MS). In order to compare chemical composition of the tested samples, the headspace (HS) GC with the MS detection was also performed.

The commercially available *Cistus incanus L.* samples tested in our study showed significant qualitative and quantitative differences in composition of the volatile fraction. An obvious conclusion is that composition of herbal medicines can distinctly vary both in qualitative and quantitative terms, thus affecting the health benefits thereof. Thus, it is necessary to define appropriate quality requirements and analytical methods, which would allow standardization of chemical composition of herbal food products, providing a repeatable efficacy and safety of their usage.

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POSTER SESSION I

WEDNESDAY, JUNE 1st, 2016

CHAIRPERSONS:

Anna Bodzoń-Kułakowska

and Ana Protić

1.

Pesticide residues in surface and drainage waters of agriculture intensive area at El-Behira province, Egypt

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The organochlorines OCs) and organophosphorus (OPs) pesticide residues in surface and drainage waters having intensive agriculture activities were determined to evaluate their potential pollution and risks for human health. Pesticide residues were extracted from water samples by solid phase extraction method (SPE) and Chromatographic Separation of the OCs and OPs pesticide residues were achieved using HP-608 fused silica capillary column ,30 m X 0.53 mm i.d., 0.5 um film thickness and HP- 5 MS capillary column, 30m x 250 um ID and 0.25 um film thickness , respectively . The OCs and OPs pesticide residues detection were done by GC-ECD and GC-ITD , respectively . were more than 85 percent at a fortification level of 5 ng/ml . Detection limit of compounds ranged between 10 to 30 ng/ml . The OCs residues detected in both drainage and drinking waters were B-HCH , gamma HCH , heptachlorepoxide , p,p -DDE , p,p -DDD, dieldrin , endrin aldehyde , endosulfan-sulfate and endrin ketone . On the other hand , the OPs residues , ethoprophos , diazinon and fenthion were only found in drainage water . Water samples collected during summer season were more polluted with pesticide residues than that of winter season. Heptachlorepoxide , p,p-DDE , dieldrin and p,p-DDD residues were more higher than the recommended permissible limits in drainage waters . Concentration of OCs residues found in the surface waters were in the acceptable limits of WHO . In conclusion drainage water was more polluted and contains more pesticide residues than the drinking water . This means that the discharging of agricultural wastes in this area of study must be controlled to protect the environment and human health from pollution of these pesticide residues .

Keywords: Organochlorine pesticide (OC) residues in water , Organophosphorus pesticide residues(OP) in water , SPE , GC-ECD , GC- ITD .

2.

Synthesis of deuterium labeled denatonium cation and its application in the quantitative analysis of Bitrex® by liquid chromatography-mass spectrometry

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Denatonium benzoate, known as Bitrex®, is a an extremely bitter compound used commonly to denature industrial alcohols and to make potential harmful household products extremely unpalatable [1]. Several methods of quantitative analysis of Bitrex have been applied to determine its level in different samples [2], however they are time-consuming and may suffer from errors inherent to the extraction process during sample preparation [1]. Recently, liquid chromatography-mass spectrometry (LC-MS) has become a method of choice in the rapid analysis of chemicals, however for the quantitative analysis, application of isotopically labelled standards is required.

Here we present a rapid and cost-efficient method of deuterium labeling of Bitrex via H/D exchange of its hydrogen atoms in CH_2 group directly bounded to quaternary nitrogen atom which occurs during a 60 minutes incubation in 1% *N,N,N*-triethylamine (TEA) solution in D_2O (Fig. 1A). The proposed strategy does not require derivatization reagents nor de-novo synthesis of denatonium benzoate from deuterated substrates.

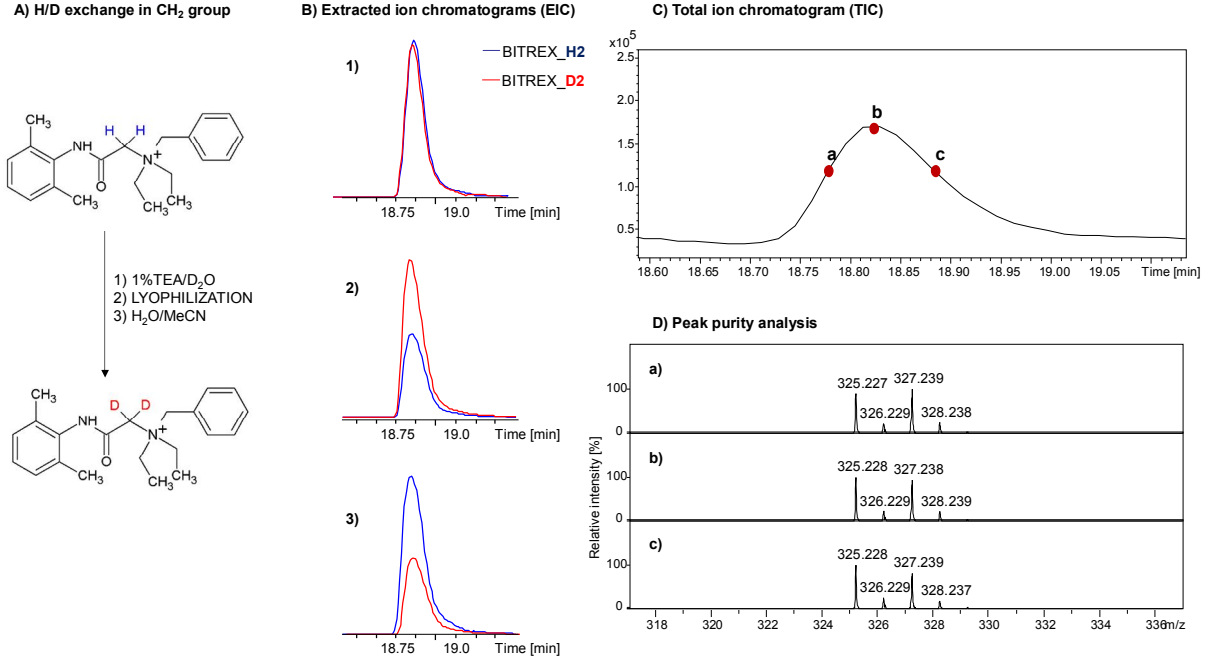

Fig. 1. Schematic presentation of isotopic exchange of the hydrogens in CH_2 group (A), extracted ion chromatograms (B) of deuterated and non-deuterated denatonium benzoate samples mixed in different ratios: 1) 1:1; 2) 2:1 and 3) 1:2. The total ion chromatogram peak at Rt 18.83 min was selected for peak purity analysis (C). The ESI-MS spectra presented for points a-c of the total ion chromatogram (D).

It was found that the introduced deuterons do not undergo back-exchange under acidic aqueous solutions. The co-elution of deuterated and non-deuterated forms on RP column was also confirmed (Fig. 1B-D) [3]. The presented isotopically labeled betaine is proposed as a new internal standard in the quantitative analysis of Bitrex in commercially available household products by LC-ESI-MS.

This work was supported by a grant No. UMO-2013/09/B/ST4/00277 from the National Science Centre, Poland.

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3.

Multidimensional (3D/4D-QSAR) probability-guided pharmacophore mapping: Investigation of activity profile for series of drug absorption promoters

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The rational production of the desired compound pharmacological profile is enormously challenging issue that still lacks a general approach. The computer–assisted drug design (CADD) is regarded as the art of specifying molecules of potential therapeutic values working as preliminary stage namely 'pre-synthesis' or 'intuitive roadmap' on the path towards the *the production of properties*. The elementary idea underlying the CADD for the robust identification of hit→lead→drug candidate is the comprehensive projection of the compound topology and/or topography into the chemical property space. A variety of the CADD methodologies, in particular multidimensional quantitative structure–activity relationships (mD–QSAR) procedures employ implicitly or explicitly the similarity principle where *compounds with similar structure are expected to have similar biological activity.*

A number of modern drugs are not available to the patients due to their poor aqueous solubility and permeability. Generally, modification/optimization of poor permeability through membranes can be solved by selection of appropriate excipients to function as transporters (surfactants or pharmaceutical complexing agents, permeability enhancers) being components of a dosage form. These excipients that increase absorption of drugs to blood circulation are known as intestinal absorption promoters in oral drug formulations and transdermal penetration enhancers in transdermal therapeutic systems.

Cholic acid is one of the most important human bile acids. Bile acid derivatives/analogues are an important class of compounds with a range of pharmacological activities. Bile acids could be easily modified by derivatisation of the functional groups on the steroid nucleus. Cholic acid derivatives were studied also as transdermal penetration enhancers.

The principal objective of the current investigation was 2–fold. First of all, it is of interest to compare the impact of the coding molecular systems on the efficiency of structure–activity performance using 3D (CoMFA and CoMSA) and 4D (standard and neural formalism) methods on the ensemble of drug absorption promoters. Additionally, we concentrated on systematic model space inspection with splitting data collection into training/test subsets to monitor statistical estimators performance in the effort for mapping of the *probabilistic* pharmacophore geometry using stochastic model validation (SMV) approach. The automated variable reduction with IVE–PLS procedure represents a sieve for detecting only those descriptors, which have prescribed the greatest individual weighting to the observed cholic acids analogue activity. A 'pseudo–consensus' 4D–QSAR methodology was used to extract an 'avarage' 3D–pharmacophore by exploration of a various data subpopulations which embodies the *quantity for quality argument* to indicate the relevant contributing factors of the cholic acid absorption activity.

4.

Present state and future perspectives of using countercurrent chromatography (CCC) in phytochemical research

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Introduction: The separation of high purity compounds from complex mixtures is widely used in the pharmaceutical, cosmetic and also food industry. Among various separation techniques countercurrent chromatography (CCC) becoming attractive due to its high resolving power. The method of countercurrent chromatography is based on selective division of separated substances (relative to their partition and solubility coefficients) between two immiscible liquid phases. The stationary phase is maintained in place by simple or complex impact of a centrifugal or gravitational field. In 1940 Craig and Post designated the first apparatus for countercurrent chromatography (CCC) and separated first compounds with similar values of the partition coefficient. In 1960 Yoichiri Ito defined this liquid chromatography as countercurrent.

Thesis aim: The evaluation of countercurrent chromatography in the process of separation of various biologically active substances.

Materials and methods: Overview and analysis of selected research and review papers concerning the use of countercurrent chromatography in the separation of biologically active compounds. The following databases have been used: PubMed, Embase, Web of Science, Medline, Science Direct (Elsevier) available from Main Library of the Medical University of Silesia.

Results: Selected papers regarding the use of countercurrent chromatography in the process of separation of biologically active compounds have been reviewed. Literature review indicates that both HSCCC and HPCCC methods are useful in the resolution of the following bioactive compounds: betanins, anthocyanins, coumarins, terpenoids, and proteins. This allows to obtain highly purified biomolecules in a novel and highly specialized way.

5.

Application of TLC-densitometry in pharmaceutical analysis of finasteride

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Finasteride is a synthetic steroid compound which is used as a surgical alternative for treatment of prostatic hyperplasia.

A new, simple in use and economical TLC-densitometric method in normal and reversed phase system (NP-TLC and RP-TLC) has been developed and validated for the identification and quantitative determination of finasteride in bulk drug and in tablet formulation containing finasteride as an active component. NP-TLC analysis was performed on aluminum plates precoated with silica gel $60F_{254}$ as the stationary phase using chloroform-acetone (40:10, v/v) as mobile phase. In the case of RP-TLC system, a mixture consisted of dioxane-water in volume composition 35:15 (v/v) and silica gel RP-$18F_{254}$ plates were optimal. Densitometric analysis was carried out at λ=212 nm in both cases. The proposed NP-TLC and RP-TLC densitometric methods were validated according to ICH guideline and other validation requirements in terms of its specificity, linearity, precision, accuracy, robustness and sensitivity. All validated parameters are satisfactory including the limit of detection (LOD) and limit of quantification (LOQ). The percent content of finasteride in marketed tablet formulation was found to be 99.0 % of label claim. The developed TLC-densitometric methods can be successfully used in quality control of finasteride in bulk material and also in tablet formulation.

Our study confirms that thin-layer chromatography combined with densitometry is a reliable, precise and cost-effective analytical tool which allows determine the content of biologically active steroid namely finasteride in tablet dosage form.

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6.

TLC-densitometric analysis of clobetasol propionate in pharmaceutical preparation

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Clobetasol propionate is one of the most important bioactive compounds important for treatment of dermatitis. It is widely used in form of cream or as lotion containing 0.5 mg/mL of clobetasol propionate. Medical importance of this substance indicates that there is a need to find a rapid analytical method for the quality control of pharmaceutical preparations containing of clobetasol propionate. Therefore, the main aim of this work was to develop the optimal chromatographic conditions, such as mobile phase composition and proper chromatographic plates enabling TLC-densitometric analysis of clobetasol propionate in form of lotion. Among different chromatographic systems which have been studied in this paper, the best are: acetonitrile-methanol 38:12 (v/v) and silica gel RP-18F_{254} in the case of RP-TLC analysis. For the purpose of adsorption thin-layer chromatography (NP-TLC) a mixture of toluene-methanol in volume composition 40:10 (v/v) and silica gel 60F_{254} can be recommended. Densitometric analysis was carried out at λ=246 nm in both cases.

The results performed in this study confirm that the developed method in NP-TLC and RP-TLC systems in combination with densitometry is a good tool in quality control of pharmaceutical formulation containing clobetasol propionate. Thus, it can be successfully applied in pharmaceutical analysis of this steroid as an alternative method to other required by Pharmacopoeia.

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7.

LC-MS, GC-MS and NMR based untargeted metabolomics in searching for urine biomarkers of bladder cancer

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Bladder cancer (BCa) constitutes ninth type of cancer in terms of cancer incidences worldwide. The diagnosis of BCa is based either on cystoscopy, ultrasound scan, computed tomography (CT) urogram, magnetic resonance imaging (MRI) scan, intravenous urogram (IVU) or histopathologic evaluation of biopsy sample. All of mentioned diagnostic methods requires the use of specialist equipment operated by a professional, may cause patients' discomfort and most of all – are adopted when disease symptoms are observed, mostly at the late stage of the disease. Therefore, specific and non-invasive diagnostic method for early diagnosis of BCa is needed. Among possible approaches, metabolomics seems to be a great tool in searching for new potential biomarkers of BCa and explanation of its pathomechanisms on molecular level. Therefore, urine metabolic fingerprinting was utilized in order to determine metabolites that could become potential biomarkers of BCa.

Urine samples obtained from BCa patients (muscle invasive, high grade BCa, n=24) and healthy volunteers (n=24) were analyzed with the use of 3 complementary analytical techniques: high performance liquid chromatography (in RP and HILIC mode) coupled with time of flight mass spectrometry detection (HPLC-TOF/MS) in positive and negative ionization modes, gas chromatography hyphenated with triple quadruple mass spectrometry detection (GC-QqQ/MS) in a scan mode and nuclear magnetic resonance spectroscopy (^{1}H NMR). Applied analytical methods were previously optimized at the Department of Biopharmaceutics and Pharmacodynamics at MUG (in case of HPLC/MS and GC/MS) and at Groupe de RMN Biomédicale, Université Paul Sabatier in case of ^{1}H NMR. After data treatment (deconvolution, filtration and normalization) statistical analysis was applied to select metabolites that represented statistically significant differences between compared groups. Finally, the identification of selected metabolites was performed with the use of publicly available databases allowing for creation of a list of putative biomarkers. The selected metabolites (e.g. uric acid, hippuric acid, glutamine, phenylacetylglutamine, pipecolic acid, acetylspermidine, tyrosine, dodecanamide or hydroxytryptophan) can play crucial role in pathogenesis of BCa.

The obtained results suggest that urine metabolic fingerprinting could be a powerful tool for biomarkers' investigation. Differences in metabolism, specific for BCa, may provide non-invasive diagnosis of the disease. Nevertheless, it should be emphasized that obtained results are preliminary and require validation on larger set of samples in order to confirm diagnostic value of selected metabolites.

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8.

The hydrogen-deuterium exchange in betaine moiety does not affect chromatographic behaviour of peptides labeled with quaternary ammonium derivatives

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The isotopically labeled standards for mass spectrometry are expected to present the same retention during chromatographic analysis as regular analytes. Therefore there is a preference for stable heavier atoms (^{13}C, ^{15}N, ^{18}O) exchange, as there is practically no isotopic effect and such isotopologues co-elute during HPLC analysis. Despite the retention time differences, the application of deuterated standards is an attractive option [1] due to low cost and relatively simple synthesis, even if additional operations are needed in proteomics studies [2].

We developed a simple procedure for hydrogen-deuterium exchange (HDX) of α-C hydrogens in *N,N,N*-trialkylglycine residues in peptides [3]. The process occurs in 1% aqueous triethylamine and the modification is not reversible under acidic conditions.

As the chromatographic separation of native and deuterium-labeled peptides depends on localization of deuterons [4], a series of model peptides containing modified betaine was subjected to HDX in methylene group (Fig.1) and the precision of co-elution in LC-MS was analyzed. We examined the modified peptides using on-line reversed phase (RP) and hydrophilic interaction (HILIC) LC-MS (Table 1).

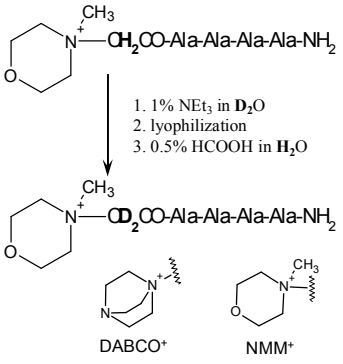

Figure 1. Triethylamine-induced HDX in betaine moiety

Table 1. Experimental conditions for LC-MS analysis of labeled peptides

	Mobile phase A	Mobile phase B
	gradient 0 – 25% B in A in 20 min, 0.1 ml/min	
RP-HPLC (C18)	0.1% HCOOH in H_2O	0.1% HCOOH in ACN
	5 mM $HCOONH_4$ in H_2O (pH 3.2)	acetonitrile (ACN)
	5 mM $HCOONH_4$ in H_2O (pH 6.3)	acetonitrile (ACN)
	5 mM NH_4HCO_3 in H_2O (pH 8.4)	acetonitrile (ACN)
	gradient 5 – 50% B in A in 20 min, 0.1 ml/min	
HILIC (cross-linked diol)	5 mM $HCOONH_4$ in ACN:H_2O (95:5)	5 mM $HCOONH_4$ in H_2O (pH 3.2)
	5 mM $HCOONH_4$ in ACN:H_2O (95:5)	5 mM $HCOONH_4$ in H_2O (pH 6.3)
	ESI-MS Bruker micrOTOF-Q mass spectrometer operated in positive ion mode	

In all experiments we observed identical retention times for native and deuterated peptides as well as the preservation of isotopic distribution through chromatographic peak. In our case, there is no difference in isotopic effect in analyzed pH range, contrary to results of Di Palma *et al.* [5]. It is worth noting that although the order of elution of the peptides labeled with quaternary ammonium group is in most cases reversed on HILIC phase as compared to RP-HPLC, in both systems peptides elute at relatively low concentration of stronger solvent, allowing for short analysis time.

According to our results, the betaine moiety not only introduces permanent positive charge, thus facilitating MS analysis of traces of peptides [6], and allows for mild HDX conditions, but also provides the deuterons with hydrophilic environment, which may be responsible for reduction of isotopic effect.

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9.

Untargeted metabolomics with the use of LC-TOF/MS and GC-MS for selection of tentative markers of renal cell carcinoma

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Kidney cancer is one of the 10 most common cancer types. Among all kidney cancer subtypes, renal cell carcinoma (RCC) is responsible for approximately 90% of cases. Due to the lack of specific symptoms and diagnostic methods, RCC is frequently diagnosed at the late stage of the disease. Therefore, development and application of new high-throughput and specific diagnostic methods is essential for early detection of RCC. In the present study urine metabolic fingerprinting was performed for understanding and explanation of RCC pathomechanisms.

Urine samples collected from RCC patients and healthy volunteers were analyzed with the use of HPLC-TOF/MS in positive and negative ionization modes, as well as GC-QqQ/MS in scan mode. The obtained datasets were processed using deconvolution, alignment, normalization and filtration steps. Afterwards, uni- and multivariate statistical analyses were performed. Statistically significant metabolites were selected according to adjusted p value (FDR p value < 0.05) and variable importance into projection (VIP) value > 1. The identification of selected metabolites using NIST, HMDB, METLIN, KEGG and CEU Mass Mediator databases allowed for creation of a list of putative markers and related biochemical pathways which they are involved in. Selected altered metabolites were found to be involved in amino acid, purine, lipid and glucose metabolism as well as TCA cycle.

The obtained results suggest that urine metabolic fingerprinting is a powerful tool which might be useful in research for RCC diagnosis and eventual further explanation of its molecular pathomechanisms..

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10.

Determination of antioxidant properties of selected cruciferous plants

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Cruciferous plants is the family to which they belong among other cruciferous vegetables, leafy and oilseeds and spices, for example. broccoli, Brussels sprouts, cabbage, radish, rapeseed, kale, mustard, watercress, arugula and horseradish. As used in the diet of cruciferous vegetables can prevent the occurrence of cancers, due to the high content of antioxidants.

Antioxidants have anti-viral, anti-bacterial, anti-cancer, slow down the action of enzymes[1-3] An important role in preventing cancer and play polyphenols glukobrassycyna glucoraphanin and, in particular, their decomposition products successively, sulforaphane and indole-3-carbinol.

In some cruciferous plants was marked antioxidant capacity and contents poifenoli techniques using UV-Vis spectrophotometry methods based on a radical reaction with DPPH and ABTS• •, and utilizing oxidation and reduction reactions: FRAP and CUPRAK.

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11.

Corrosion in continuous bio-degradation of sulfur-containing, volatile organic compounds

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Environmental regulations are continuously pushing industry for emission reduction of NOx, CO, H_2S and other poisonous low-molecular weight gases. Volatile organic compounds (VOC) represented by carbon-based chemicals like vinyl acetate, ethyl acetate, styrene or dimethylsulphide (DMS), however less known to public, play significant role in air poisoning. Industrial emission of those substances is much lower in comparison to gases like NO_x, but their high toxicity, reactivity and consequent severe interference with living organisms puts them at the top of a list of industrial pollutants. Effective removal of VOC from off-gases is a challenging process due to very low concentration of these toxic compounds.

The VOC mixture (as a liquid) was injected to the flow of air and introduced to the reactor co-currently with circulating water phase (see Table 1 for water composition). The VOC mass load varied between 0.07 g/m^3 and 1.2 g/m^3 which translates to concentration of DMS in the inlet gas in range of 3-8 ppm_{mol} which is typical for low polluted gas streams. Biodegradation was carried out at a temperature of 27±2ºC and a pH of 7.0±0.5. The pH was controlled and adjusted automatically by addition of 10% KOH and 10% KH_2PO_4.

Results demonstrate the efficacy of utilizing an industrial-grade, on-line corrosion monitoring technique, integrating multiple electrochemical techniques such as Harmonic Distortion Analysis (HDA), Low Frequency Impedance (LFI) and Electrochemical Noise (ECN). Results also demonstrated that the real-time monitoring described herein provided excellent measurement accuracy even under conditions of very low corrosion rates, as evidenced by the close agreement in general corrosion rate values between online measurements and those obtained through the traditional mass loss technique. Furthermore, these online corrosion measurements could be used to determine the effects of process variables on the severity of corrosion. This result suggests that real-time corrosion measurement can be used to control and minimize corrosion in the bio-processing of VOCs.

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12.

Synthesis and physico-chemical properties of derivatives of graphene oxide as a potential drug carriers

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Synthesis of GO-X is based on the nucleophilic substitution ofaminoacids to graphene oxide nanoparticles. In the frst step, aminoacid compaunds was transformed into methyl ester hydrochloridein order to protect the C-terminal end and the N-terminal end ofthe amino acid. The obtained product, activated in situ, wasused for the formation of amido binding between aminoacids and GO.

The synthesized GO-X was characterized by Fourier transforminfrared spectroscopy, X-ray photoelectron spectroscopy, and scanning electron microscopy.

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13.

Lipophilicity of isomeric *N*-methylquinolinesulfonamides

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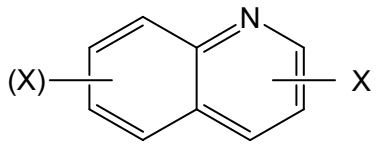
Sulfonamide group containing azine derivatives are of interestfor their biological activity. Several of them are potential diuretic agents.

Lipophilicity is an important factor determinating the biological activity of drugs. RP TLC is very useful method for determination of lipophilicity. We used this method in our study of seven isomers of N-methylquinolinesulfonamide:



X = 2-,3-,4-,5-,6-,7- or 8-$SO_2NH(CH_3)$

TLC was performed on silica gel RP-18 F_{254} plates (Merck #115389), activated by heating at 100 °C for 1 h. Mixtures of methanol-water solutions were used as mobile phases. Relationship between R_M values obtained from chromatography and partition coefficients is described by the equation R_M = a log P + b, where a and b are constants for a particular system and P is the partition coefficient.

Coefficients a and b were found in experiments for compounds with known log P values. Log P values obtained for the compounds investigated were correlated with values calculated by several theoretical methods. Lipophilicity of *N*-methylquinolinesulfonamide isomers were compared with lipophilicity of quinolinesulfonamide and *N*,*N*-dimethylquinolinesulfonamide analogs.

14.

Application of UHPLC-PDA-ESI-MS^n for phytochemical profiling of antioxidant active extracts from selected *Sorbus* species

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Genus *Sorbus sensu lato* is a broad taxon comprising over 250 species widespread in cool to temperate regions of northern hemisphere [1]. Many of *Sorbus* representatives have been sources of ethnomedicinally used plant materials with diuretic, anti-inflammatory and vasoprotective properties. As rich sources of natural polyphenols, the *Sorbus*-derived extracts have been pointed to as a potential material for development of dietary supplements effective in prevention of many oxidative-stress related diseases [2]. In our previous studies we found out that leaves and inflorescences of species representing the subgenus *Sorbus* are distinguished by the exceptional abundance of polyphenolic compounds [3,4]. We then demonstrated that the polyphenols could be further concentrated by fractionation of hydromethanolic extracts and established that the thus obtained ethyl acetate fractions exhibit the highest antioxidant potential [4]. Preliminary HPLC-PDA analyses of the active fractions revealed the considerable complexity of their composition [4] and prompted us to conduct more detailed investigation into the subject.

Therefore, the aim of the study was thorough phytochemical profiling of the most antioxidant active fractions of selected *Sorbus* species with the use of UHPLC-PDA-ESI-MS^n. The ethyl acetate fractions of hydromethanolic extracts from eight species representing the subgenus *Sorbus* were investigated. The analyses were performed on an UHPLC system (Dionex, Germany) with PDA detector and an ion trap mass spectrometer with ESI interface (Bruker Daltonik, Germany). Separations were carried out on a Zorbax SB C18 column (150 × 2.1 mm, 1.9 µm, Agilent, USA). The compounds were identified based on the comparison of their chromatographic and spectral (UV and MS^n) data with authentic standards (obtained previously in isolation studies) and/or with literature. The extracts exhibited a complex and diverse composition, containing polyphenols classified as flavonoids, phenolic acid derivatives and proanthocyanidins. Eventually, about 40 compounds were identified including kaempferol, quercetin and sexangularetin glycosides, caffeoylquinic acid isomers as well as dimeric and trimeric proanthocyanidins.

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15.

Thin-layer chromatographic identification of phenolic acids in cosmetic raw materials

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An increased interest in bioactive compounds is due to a possibility of using them as additives to cosmetics, alimentary products, and pharmaceuticals. As bioactive compounds, these substances of herbal origin are understood which appear in low quantities in the plants. A particularly interesting group of bioactive compounds are antioxidants. In cosmetics, particularly useful are phenolic acids, which are widespread in the kingdom of plants (e.g., gallic acid appears in strawberried, raspberries, or grapes, etc.).

Antioxidant properties of phenolic acids consist in eliminating reactive forms of oxygen, blocking free radicals, inhibiting the oxidase enzymes but also supporting action of the enzymes with an antioxidant action, and on chelating metal ions (e.g., iron or copper). Such properties of bioactive compounds added to the cosmetics result in preventing human skin from ageing and in extending the shelf life of cosmetics.[1,2]

In our study, we selected a simple and cost-friendly thin-layer chromatographic technique to identify the following phenolic acids: caffeic, ferulic, gallic, and coumaric acid in the commercially available cosmetic raw materials (such, as extracts derived from red wine, grape skins, pomegranate peels and juice, green tea, etc). Moreover, caffeic acids was quantified in the scrutinized samples.[3,4]

In the course of this study, it was established that the highest contents of phenolic acids are in the cosmetic raw material obtained from green tea.

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16.

Functionalization of quinoline derivatives by Reimer-Tiemann reaction; the GC-MS and TLC investigation

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Aldehydes are the main precursor of very important chemical reactions. There are a lot of synthetic routes that allow to obtain aldehydes. They can be obtained by the electrophilic aromatic substitution (S_EAr) route such as Reimer-Tiemann reaction. This is a three steps reaction. The first step consists of generation of electrophilic carbenes: CX_2 (X = halogen). During second step is formation of C_{Ar}-C_{carben} bond based on S_EAr substitution. The final third step leading to aldehydes is the hydrolysis of C-X (X = halogen) bond of C_{carben} fragment [1, 2]. This type of reaction has been used to transformed variety of phenols, and many electron rich aromatic or heteroaromatic compounds [3].

However, quinoline was firstly isolated by F.F. Runge in 1834 during the extraction of coal tar [4]. Currently, quinoline and their derivatives have found a wide range of applications. They are a valuable material for the preparation of dyes and pharmaceuticals. Quinoline derivatives exhibit biological activity against malaria and possess antifungal, antibacterial, anti-asthmatic and anti-inflammatory properties. They also reduce blood pressure [5, 6].

Despite their potential value, their synthetic protocols are poorly characterized according to literature data. Some characterizations are not clear or contained mistakes. Some authors announced quinoline aldehyde structures with newly formed $C_{quinoline}$-C_{carben} bond in 7 position (phenol ring), instead of 5 (prefer activated position on phenol ring). We obtained and characterized both regioisomers of 8-hydroxyquinoline derivatives by the combination of NMR, GC-MS and TLC and electronic absorption spectroscopy.

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17.

Chemometric analysis of some histamine H_1- and H_2-receptor antagonists on the basis of chromatographic data and molecular descriptors

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Chemometric analysis was performed on 18 compounds with histamine H_1- and H_2-receptor affinity. Thin layer chromatographic data and physicochemical parameters of the examined compounds were applied in this study.

Glass TLC silica gel 60 F_{254} plates (20×20 cm, Merck, Darmstadt, Germany) were used in two developing solvents as H_1-, H_2-antihistaminic interaction models:

a) acetonitrile:water (80:20, v/v)

b) acetonitrile:methanol:water (40:40:20, v/v/v).

Additionally, the stationary and mobile phase were modified with a solution of aspartic acid (L-Asp) and a solution of an analogue of aspartic acid (propionic acid).

The plates were scanned densitometrically at 254 nm by means of a Desaga CD 60 densitometer with Windows-compatible ProQuant software (Desaga, Germany).

The semiempirical method AM1 (HyperChem v. 7.0 program) and ACD/Labs v. 8.0 program were employed to calculate a set of physicochemical parameters for the investigated compounds. The pK_i values of H_1- and H_2-receptor ligands were collected from the literature and used for generating QSAR models. The correlations obtained for the compounds studied represent their interactions with the proposed chromatographic models. The good multivariate relationships obtained by means of regression analysis can be used for predicting the quantitative effect of biological activity of different compounds with histamine H_1- and H_2-receptor affinity. Leave-one-out (LOO) and leave-N-out (LNO) crossvalidation methods were used to judge the predictive power of final regression equations.

This study was supported by Medical University of Lodz, Poland, Research Program No 503/3-016-03/503-31-001.

18.

Chemometric analysis of some dopamine receptor agonists and antagonists on the basis of chromatographic data and molecular descriptors

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Chemometric analysis was performed on 23 drugs with affinity for dopamine receptors. A set of physicochemical parameters calculated by HyperChem 7.0 and ACDLabs 8.0 programs and thin layer chromatographic data were applied in (Q)SAR analysis. Chromatography was performed on glass TLC silica gel 60 F_{254} plates (20×20 cm, Merck, Darmstadt, Germany) impregnated with a solution of L-aspartic acid and a solution of an analogue of aspartic acid (propionic acid) with two mobile phases: a) acetonitrile:water (80:20, v/v) and b) acetonitrile:methanol:water (40:40:20, v/v/v). The systems were chosen as models of drug-dopamine receptor interaction. The pK_i values of dopamine receptor ligands, including agonists and antagonists, were collected from the literature and used for generating these models. Relationships between chromatographic data, molecular descriptors and biological activity data were found by means of stepwise multiple linear regression (MLR) and stepwise discriminant analysis (SDA).

This study was supported by Medical University of Lodz, Poland, Research Program No 503/3-016-03/503-31-001.

19.

Determination of acrylamide in real samples

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Frying or baking of food products improves their taste, but decrease their nutritional value. This is a result of thermal processing of food due to decomposition of some substances (e.g. ascorbic acid), or the formation of new compounds that may affect our body. The second group includes acrylamide (AA) among others, which has neurotoxic, mutagenic and carcinogenic properties - AA binds to hemoglobin and DNA [1]. It is formed as a product of the Maillard reaction between amino acids (mainly asparagine) and reducing sugars such as glucose or fructose. The Maillard reaction is triggered in when food is heated above 120 °C [2].

Acrylamide due to the harmful properties must be monitored. Usually it is done using different chromatographic techniques including high performance liquid chromatography, gas chromatography and liquid chromatography coupled with mass spectrometry or tandem mass spectrometry detection [3]. However, these techniques are in general expensive, time-consuming and laborious.

It is known that AA has quenching properties. It is interesting, that two mechanisms of quenching occurs which can be distinguished by means of fluorescence spectroscopy with liquid nitrogen. This method is simple, cost-effective and very sensitive technique compared with chromatography methods. In static quenching non-fluorescent complex is formed. In second mechanism, quenching occurs as a result of the collision of quencher with fluorescent probe (dynamic/collisional quenching) [4]. If we freeze the sample, we can eliminate a collisional quenching. This property will be used for the construction of advanced chemometric calibration models enabling quantification of analyte under the presence of additional fluorescence interferents [5].

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POSTER SESSION II

THURSDAY, JUNE 2nd, 2016

CHAIRPERSONS: Jelena Trifković

and Agata Kot-Wąsik

1.

Speciation and determination of trace and ultratrace amount of arsenic and selenium on the graphene oxide decorated with cerium oxide

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Heavy metal ions are one of the harmful substances in environment (water, air, plants). They can be created in environment by a mine and car industry or a metallurgical process. Because the heavy metal ions are cancerous the concentration of them are required on trace levels.
The aim of the work was synthesised a new nanocomposide based on the graphene oxide (GO) prepared using modified Hummers method [1]. The next step of synthesis included the surface modification of GO using cerium(III) nitrate [2]. The structure of graphene oxide decorated with cerium nanoparticles (GO/CeO_2) was investigated by spectroscopy and microscopy technique. The structural research confirmed that the GO was covered by cerium nanoparticles (CeO_2) on the whole surface.
Further researches were based on investigation the influence of pH, contact time, sample volume, influence of coexisted ions and humic acid (HA) on the recovery of determined elements. The As(V), Se(IV), Cu(II) and Pb(II) ions were adsorbed by functional groups of GO/CeO_2 and the methodology dispersive micro-solid phase extraction with final measurement energy dispersive X-ray fluorescence spectrometry as non-destructive and eco-frendly technique (DMSPE/EDXRF) was developed. The obtained detection limit (LOD) are 0.072, 0.100, 0.175 and 0.074 for As(V), Se(IV), Cu(II) and Pb(II), respectively. The proposed methodology DMSPE/EDXRF was applied to determine the selected heavy metal ions in the natural water samples.

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2.

Novel insights into pH-dependent retention behavior of analytes in chaotropic chromatography

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Complexity of chromatographic systems modified with chaotropic salts poses numerous challenges both for practitioners, and for those involved in developing a rigorous theoretical description of these systems. Numerous factors that affect retention – type and concentration of the chaotropic salt and organic modifier, and choice of column – have been recognized. Up until recently, however, mobile phase pH was thought to have negligible effects on retention of analytes once their full protonation is achieved. Our group has recently provided experimental evidence that even when no significant changes in analytes' ionization occur, variance in mobile phase pH can exhibit profound effects on their retention. We hypothesized that the observed effects are essentially governed by changes in organic modifier's surface adsorption and magnitude of the surface potential created by chaotrope's adsorption on the stationary phase.

To further test this hypothesis, we profiled the retention behavior of 13 structurally diverse analytes under chromatographic conditions chosen to assure full protonation of the solutes: pH was varied from 2 to 4, sodium hexafluorophosphate concentration from 1 mM to 50 mM, while acetonitrile content in the mobile phase was kept constant at 40%. To discern the contribution of changing ionic strength (I) to variance in retention occurring as function of pH, two sets of experiments were performed – one, in which I was held constant, and another where it was allowed to vary with concentration of chatrope and additives for mobile phase pH adjustment. The obtained data was modeled using the extended thermodynamic approach by Cecchi *et al*.

The obtained results quantitatively support our previous findings regarding the pH-dependent increased affinity of analytes for the stationary phase, caused by differential adsorption of the organic modifier and chaotrope. Furthermore, difficulties in modeling this effect are highlighted. Insights offered by the extended thermodynamic treatment of retention data provided a basis for the development of a physically well founded, empirical model which can be used to predict retention in the studied systems, based on analyte's chemical structure alone. The developed model incorporates both parameters of the chromatographic system, and molecular descriptors which account for key phenomena affecting retention; namely, interactions with the electric double layer and ion pair formation. It, therefore, presents a potentially very useful tool in both fundamental understanding of chaotropic chromatography and its practical application.
in amental understanding of chaotropic chromatography and its practical application.

3.

A comparative study of chromatographic behaviour and lipophilicity of selected imidazoline derivatives

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Chromatographic behavior and lipophilicity of 20 selected imidazoline derivatives was examined by thin layer chromatography using CN, RP-2, RP-8 and RP-18 stationary phases and a mixture of methanol, water and ammonia as mobile phase.

In all the examined chromatographic systems, linear relationships were established between retention parameters and the volume fraction of the methanol in mobile phase ($r>0.985$, 0.978, 0.981, 0.988 for the CN, RP-2, RP-8 and RP-18, respectively). The highest correlation between the obtained R_M^0 values was observed for RP-2 and RP-8 stationary phases. The experimental lipophilicity indices (R_M^0, m and C_0) obtained from the retention data were used in a correlation study with the calculated logP values. Experimentally determined R_M^0 values for all the investigated chromatographic systems exhibited the highest correlation with the calculated ClogP values (r: 0.880, 0.872, 0.897 and 0.889 for the CN, RP-2, RP-8 and RP-18 stationary phases, respectively). The performed QSRR analysis showed that frequency of C-C at topological distance 1 and CATS2D Lipophilic-Lipophilic at lag 01 were important descriptors with an influence on the R_M^0 values in all the examined chromatographic systems, while the differences in the retention behavior of compounds on the examined stationary phases can be distinguished, based on their specific geometrical, electronic and constitutional properties.

4.

Determination of microbiological activity of hop extracts from different varieties of *Humulus lupulus* by TLC + direct bioautobiographic method

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History of beer brewing dates back to 4000 years BC. The first mention of this brewing comes from Mesopotamia, inhabited by the culture of the Sumerians. The first beer was prepared with bread and water. A small amount of ethyl alcohol was created as a result of alcoholic fermentation. Hop as a beer component for the first time was used by the ancient Babylonians.

The content of ethyl alcohol and compounds found in hops allows for longer storage brewed beer.

The aim of our work was comparison of bacteriostatic/antibacterial properties of various hops varieties (*Humulus lupulus* L.) and in next step, isolation of biologically active compounds/groups of compounds from examined hop extracts. There was two solvents used to extraction process: water and ethyl alcohol. Extracts were prepared from two commercially available types of granulates: T-90 (qualitative and quantitative very similar to cone hops) and T-45. Water extracts were prepared using percolation process, choice of method was due to similarity to hopping beer. Ethanolic extracts were obtained by continuous extraction in Soxhlet apparatus. The next, both types of extracts were evaporated and dissolved again to needed volume.

Chromatographic separation was performed on TLC systems: silica/mixture of organic solvents, under earlier found conditions. The samples were introduced as narrow bands (automatic applicator with evaporation of solvent) on silica and TLC plates were developed and dried.

Bioautographic visualization method allowed to find separated fractions of extracts containing substances with bacteriostatic/antibacterial properties.

After isolation (scraping the important bands and washing out it content from bed) of found fractions, the next planned step is re-chromatography in various chromatographic systems and after bioautographic confirmation of its biological properties, prepared to mass spectrometry analysis.

5.

Application of TLC, HPLC and GLC in the analysis of metronidazole and secnidazole

Lina Yu Klimenko, Galyna L. Shkarlat, Oksana V. Shovkova, Zoia V. Shovkova

6.

Studying the changes of excise duty components in diesel oil samples under influence of a reducing agent using gas chromatography with nitrogen chemiluminescence detector

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In EU countries, diesel oil is spiked with chemical substances to indicate tax level and further use. In Poland, diesel oil designated for regular transport contains Solvent Yellow 124 (a marker compound) and Solvent Red 19 or 164 (a red dye) [1]. Even though these substances are considered to be stable, there are many illegal attempts to modify basic physico-chemical properties and thus to eliminate their role as specific fuel markers.
The major goal of this study is to gain knowledge of changes in the content of excise duty components in diesel oil samples induced by a reducing agent.
In the course of experiment 36 different diesel oil samples were analyzed. To describe the studied process, gas chromatographic fingerprints registered with nitrogen chemiluminescence detector were considered since the selected excise duty components contain diazo group(s). In total, 144 fingerprints were collected: (1) for 36 raw diesel oil samples, (2) 36 for their methanol extracts, (3) 36 for diesel oil samples after influence of a reducing agent, and (4) 36 for their methanol extracts. It is important to emphasize that each diesel oil same sample is characterized by four chromatographic fingerprints.
In order to identify differences in the chemical composition of diesel oil samples partial least squares discriminant analysis extended with variable selection based on the selectivity ratio [2-4] was used.

Acknowledgement
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7.

Investigation of micro- and nanostructures spontaneously formed in monocomponent proteinogenic amino acid solutions

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The research presented in paper [1] showed that the amino acids dissolved in aqueous organic solvents spontaneously undergo two parallel processes of chiral conversion and peptidization. As a result of peptidization, peptides are formed giving the nano- and microstructures. Some of spontaneous reactions running in living organisms and resulting in formation of novel peptides are recognized as producing erroneous and harmfully folded proteins. As a result, deposits of such reaction products in form of insoluble fibers are formed, which are considered as a cause of many grave health conditions [2].

In the reported experiment, we focused our attention on the monocomponent amino acid systems: *L*-phenylalanine (*L*-Phe), *L*-methionine (*L*-Met) [3], *L*-phenylglycine (*L*-Phg) and *L*-cysteine (*L*-Cys) [4] dissolved in 70% aqueous methanol. The main tool of investigating peptide micro- and nanostructures was scanning electron microscopy (SEM). As auxiliary techniques, high-performance liquid chromatography (HPLC), and mass spectrometry (MS) were applied. Observations of the oscillatory peptidization reactions of amino acids were possible with use of HPLC. With MS, we confirmed the presence of the spontaneously formed peptides in the investigated solutions.

The obtained results demonstrate that the investigated amino acids can undergo an spontaneous oscillatory condensation, with a consequence that these compounds may form peptide nano- and microstructures in an abiotic system. Furthermore, an evident difference among the peptides originating from different amino acids apparently is due to their different molecular structures.

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8.

The ways of detecting counterfeit plant protection products

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The presence on the market of counterfeit and illegal plant protection products (PPPs) has become a global problem in recent years. Data published by the European Crop Protection Association (ECPA) show that more than 10% of the world pesticide market are composed of illegal and counterfeit products. There are services in the member states of the European Union (EU) whose a one of main goal is an official quality control of plant protection products introduced to the market. In Poland, this task is performed by the Main Inspectorate of Plant Health and Seed Inspection. Samples of plant protection products collected in the official quality check of pesticides by inspectors of the Main Inspectorate of Plant Health and Seed Inspection are sent to the Quality Research Laboratory of Plant Protection Products in the Institute of Plant Protection National Research Institute, Sośnicowice Branch. It is for conducting laboratory studies whose aim is to check whether the samples meet the technical requirements established in the process of their registration. Introducing the illegal and counterfeit pesticides on the market has resulted in the need for steady improvement of methods and using different analytical techniques in order to verify the origin of the delivered to the laboratory samples of PPPs. The subject of research were chemical PPPs which came from all over the country in the years 2011 - 2015 in the official control and individual orders. There were used a few techniques to determine active substances such as: gas chromatography (GC-FID, GC-MS) and liquid chromatography (HPLC, RR-HPLC, LC-MS/MS). The following physicochemical parameters such as: stability of suspension (MT 184 CIPAC K), stability of emulsion (MT 36.3 CIPAC K), wetting time (PN-87/C-04662), sieve analysis (MT 185 CIPAC K), pH (MT 75.3 CIPAC J), density (MT 3 CIPAC F), dissolution degree and solution stability (MT 179 CIPAC H) were examined in the formulations.

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9.

Fatal poisoning of 3-MMC

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The issue of sudden deaths due to acute 3-MMC (3-Methylmethcathinone) poisoning is presented in the report. The analysis included case autopsied. A 20-year-old woman was found dead after her suicide. Biological material were delivered to the Toxicological Laboratory ToxLab placed in Katowice, during the autopsy were subjected to chemical-toxicological analysis. Samples analyzed by performance liquid chromatography coupled with mass spectrometer and PDA detector (LC-PDA-MS). Analysis of blood samples present concentration of the 3-MMC were 800 ng/ml. Analysis of urine present 3-MMC concentrations were 150ng/ml. Analysis of stomach contents samples present concentration of the 3-MMC were 5,5 μg/ml.

10.

Determination of caffeine content in fat-burning supplements

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Obesity is a chronic condition, which increasingly gains ground among the inhabitants of fast-developing countries. BMI values of 25-29.9 kg/m^2 are classified as overweigh range, values above 30 kg/m^2 fall within the obese range, and values greater than 40 kg/m^2 are typically referred to as severe obesity. Obesity is closely related to lifestyle. Extended working hours are conducive to having so called “quick meals”, snacking and having little physical activity. It is often the case that in order to maintain focus for longer periods of time, one reaches for caffeine, often consumed in the form of coffee or tea. It is caffeine that is responsible for alertness, reducing weariness, and decreasing the need for sleep, but its large doses actually conduce to the reduction of body mass. Both scientific evidence and historical reports show that among a healthy population of adults, the consumption of caffeine limited to 400mg a day does not cause negative health effects. Due to the fact that the pharmaceutical market is currently lacking an ideal product for reducing body mass, manufacturers are competing in producing new sets of substances registered as food supplements. The consumption of caffeine, mainly in form of food supplements aimed at reducing the body mass, should not cause dangerous side effects as long as it does now exceed the recommended dose.

11.

Determination of ziprasidone and its impurities by thin-layer chromatography

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Ziprasidone belongs to the second generation of so-called atypical antipsychotic drugs, having the G protein-coupled receptor binding profile. Five ziprasidone impurities representing degradation products of ziprasidone (impurities II, III, and V) or originating from the synthesis (impurity I and IV) are the compounds significantly different in polarity. A thin-layer chromatographic method for simultaneous determination of ziprasidone and its main impurities was developed and validated. Separation of the examined compounds was performed on chromatographic plates precoated with silica gel $60F_{254}$ and using toluene-methanol-glacial acetic acid, 7.5:0.5:0.5 (v/v/v) as mobile phase. The ascending development mode was performed in the twin-trough chromatographic chamber, which was presaturated with mobile phase vapors for 15 min. The developed chromatographic plates were dried in air and densitometrically scanned at the wavelengths of 250 and 320 nm. Regression coefficient ($r \geq 0.992$), recovery (94.94-106.70%), precision (RSD 0.41 to 8.45 %), limit of quantification of impurities (25 ng band-1 equivalent to the 0.14% impurity level), and robustness were validated and found satisfactory. The developed method is convenient for quantitative analysis and the purity screening of ziprasidone in pharmaceutical formulations.

12.

Chromatographic methods for qualitative evaluation of oxidative stress markers in urine samples

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Oxidative stress is regarded as an imbalance in oxidative status of living organism between antioxidant defense and the production of reactive species (e.g. free radicals). It occurs when certain pathological conditions like environmental pollution, stress or limited access to natural sources of antioxidants (e.g. provided with food) influence the system being studied. Reactive species interact with basic components of organisms, i.e. DNA, lipids and proteins. Products of these interactions are considered as oxidative stress markers. The most studied oxidative stress markers are 8-hydroxy-2'-deoxyguanosine, isoprostanes, and dityrosine formed as a result of DNA, lipids and proteins damage. Increased levels of these compounds indicate imbalance in the oxidative status. They can also provide information about increased risk of certain diseases, since reactive species influence regular biochemical pathways [1].

Urine is a product of blood filtration that can describe biochemical changes and as biofluid sample has a number of desired properties. It is (i) less complex matrix with respect to organic and inorganic compounds compared with blood or plasma fluids, (ii) more stable with respect to increase of oxidative markers formed in the course of collection and storage of samples, and (iii) relatively easy to collect.

There are only a few oxidative stress markers bearing in mind 4000 compounds found in urine metabolom [2]. Their quantitation requires separation form the sample matrix, and thus chromatographic techniques are valuable for such studies. In this work we focus of the presentation of methods of preparation and the chromatographic analysis of urine samples of such oxidative stress markers as 8-hydroxy-2'-deoxyguanosine, isoprostanes, and dityrosine.

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13.

Thermal stability of silica based stationary phases for liquid chromatography by vibrational spectroscopy techniques

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Searching for novel approaches to the separation problems reveals numerous limitations related to the instrumentation and materials. Special techniques, like supercritical fluid chromatography, ultra-fast liquid chromatography or high-temperature liquid chromatography set extraordinary requirements when it comes to the instrumentation and employed materials. Many authors underline miscellaneous advantages of silica material as a support material for the chromatographic bed. The high mechanical strength, the rigidity, the large achievable surface areas and the ability to modify the surface chemistry in a highly controlled way, makes silica ideally suitable as packing material in chromatographic columns [1]. At the same time, in the case of silica based stationary phases, higher temperature (60 – 80ºC) accelerates stationary phase degradation in the mobile phase even with neutral pH much more than does increased buffer concentration [2-5].

For a number of reasons, chromatographers want to improve chemical and thermal stability of the chromatographic bed [4,6]. Several authors suggest tests based on chromatographic retention and selectivity combined with the programmed column aging for the stability evaluation. Results obtained in the course of chromatographic tests usually concern temperature (extend) ranging up to 150ºC. Usually much less then 100ºC [5]. Heating the reversed-phase liquid chromatographic beds causes also changes in the intensity of vibrational spectra bends attributed to the particular fragments of covalently bonded ligands. Changes in vibrational spectra of the chromatographic bed sample resulting from heating at temperature above 150ºC indicate the decrease of the alkyl ligands content and, in some cases, occurring the bends attributed to structural fragments of carbonyl, vinyl or aromatic functionalities. Changes in *Raman* scattering intensity, particularly in the range from 3000 to 2800 cm^{-1} may indicate a loss of the stationary phase ligands or even of their chemical transformation occurring during the aging process.

Raman spectrometry is a promising alternative to chromatographic tests performed in the study on the stability of chromatographic beds for reversed-phase liquid chromatography.

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14.

RP-18 thin layer chromatographic study of selected biological and physicochemical properties of substances

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This paper is a part of broader research on the application of Thin Layer Chromatography to prediction of bio-availability of compounds. 21 Randomly selected drugs and excipients of different molecular structures (propylparaben, methylparaben, benzophenone-3, methylbenzylidene camphor, triclosan, diethylamino hydroxybenzoyl hexyl benzoate, nifuroxazide, thioridazine, quetiapine, sulpiride, perazine, zuclopenthixol, flupentixol, telmisartan, hymecromone, capeticabine, acenocumarol, drotaverine, emedastine, atropine, clemastine) were subjected to thin layer chromatography on the RP-18 stationary phase using 30:70 (v/v) binary mixtures of pH 7.4 phosphate-buffered saline – organic modifier (acetonitrile, tetrahydrofuran, 1,4-dioxane, methanol, ethanol or isopropanol) as mobile phases. Selected physicochemical and biological properties of these compounds: lipophilicity (log P), blood and brain barrier penetration ability (log BB), skin permeability (log K_p), human intestinal absorption (%HIA) and plasma protein binding ability (%PPB) were predicted *in silico* using Pre-ADMET server available *via* the Internet. The relationships between the retention of compounds listed above and their predicted properties were investigated. It was concluded that log P, log BB and %PPB of compounds investigated throughout this study are in better agreement with their retention than log K_p and %HIA and that methanol, acetonitrile and ethanol as the organic modifiers give much better correlations than 1,4-dioxane, tetrahydrofuran or isopropanol.

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15.

RP-18 thin layer chromatographic investigations of lipophilicity of selected steroid hormones

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Retention parameters R_f and R_m were obtained for 16 steroid hormones and other drugs, containing the steroid moiety (cortisol, hydrocortisone acetate, methyltestosterone, progesterone, testosterone propionate, testosterone heptanoate, cortisone acetate, prednisolone, estrone, estradiol benzoate, desoxycorticosterone acetate, tibolone, spironolactone, eplerenone, digoxin, dexamethasone) by thin-layer chromatography on the RP-18 stationary phase using pH 7.4 phosphate-buffered saline - acetonitrile 30:70 (v/v) mixture as the mobile phase. R_f and R_m values were correlated with lipophilicities calculated *via* different algorithms (ALOGPs, AClogP, milogP, ALOGP, MLOGP, XLOGP2, XLOGP3, ACDLab) and with experimental $logP_{o/w}$ obtained from the literature sources. Analysis of correlations of R_m values with $logP_{exp}$ proved that the single chromatographic run approach used throughout this study gives sufficiently good result (R = 0.95) and using R_m^0 values extrapolated to zero concentration of the organic modifier is not necessary.

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16.

Chromatographic investigations of plasma protein binding of selected drugs

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21 Structurally diverse drugs (acetaminophen, acyclovir, amoxicillin, aspirin, bromazepam, carbamazepine, chlorpromazine, cimetidine, clonidine, diazepam, famotidine, ibuprofen, indomethacin, naproxen, phenytoin, piroxicam, propranolol, ranitidine, spironolactone, trazodone, zolpidem) were investigated by single-run RP-18 TLC using pH 7.4 phosphate-buffered saline - acetonitrile 30:70 (v/v) mobile phase. R_m values determined for these drugs were correlated with their experimental plasma protein binding ability (%PPB) taken from the literature sources. Linear correlations of R_m (RP-18 TLC) with %PPB explained 64% of the total variance. Moderate as they might appear, the results of the RP-18 TLC experiments prove that this cheap and readily available technique may be a good starting point to obtain chromatographic descriptors useful in generating more complex and (hopefully) predictive models of the compounds' ability to bind to plasma proteins.

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17.

The development of the conditiond for thin-layer chromatographic separation and the detection of bioactive flavonols and depsides in plants

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Phenolic compounds are the most numerous group of chemical compounds in the world of plants. Many scientific studies have shown that they posses a wide range of biological and pharmacological activities. Plant phenolics show antimutagenic, antiproliferative, antioxidant, antiinflammatory, antibacterial and antiviral properties [1-3].
Similarities of chemical structures and physicochemical properties between catechins and depsides cause major difficulties in isolating these compounds from plant materials. The aim of this work was to develop the conditions for the chromatographic separation of (-)-epicatechin, (+)-catechin, cynarin, epigallocatechin gallate, chlorogenic acid, cichoric acid and rosmarinic acid.
As an analytical method thin layer chromatography (TLC) was used. The mixture of compounds were separated by using cellulose plates in 18 developing systems, silica gel plates in 19 developing systems and HPTLC $RP18F_{254}$ plates in 4 systems. The data obtained were used for the simulation analysis in two dimensial chromatography. The optimized separation conditions were used to identify these compounds in the herb and roots of *Lamium album* L., herb of *Echinacea purpurea* (L.) Moench, roots of *Taraxacum officinale* Web. and rhizome of *Polygonum bistorta* L.

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18.

Finding a set of orthogonal solvents for effective plant extraction by chemometric analysis of chromatograms

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Extraction of plant material is a crucial part of the phytochemical study and changing the extraction solvent can change the chemical composition of the extract dramatically. Instead of finding the one optimal solvent, which is often far away from ideal extraction performance, we propose a methodology to choose an "orthogonal" set of several (as few as possible) solvents for separated extraction of the material. These extracts can be merged (mixed) after this process, resulting in extract which is more rich for active plant ingredients belonging to various classes. The choice of orthogonal set is done by chemometric analysis of chromatograms obtained for the same plant material, but various solvent used for extraction. The idea consists of: chromatogram preprocessing (if needed: denoising, baseline removal, warping), principal component analysis, identifying most "extraction-sensitive" peaks by inspection of loading vectors for several first PC (plotting the variance of k first loading values at one wavelength, as a function of the wavelength; for example for 4 PCs, four loading values are taken for each wavelength and the variance of these values is plotted in function of wavelength) and then principal component analysis on heights of identified peaks. As we present the application of this approach to fingerprinting of fruits and roots of *Angelica sylvestris* L. From 17 investigated solvents we chose benzene and toluene as most orthogonal extractants. Other solvents were intercorrelated, so the third solvent can be freely chosen from them.

19.

Photocatalytic degradation study of tiapride by ESI-LC-MS method

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Ultraviolet and visible radiation are one of the most important factors affecting the stability of drug substances. Formed photoproducts may be more toxic than parent molecules, what is problematic also from the environmental point of view. However, radiation could be also used in wastewater treatment to remove pharmaceuticals especially with the use of catalytic agent. In this context, photocatalytic degradation of drugs can be very useful in environmental research as well as in the stability study of pharmaceuticals.

In this study the photocatalytic degradation of tiapride – an atypical antipsychotic drug acting as a selective D_2/D_3 receptor antagonist with the use of titanium dioxide as a catalyst was performed. Water solutions of tiapride hydrochloride were irradiated (0 – 400 min) with the use of solar simulated radiation (UV-VIS), and then analyzed with the use of UHPLC-DAD/ESI-Q-TOF coupled system. Reversed phase chromatographic column (RP-18) and gradient elution of mobile phase consisting of acetonitrile and water with addition of 0.1% formic acid were used. Mass spectrometer was set in a dynamic range and auto MS/MS mode to enable simultaneous qualitative and quantitative analysis. Total time of the LC-MS analysis was 9.5 minutes.

As a result of the above study, four main photoproducts were identified. Three of them were the effect of photolysis reaction and one of photooxidation process. It was also found that the photocatalytic decomposition of tiapride yields the second-order kinetics reaction (k = 0.0011 min^{-1}, $t_{1/2}$ = 90 min). The comparison of the reaction kinetics between the samples with and without addition of titanium dioxide revealed that the presence of the catalyst accelerates the photolysis reaction over four times.

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