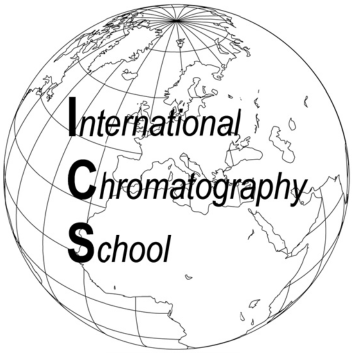
UNIVERSITY OF ZAGREB
FACULTY OF CHEMICAL ENGINEERING AND TECHNOLOGY

19th International Chromatography School



BOOK OF ABSTRACTS

10th – 11th June 2019
ZAGREB, CROATIA

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FINAL PROGRAM

Monday, 10th June 2019

09.00-09.10 Opening of the 19th International Chromatography School
(The Great Lecture Hall at Faculty of Chemical Engineering and Technology, Marulićev trg 20)

Š. Ukić (President of the 19th ICS, HR): *OPENING REMARK*

T. Bolanča (Dean of FCET, HR): *WELCOME REMARK*

09.10-09.40 J. Weiss (Thermo Fischer Scientific, GE; University of Innsbruck, AT):

EMERGING CONTAMINANT ANALYSIS BY LIQUID CHROMATOGRAPHY AND MS DETECTION METHODS

09.40-10.10 M. Cvetnić (University of Zagreb, Faculty of Chemical Engineering and Technology, HR):

DETERMINATION OF DISINFECTION BYPRODUCTS BY IC

10.10-10.50 I. Igrec (Croatian Meteorological and Hydrological Service, HR):

ION CHROMATOGRAPHY MEASUREMENT DATA FOR PREDICTING AIR QUALITY

10.50-11.30 Coffee break

11.30-12.00 M. Đapo-Lavić (University of Mostar, Faculty of Education, BA):

THE EFFECTS OF CHLORIDE FROM WASTEWATER ON THE SPREČA RIVER

12.00-12.20 B. Čeh (Labena, HR):

BIA SEPARATIONS CRO ANALYTICAL LABORATORY

12.20-12.50 A. Ćorović (SP Laboratorija, RS):

NITRATE CONTENT IN GREEN LEAFY VEGETABLES AND ITS POSSIBLE IMPLICATIONS TO HUMAN HEALTH

12.50-14.30 Lunch break (organized by participants themselves)

Monday, 10th June 2019

14.30-15.00 D. Kikić (University of Zagreb, Faculty of Agriculture, HR):

IC AND ZEOLITES AS TOOLS FOR PROTECTION OF AGROECOSYSTEMS

15.00-15.30 M. Livio Jeličić (University of Zagreb, Faculty of Pharmacy and Biochemistry, HR):

IN SILICO PREDICTION OF DRUG PROPERTIES AND HPLC ASSAY METHOD DEVELOPMENT FOR NEW AMINOSALICYLATE/FOLIC ACID FORMULATION

15.30-16.00 M. Markeš (BIOCentre, HR):

DIVERSITY OF PREPARATIVE LIQUID CHROMATOGRAPHY

16.00-16.30 Coffee break

16.30-17.00 I. Tomaz (University of Zagreb, Faculty of Agriculture, HR):

CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF NATURALLY OCCURING POLYPHENOLIC AND VOLATILE COMPOUNDS

17.00-17.40 T. Kovalczuk (LECO Intrumente Plzeň, CZ):

TOF MS: BEYOND THE LIMITS OF CONVENTIONAL GC-MS TECHNIQUES – DISCOVER & IDENTIFY HITHERTO HIDDEN COMPOUNDS

17.40-18.10 L. Dončević (University of Zagreb, Faculty of Sciences, Department of Chemistry, HR):

MULTIDIMENSIONAL CHROMATOGRAPHY USING LIQUID HANDLING PLATFORM

Tuesday, 11th June 2019

09.00-09.20 G. Dinter (Labtim Adria, HR):

MS TROUBLESHOOTING STRATEGIES

09.20-09.40 L. Hollosi (Thermo Fischer Scientific, AT):

SCREENING FOR KNOWN AND UNKNOWN COMPOUNDS WITH GC COUPLED HIGH AND LOW RESOLUTION MASS SPECTROMETRY – OPPORTUNITIES AND BOUNDARIES

09.40-10.20 L. Ivanjko (Shimadzu, HR):

INCORPORATION OF ARTIFICIAL INTELLIGENCE IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SYSTEMS – NEW NEXERA LC-40 SERIES

10.20-11.00 Coffee break

11.00-11.30 S. Zubčić (Agency for Medicinal Products and Medical Devices, HR):

DEVELOPMENT AND VALIDATION HPLC-UV-MS METHOD FOR IDENTIFICATION AND QUATIFICATION OF PHOSPHODIESTERASE 5 INHIBITORS IN DIETARY SUPPLEMENTS FOR ERECTILE DISFUNCTION

11.30-12.00 V. Stankov (Sample Control, HR):

DETERMINATION OF TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD) IN COSMETICS BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION MASS SPECTROMETRY (UHPLC-MS/MS) USING MODIFIED QuEChERS METHOD

12.00-12.30 D. Marinković (SP Laboratorija, RS):

REVIEW OF RESULTS OBTAINED FOR ACRYLAMIDE IN BISCUITS

12.30-14.00 Lunch break (organized by participants themselves)

14.00-14.30 M. Bulajić (Inspecto Ltd., HR):

MATRIX EFFECT IN QUANTITATIVE ANALYSIS OF PESTICIDE RESIDUES IN FOOD BY GC-MS/MS

14.30-15.00 G. Jurak (Andrija Štampar Teaching Institute of Public Health, HR):

MULTIRESIDUE PESTICIDE ANALYSIS USING GC-MS/MS

15.00-15.30 K. Vinković (University of Zagreb, Faculty of Science, Department of Chemistry, HR):

BISPHENOL A ANALYSIS DURING THE ANAEROBIC DEGRADATION OF PAPERS WITH THERMOCHROMIC PRINTS IN SOIL

15.30- **Closing of 19th International Chromatography School**

Sample delivery for 18th Proficiency Test

Award of the certificates

PREFACE

Welcome to 19th ICS

Chromatography is one of the most versatile analytical techniques nowadays, most probably due to the fact that it simultaneously enables separation, identification, and quantification of diverse analytes, commonly in very complex matrices. Even more, numerous new inventions and improvements in a field of chromatography, followed by related applications, support significantly the continuous growth of its popularity. All this progress has been accomplished because there was an understanding of physico-chemical principles of chromatographic process. Therefore, as an expert in chromatography, each participant also needs to understand these principles and to learn how they are implemented into their daily practice.

International Chromatography School started and is still guided by idea to be a bridge that connects chromatographic theory and every-day practice; it provides an unbiased education based on scientific facts and long experience. The Organizers hope that holistically oriented policy of International Chromatography School (no registration fees) will contribute in higher accessibility of chromatographic information and thus make the difference. The School is an excellent for those who follow the global mainstream of lifetime learning as well, providing an insight to numerous new technical solutions and trends in a field of chromatography. Last, but not least, it is very likely that solutions for many your actual chromatographic problems can be found in some of presented lectures or that you can reach them through discussion with other school participants.

Therefore, welcome to the 19th International Chromatography School, collect the provided knowledge, meet new colleagues and make new friends!

Assoc. Prof. Šime Ukić
President of the Organizing Committee

We would like to thank sincerely all the lecturers, sponsors and donors for their contributions at 19th ICS.

LECTURES

EMERGING CONTAMINANT ANALYSIS BY LIQUID CHROMATOGRAPHY AND MS DETECTION METHODS

J. Weiss[1,2]

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The environmental fate and ecotoxicology of contaminants of emerging concern (CECs) need to be prioritized due to their bioaccumulation potential as well as suspected detrimental effects on wildlife and humans. CECs consist of a wide range of compounds, including endocrine-disrupting chemicals (EDCs), personal and pharmaceutical care products (PPCPs), drugs of abuse (DA), brominated flame retardants, perfluorinated alkyl compounds, polar pesticides, and disinfection by-products that have been shown to cause developmental and reproductive disorders in aquatic organisms. There is an urgent need to better understand the fate of CECs in wastewater treatment plants (WWTPs), since these facilities were not designed to efficiently remove CECs. Therefore, CECs are eventually released into the environment via WWTP effluents or bio-solids. Given the complexity of wastewater matrices, extensive sample clean-up and preconcentration steps, as well as sensitive detection methods, are necessary to detect and quantitate CECs found at trace levels. In many cases, SPE has to be employed as a sample preparation step. In this presentation, innovative methods are shown for the quantification of CECs from different chemical classes (hormones, HAAs, polar pesticides, perfluorinated alkyl compounds, PPCPs, etc.) in drinking and wastewater.

DETERMINATION OF DISINFECTION BYPRODUCTS BY IC

M. Cvetnić[1], M. Markić[1], N. Kolar Jukić[2], T. Bolanča[1]

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Disinfection of drinking and wastewater is required to protect the public from pathogenic microorganisms. When the disinfectants, such as chlorine, chloramines, chlorine dioxide, and ozone, are added, they reacts with inorganic or organic matter and produces disinfection byproducts (DBPs), react with inorganic or organic matter. Common DBPs produced in drinking water include chlorate, chlorite, bromate, trihalomethanes, and haloacetic acids (HAAs). They potentially pose a health threat and because of that are regulated. Type and number of DBPs differ with the presence and amount of different inorganic and organic matter, the water temperature and pH, and the dose of disinfectant.

Well-known DBPs contain chlorine and bromine, but most recently, emerging DBPs have been found to contain iodide. According to research studies, the toxicity of DBPs is in this order: DBP (I) >> DBP (Br) > DBPs (Cl). Ion chromatography (IC) is an established technique worldwide for monitoring water, including surface, ground, drinking, and wastewaters. By EPA Methods (EPA Method 300.0 and 300.1), IC is used for monitoring inorganic anions, fluoride, chloride, bromide, nitrite, nitrate, sulfate, phosphate but also oxyhalides, including chlorite, chlorate, and bromate.

HAAs are also part of DBPs that are found to increase the risk for cancer, are generated during the water chlorination process. Of the nine known species of HAAs (HAA9) found in the treated water, five (HAA5) are currently regulated: Monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA). EPA Methods (552.1, 552.2, and 552.3) use gas chromatography with electron capture detection (GC-ECD) to determine the level of HAAs in drinking water. The drawback of these methods is the use of derivatization and multiple extraction steps which can take a total 30 hours to complete the analysis. EPA Method 557 uses ion chromatography (IC) coupled with mass spectrometry (IC-MS/MS), providing sensitive and rapid detection without sample pretreatment. Also, the 2-D IC method for HAAs is a viable alternative to EPA methods 552.3 and 557.

In order to deal with these challenges, significant work has been done utilizing matrix elimination and/or pre-concentration in order to minimize interference and achieve lower detection limits. With simple adjustment of the IC system you can monitor DBPs and protect human health.

ION CHROMATOGRAPHY MEASUREMENT DATA FOR PREDICTING AIR QUALITY

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The modernization of the national network for air quality monitoring aims to support the legislative framework for air quality and environmental protection. The main goal of such modernization is to establish an integral air quality control system that is compliant with all European and national criteria regarding environmental protection and human health. In regard to chemical analysis, this entails implementation of some new methods for measurements of atmospheric precipitation chemistry in order to monitor and better understand air quality. In that light, ion chromatography is the main tool for conducting chemical analysis of precipitation, the result of which quantifies air pollutants that can be used to track and predict the source of air pollution. Currently, the atmospheric precipitation chemistry and specification of main ions in particulate matter is being conducted, which includes deposition of main ions, mainly sulfur and nitrogen, base cations and pH. The new system has been obtained so as to expand the measurement of air pollution parameters, such as carbohydrates, amine and organic acids. For scientific purposes, ion chromatography is the most valuable and suitable tool for scanning the precipitation and PM eluates for various water soluble components – it could give us a new insight into air pollution and enable us to acquire more measurement data for air quality prediction.

THE EFFECTS OF CHLORIDE FROM WASTEWATER ON THE SPREČA RIVER

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One of the important activities of the "Sava River Watershed Agency", Sarajevo, as defined in Article 156 of the Law on Waters of the Federation of Bosnia and Herzegovina ("Official Gazette of the Federation of Bosnia and Herzegovina" No. 70/06), is a water monitoring organization in the Sava River Basin in the Federation of Bosnia and Herzegovina. The Sava River Watershed Agency, Sarajevo, since 2005 has been organizing surface water monitoring in the Sava River Basin in the Federation of Bosnia and Herzegovina and since 2011 has been aligned with the guidelines of the EU Water Framework Directive (2000/60/EC) with the aim of establishing water status in the Federation of Bosnia and Herzegovina in the water area for which it is competent. By monitoring the deviation of the selected parameters from the required classes or environmental quality standards for the priority substances in the subsoil, it is evident that the riverbed of the river Bosnia has the worst quality of water from all the controlled pools in the Sava River Basin in the Federation of B&H.

One of the main sources of pollution is urban wastewater, which without direct treatment is directly discharged into the watercourse, with several exceptions, where there are facilities for purification and purification of municipal wastewater. There are also unprocessed industrial wastewaters, and their impact is particularly pronounced in the Tuzla industrial pool, which negatively affects Spreča River quality.

Several major industrial plants in the mining, energy, and chemical industries are releasing wastewater into the Spreča River. Wastewater is discharged into the recipient mainly directly without prior purification or partially treated. Spreča River is therefore exposed daily to industry and urban wastewater pressures, resulting in persistent disturbances in plant and

animal associations or loss of biodiversity. In addition, this is also a danger to human health. Long-term studies have shown that a large number of parameters outside of the required classes, outside the environmental quality standard for priority substances, are located in the exploration areas of the Spreča River. One of these parameters is chlorides. The determination of chloride content was carried out in accordance with standard BAS EN ISO 10304–1:2010 "Water quality. Determination of dissolved anions by liquid chromatography of ions – part 1. Determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate and sulphate".

It is known that elevated chloride concentrations have a toxic effect on aquatic organisms, leading to a decrease in biodiversity. Since biological parameters of water quality, especially phytobenthos, sensitive to chloride exposure, the aim of this paper is to examine the effect of secondary salinization on river ecosystems. To monitor the effects of chlorides, algae are used as bioindicators because they are the most sensitive.

Keywords: wastewater, chloride, ion chromatography, secondary salinization, algae, bioindicators

BIA SEPARATIONS CRO ANALYTICAL LABORATORY

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BIA Separations CRO is a research GMP/FDA laboratory that deals with the development of chromatographic methods for the pharmaceutical and biotechnology industry. It is research laboratory providing high quality services in the field of chromatography, dissolution testing and molecular biology. We are working with pharmaceutical industries from the region and from the EU and SAD. In our lecture we will present the GMP/FDA concept of work and what is important when it comes to pharmaceutical industry.

NITRATE CONTENT IN GREEN LEAFY VEGETABLES AND ITS POSSIBLE IMPLICATIONS TO HUMAN HEALTH

A. Ćorović, D. Marinković, Đ. Vujić, M. Obradović, R. Trifković, B. Marošanović

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Nitrate is an approved food additive as well as a naturally occurring compound that can be found in soil, water and food as a part of nitrogen cycle. Vegetables can be considered as the main source of dietary nitrate intake. Green leafy vegetables are foods that contain considerable amounts of nitrate which can have both positive and negative effects on human health. The presence of nitrate is often associated with toxic effects of methemoglobinemia and the possibility of causing an endogenous formation of carcinogenic N-nitroso compounds. On the other hand it is also associated with beneficial health effects, since nitrate represent an important alternative pathway to bioactive NO (nitric oxide) and its important physiological roles in vascular and immune function.

Most European countries have regulated the nitrate content in food. Commission Regulation EU No 1258/2011 amending Regulation EC No 1881/2006 regulates maximum levels for nitrates in certain type of lettuce, spinach and rucola, and these have also been adopted by Serbian Regulation.

Our objective was to determine nitrogen content of commercial leafy vegetables samples commonly grown in Serbia analysed in the past 5 years and to compare them with relevant legislation. Furthermore, we wanted to determine whether there is a significant difference in nitrate accumulation in these vegetables concerning sampling period of the year.

Prior to analysis, the fresh plant samples were minced and homogenised and samples were prepared according to SRPS EN 12014-2:2008. Analyses were carried out on the Ion Chromatograph with UV detection. For the separation we used Ion Pac AG14 column (4×250 mm) and Ion Pac AG14 guard column (4×50 mm).

We compared results of 156 samples (70 samples of fresh lettuce, 24 samples of Iceberg lettuce, 44 samples of fresh spinach and 18 samples of rucola) analysed during two seasonal periods (April-October and October-April), during the past five years. The results varied significantly from 64 mg/kg in fresh lettuce to 4932 mg/kg in rucola. All of the obtained results were in compliance with legislation. The results showed that nitrate concentration is significantly higher in almost all of the analysed samples during the second seasonal period (October-April). Rucola contains the highest concentration of nitrate and can therefore influence nitrate intake.

In human organism, as many other essential mineral substances, nitrates also possess a positive and negative role. Which of these roles will prevail depends mainly on the intake. Excessive nitrate intake is in connection with risk for emergence of diseases. Therefore, it is very important to widen the awareness of the public on the appropriate dietary habits as well as to follow the principles of good agricultural practice.

IC AND ZEOLITES AS TOOLS FOR PROTECTION OF AGROECOSYSTEMS

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Facing the challenges of many global trends (overpopulation, climate changes, hunger, natural disasters, *etc*.) agriculturists have two important tasks: to insure a food security and to preserve natural resources. In order to achieve increase yield of cereals, all efforts are insignificant without nitrogen application, which is now, in modern agriculture an indispensable practice. That practice leads to many environmental problems (surface and ground water eutrophication, acidification of soils, air quality degradation) which require new remediation approaches for soils and waters. Zeolites, natural or synthetic hydrated aluminum silicates, due to their ion-exchange properties are recognized as effective tool for binding of hazardous inorganic components in soil and water systems. The aim of this research was to determine the influence of increased rates of natural zeolite (Clinoptilolite) on ionic composition of water contaminated by nitrogen fertilizers.

The research included three laboratory experiments (with two different nitrogen fertilizers in three different production formulas: NPK 7-20-30, NPK 15-15-15, and KAN) which were consisted of five treatments in three repetitions, a total of 45 water samples. Each experiment involved: control treatment (water of Bliznec stream), artificially contaminated water (water of Bliznec with addition of certain nitrogen fertilizer) and three treatments of contaminated water treated with different amounts of zeolite (5, 10, 15 g / 200 ml water). Water, fertilizers and zeolites were in contact two weeks.

After two weeks samples were filtrated in two steps: firstly through filter paper and then through cartridge (OnGuard IIP, 1cc, Dionex, Sep-Pak Vac and OASIS HLM, Waters). Anions separation and detection were performed on Doinex ICS-1000 system: separator column [Ion Pac AS 17 (4×250 mm) Dionex]; KOH eluent with gradient elution: (A) 10 mmol L^{-1} KOH from 0-5.5 min, (B) 10-35-10 mmol L^{-1} KOH from 5.5-16.6-18 min, respectively, and (C) 10 mmol L^{-1} KOH from 18-30 min (total separation time 30 min); flow rate: 1.0 mL min^{-1}; detection: suppressed conductivity. Cation separation and detection were performed on Doinex

ICS-1000 system as well: separator column [Ion Pac CS 16 (5×250 mm) Dionex]; MSA eluent (30 mM) constant during analysis (total separation time 30 min); flow rate: 1.5 mL min^{-1}; detection: suppressed conductivity.

Statistical analyses of differences in ion concentrations according to zeolite application were computed by analysis of variance (ANOVA) using SAS Institute 9.1.3 and mean values were separated by Fisher's LSD test at $P \leq 0.05$.

Results indicate the capability of Clinoptilolite to ion exchange and its applications in remediation of contaminated water but also that ion chromatography method is suitable method for determination of ion contamination in water. Concentrations of $N\text{-}NH_4$, $N\text{-}NO_3$, and SO_4^{2-} significantly increased compared to control application of all nitrogen fertilizers. Additionally application of NPK fertilizers contributed to significant increment of Cl^- and PO_4^{3-} ions. Compared to the concentrations in contaminated water samples, which were 68.9 mg $N\text{-}NH_4/L$ (in treatment with NPK 7-20-30), 99.0 mg $N\text{-}NH_4/L$ (in treatment with NPK 15-15-15), and 183.3 mg $N\text{-}NH_4/L$ (in treatment with KAN), applications of different doses of Clinoptilolite significantly decreased $N\text{-}NH_4$ presence in water in range from 19.6 to 25.0 %. Also, significant decrement of PO_4^{3-}, SO_4^{2-} and Cl^- ions in water was recorded just in treatment with the highest dose of zeolite. Only concentrations of $N\text{-}NO_3$ were not significantly changed after Clinoptilolite applications. Further detail investigations can be focused on determination of sufficient zeolite dose which will lead to decrement of ions concentration on level present in natural and non-contaminated conditions.

Keywords: Clinoptilolite, ion chromatography, nitrogen fertilizers, remediation, water

IN SILICO PREDICTION OF DRUG PROPERTIES AND HPLC ASSAY METHOD DEVELOPMENT FOR NEW AMINOSALICYLATE/FOLIC ACID FORMULATION

M. L. Jeličić, E. Brusač, D. Amidžić Klarić, B. Nigović, A. Mornar

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In silico prediction of drug metabolism and fate is a growing approach in drug development. Various studies should be performed as early as possible in the drug development process to get a brief insight into molecule characteristics, such as absorption, distribution, metabolism and excretion (ADME). Methods used to examine molecule characteristics such as lipophilicity, solubility and plasma protein binding require active pharmaceutical ingredient to be synthesized and analyzed and that is, in first place, expensive, but also time consuming. *In silico* approach offers fast and inexpensive way to obtain information about the specific molecule through many *on-line* prediction platforms.

Inflammatory bowel disease is a chronic inflammatory state that can affect the entire gastrointestinal tract, commonly known as Crohn's disease, or it can take place mainly in the colon, which is known as Ulcerative colitis. Due to damaged sidewalls of intestine folic acid is malabsorbed, and as consequence, leads to development of megaloblastic anemia. Novel fixed dose combination of one aminosalicylate (mesalazine, sulfasalazine, olsalazine and balsalazide) and folic acid could be used in the treatment of inflammatory bowel disease to simplify the therapy and to make sure that folic acid is compensated, especially in the case of sulfasalazine and olsalazine, which additionally inhibit its absorption.

To characterize active pharmaceutical ingredients, lipophilicity and ADME parameters should be investigated and therefore correlated with in silico predicted values to investigate their applicability. Furthermore, to control the quality of proposed fixed dose combination, analytical methods should be developed.

Experiments were done on HPLC-DAD system using high throughput methods and various columns from conventional octadecylsilyl column to biomimetic chromatographic columns such as: immobilized artificial membrane (IAM), human serum albumin (HSA) and α-1 acid glycoprotein (AGP). Experimental results were correlated with predicted parameters such as lipophilicity, plasma protein binding and Caco-2 permeability from 14 *on-line* platforms. Six of them showed good correlation with experimentally obtained values (*R* from 0.89 to 0.96), implying that *in silico* prediction has great potential in screening of similar properties in drug discovery.

Second part of the research was to develop a single RP-HPLC-DAD method for determination of assay in proposed fixed dose combination of each representative of aminosalicylate group and folic acid. The method was developed on Waters XBridge Phenyl (150×4.6 mm, 3.5 µm) column with gradient elution using 0.2% (*v/v*) HCOOH in water as mobile phase A and 0.2% (*v/v*) HCOOH in methanol as mobile phase B. The method was validated according to ICH Q2(R1) guideline where all obtained validation parameters were within the acceptable ranges. Therefore, according to obtained results, it can be confirmed that the method is suitable for future research.

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DIVERSITY OF PREPARATIVE LIQUID CHROMATOGRAPHY

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Preparative liquid chromatography is used for purification and collection of targeted molecule(s) from other components present in starting material. The aim is to purify the molecule quickly, obtain a high yield and good purity so that purified sample can then be used for further research purposes.

Like in analytical liquid chromatography, preparative separations use all types of liquid chromatography (SEC, IEX, RP, affinity chromatography etc.) but in this case, selection of chromatography type depends not only on chemical properties of starting material and target molecule but also on final objective of the purification – final purified product must be compatible with further applications. Separation also varies in specificity – from targeting one specific molecule, over purifying couple of target molecules up to non-specific separation of whole starting sample into a number of fractions. In addition, separation is carried out in wide range of quantity – from couple of micrograms for research purposes up to kilograms at production scale. All this results in very wide application area.

This lecture will illustrate this diversity of preparative liquid chromatography by including some applications performed in laboratory for downstream processing in BICRO BIOCentre.

CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF NATURALLY OCCURING POLYPHENOLIC AND VOLATILE COMPOUNDS

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Different plants contain more than 1000 different chemical compounds that can be divided into primary and secondary metabolites. Primary metabolites are related to the anabolic and catabolic processes needed for living cells and proliferation. Examples are sugars, acids, amino acids etc. Secondary metabolites are compounds that are not directly involved in photosynthesis or breathing but are essential for the plant to survive in its environment. Among secondary metabolites, the most important are polyphenols, carotenoids and volatile compounds (VOCs). Application of analytical technique for determination of above-mentioned classes of compounds is strongly dependent upon physio-chemical properties of analytes. For the determination of polyphenolic compounds and carotenoids the most frequently applied chromatographic technique is High-Performance Liquid Chromatography (HPLC) while for the determination of VOCs Gas Chromatography-Mass Spectrometry (GC-MS) is a technique of choice. Secondary metabolites are usually contained in solid parts of plants thus the application of some extraction technique before HPLC or GC-MS analysis is mandatory. Extraction of phenolic compounds can be done by applying one of the following extraction techniques: solid-liquid extraction, ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, matrix solid phase extraction etc. VOCs can be extracted by application of solid-liquid extraction, ultrasound-assisted extraction, solid phase extraction, solid phase microextraction and stir bar sorptive extraction. Each of the mention techniques has some features and requirements which must be optimized during the development of the method.

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TOF MS: BEYOND THE LIMITS OF CONVENTIONAL GC-MS TECHNIQUES – DISCOVER & IDENTIFY HITHERTO HIDDEN COMPOUNDS

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Although the basics of Time of flight mass spectrometers (TOF MS) were established in 30s of 20th century, due to the lack of fast electronic the renewal of this technology was postponed to early 90s of last century. At this time the enormously fast, but extremely sensitive, first commercially available GC-TOF MS systems were introduced to the market. The nature of TOF MS technology, such as fast and sensitive acquisition of unskewed MS spectra, was followed by implementation advanced mathematical algorithms of data mining – automated peak find, deconvolution and scripting.

The potential of marketed fast GC-TOF MS instruments equipped with ion source not requiring its routine cleaning was later on extended by their combination with comprehensive gas chromatography (GC×GC).

The GC×GC-TOF technique enabled discovering of previously hidden sample components by greatly increased separation capability employing two columns with different separation principles connected via orthogonal modulator. Thanks to focusing of analytes in the thermal modulator, the overall sensitivity was improved over conventional one dimensional systems.

The principle of TOF MS analyzers are predestining them to fulfill nowadays demanding requirements on any analytical technique - "One run covering all analyst's requirements, such as target and non-target screening, qualitative and quantitative capabilities along with easy and fast hardware-software operation"

Within a contribution the emerging applications of GC and GC×GC-TOF MS will be shown.

MULTIDIMENSIONAL CHROMATOGRAPHY USING LIQUID HANDLING PLATFORM

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Multidimensional chromatography followed by electrospray ionization mass spectrometry was used to characterize two different samples, mycoplasma in human urine samples and lipids from vegetable. Automated liquid handling platform is a strong tool for fast and precise chromatographic separation which can provide 6 fractions of 96 samples simultaneously in less than 120 minutes. Although a state-of-the-art technology, lack of suitable and non-reusable commercially available cartridges limits such automated platform in scientific research. A necessity for specific chromatography separation has led us to modify commercial chromatographic cartridges. Cartridges were detached and filled with more suitable stationary phase for sample separation and method procedures with sufficient recovery rate were developed for both chromatographic sample separation. Quaternary methylammonium (QMA) was used as a strong anion-exchange stationary phase for fractionation of peptides extracted from mycoplasma. Besides, polar silica sorbent was used as a stationary phase to retain molecules in nonpolar matrices, which can effectively separate compounds with small variations in structure, such as lipids. Afterward, six fractions of each sample were analyzed using LC-MS system (nano-LC-ESI-qTOF) and the raw data were processed using protein database search engine. Different stationary phases can be used for the development of a new multidimensional chromatography using liquid handling platform. Commercially available cartridges can be easily reused and loaded with desirable stationary phase. Not less important, such automated platform is no longer limited and can be used for various types of samples.

MS TROUBLESHOOTING STRATEGIES

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LC-MS troubleshooting is skill that is slowly phasing out. This lecture is providing general scheme for structured step by step troubleshooting in LC-MS laboratories.

Systematic approach has many benefits such as: elimination of easy common problems, isolation of crucial parts and targeting of critical errors.

Focus will be on MS troubleshooting with some practical advices for both novice and for routine MS users.

Topics covered will include:

- Overview of Troubleshooting Strategy
- ESI sources parameters
- Single and triple Quadrupoles
- SIR vs MRM
- Ion Suppression

SCREENING FOR KNOWN AND UNKNOWN COMPOUNDS WITH GC COUPLED HIGH AND LOW RESOLUTION MASS SPECTROMETRY – OPPORTUNITIES AND BOUNDARIES

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Thermo Fisher Scientific is world leader in serving science providing high- to top-end technologies for the most challenging analytical applications. Latest developments in mass spectrometer technology enables users on one hand side simplification of analytical efforts, reducing sample preparation needs for more and more complex analytical tasks. Analysis and evaluation of hundreds of targeted compounds within shortest time and minimal method development and instrument down time has never been so easy and obvious as it is nowadays with e.g. the latest triple quadrupole technology.

On the other hand stretching the boundaries and reaching higher and higher mass resolution (>100 K) and mass accuracy (HRAM) with Orbitrap™ technology introducing more and more confidence in to measurement results besides opening up a totally new dimension of data interpretation.

In current presentation an overview will be given about the latest technology developments on GC coupled mass spectrometry at both low (triple quadrupole MS) and high resolution and mass accuracy (GC Orbitrap) demonstrating how these developments lead to real practical benefits and enormous efficiency in the practical work in routine or exploratory research projects.

In addition to that also typical targeted and non-targeted analytical workflows using the above instrumentations will be presented. As practical examples targeted analysis of food safety relevant compounds (> 400 pesticides) with in-house validated data will be shown as well as application of HRAM MS workflows for unknown compound identification in a toxicology relevant case-study.

INCORPORATION OF ARTIFICIAL INTELLIGENCE IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SYSTEMS – NEW NEXERA LC-40 SERIES

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Shimadzu released the new Nexera Ultra High Performance Liquid Chromatography series which incorporates artificial intelligence as Analytical Intelligence, allowing systems to detect and resolve issues automatically. The Nexera series makes lab management simple enabling users to easily review instrument status and achieve higher throughput.

Operators can set the Nexera to start up at a specified time, so that it can complete auto-purge, equilibration, baseline checks and system suitability in advance, and be ready for analysis before they arrive at the lab. In addition, FlowPilot ramps up the flow rate gradually, reducing the possibility of damage to columns. The Nexera also has auto-diagnostics and auto-recovery capabilities that allow it to monitor pressure fluctuations to check for anomalies.

Real-time monitoring of mobile phase levels allows lab personnel to efficiently run batches and respond accordingly if there isn't enough mobile phase before starting a run. In addition, Nexera tracks consumable usage and sends alerts when parts need replacing, allowing users to keep the system running at peak performance.

The Nexera series photodiode array detector SPD-M40 is equipped with a UV cut-off filter that excludes light in the ultraviolet range, in order to ensure more stable detection of compounds that are prone to photodegradation. Here we introduce examples of improving the quantitation of ibuprofen using the UV cut-off filter function of the SPD-M40. Since the new SPD-M40 has completely eliminated the effects of stray light during detection, it provides a wide dynamic range, low noise and high sensitivity which makes it ideal for impurity analysis in pharmaceutical products.

The Nexera's elegant, compact design saves valuable bench space. Because it uses over 80 % less electricity when on standby, it significantly reduces running costs and supports an environmentally-friendly lab.

DEVELOPMENT AND VALIDATION HPLC-UV-MS METHOD FOR IDENTIFICATION AND QUATIFICATION OF PHOSPHODIESTERASE 5 INHIBITORS IN DIETARY SUPPLEMENTS FOR ERECTILE DISFUNCTION

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In recent years, we are witnessing the increased use of dietary supplements with herbal substances. In the highly competitive dietary supplements market, there are an increasing number of products intended to help the normal sexual function of a man with the claim to be of a purely natural origin. However it was reported that a considerable number of such products contain pharmacologically active substances, which is not allowed according to the article 3 of the Regulations on Dietary Supplements. Pharmacologically active substances that can often be detected in such dietary supplements belong to a group of highly competitive phosphodiesterase type 5 inhibitors (PDE5-Is). This enzyme is crucial in regulation of male erectile function. In Croatia, registered PDE5 inhibitors are following: vardenafil, tadalafil, and sildenafil.

Need for development of analytical methods for identification and determination of vardenafil, tadalafil and sildenafil has risen due to apparent increase in number of suspected illicit supplements for sexual dysfunction. Consequently, liquid chromatography coupled with mass spectrometry (LC-MS) emerged as a method of choice. It can be described as a physico-chemical analytical technique that combines the power liquid chromatography to separate diverse components which are afterwards analyzed by means of mass spectrometry.

International Conference on Harmonisation (ICH) and European Directorate for Quality of Medicines and Healthcare (EDQM) both propose guidelines for the parameters, principles and acceptance criteria for method validation of quantitative methods. According to previously mentioned guidelines developed method was validated by the following criteria: method selectivity/specificity, response detector linearity, accuracy, method precision and robustness. The validated method is used as an official standard method in Official Medicines Control

Laboratory (OMCL) in Agency for Medicinal products and Medical Devices (HALMED) for all dietary supplements suspected of containing vardenafil, tadalafil and sildenafil.

DETERMINATION OF TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD) IN COSMETICS BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION MASS SPECTROMETRY (UHPLC-MS/MS) USING MODIFIED QuEChERS METHOD

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In the past 50 years, *Cannabis sativa* (C. sativa) has gone from a substance essentially prohibited worldwide to one that is gaining acceptance both culturally and legally in many countries for medicinal and recreational use. As additional jurisdictions legalize Cannabis products and the variety and complexity of these products surpass the classical dried plant material, appropriate methods for measuring the biologically active constituents is paramount to ensure safety and regulatory compliance. While there are numerous active compounds in C. sativa the primary cannabinoids of regulatory and safety concern are (-)-Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and their respective acidic forms THCA-A and CBDA [1].

The present method describe determination of THC and CBD in various cosmetic products using QuEChERS extraction salts (4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogencitrate sesquihydrate) by ultra-high performance liquid chromatography-electrospray ionization mass spectrometry (UHPLC-MS/MS).

THC and CBD are extracted from 0,1 g of cosmetic products (anti age moisturizer, acne serum, eye gel, rescue balm, men's after shave moisturizer, relief massage) with 20 mL of 50 % acetonitrile in 0,1 % formic acid in water and 6,5 g of QuEChERS extraction salts is added to enhance extraction efficiency.

Their identification and quantification is achieved through positive MRM (multiple reaction monitoring) mode (315.3 →193.1 (24 eV), 315.3 →123.1 (35 eV).

Separation was done with analytical column Shim-pack XR-ODS III (2.0×150 mm, 2.2 μm) and the mobile phase was 5 mM NH_4HCO_2 in 0.01 % formic acid in methanol and 5 mM NH_4HCO_2 in 0.01 % formic acid in water flowing under gradient elution.

THC was found in cosmetic products in concentration range from 0.0013 to 0.0046 % and CBD was found in concentration range from 0.088 to 1.3 %.

Limit of quantification for THC and CBD in cosmetic products was 0.001 % and recovery was in the range from 90 to 110 %.

All the analyzed cosmetic products contain THC above the Canadian legal limit of 10 μg/g and France has zero tolerance for THC.

In the European Union, it is legal to cultivate and supply cannabis plants for hemp fiber if they have low levels of THC. The granting of payments under the Common Agricultural Policy is conditional upon the use of certified seeds of specified hemp varieties; only varieties with a THC content not exceeding 0.2 % may be used (EU Regulation 1307/2013).

Reference:

1. Q. Meng, B. Buchanan, J. Zuccolo, M.-M. Poulin, J. Gabriele, D. C. Baranowski, A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products, *PLoS ONE* **13** (5) (2018) e0196396.

REVIEW OF RESULTS OBTAINED FOR ACRYLAMIDE IN BISCUITS

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Acrylamide is organic compound which forms from the naturally occurring constituents of amino acid asparagine and reducing sugars in certain foods when prepared at temperatures typically higher than 120 °C and low moisture. It forms mainly in baked or fried carbohydrate-rich foods where raw materials contain its precursors, such as cereals, potatoes and coffee beans. The main chemical process that causes this is known as the Maillard Reaction – it is the same reaction that „browns" food and affects its taste. Acrylamide is a contaminant as defined in Council Regulation (EEC) No 315/93 (2) and as such, it is a chemical hazard in the food chain. The current levels of dietary exposure to acrylamide across age groups indicate a concern with respect to its carcinogenic effects.

SP Laboratorija is accredited for acrylamide analysis in accordance to SRPS EN 16618 since Jun 2018. For detection of acrylamide is used HPLC system with MSMS detection. Method implies acrylamide extraction with water and isotopic labeled standard addition. The extract is the centrifuged and the supernatant is cleaned up with two solid phase extraction (SPE) columns. First column is used as chemical filter to retain as many matrix components as possible, and the other one is used to concentrate the extract and to obtain low limits of quantification. ISOLUTE Multimode and ENV+ SPE columns were used. After evaporation of methanol, the extract is analyzed by LC-MS/MS. For the separation is used Hypercarb column 100×2.1, 0.1% acetic acid mobile phase, at flow rate of 0.4 ml/min. Following transitions were: 72.2 > 44.30, 72.2 > 54.26, 72.2 > 55.3 for acrylamide and 75.10 > 58.10 for acrylamide ISTD. Validation of the method was done in November 2017 and recoveries were between 93% and 107% and intra-laboratory reproducibility in interval of 1.52-8.19%. Measurement uncertainty was 25% and limit of quantification was 15 μg/kg.

From November 2017 to April 2019, were analyzed 784 biscuit samples. During that time, in 20 biscuit samples, content of acrylamide was below LOQ. The highest content of acrylamide, 1743 µg/kg, is recorded in a sample of integral biscuits with plum in December 2017. The observation of the average acrylamide content in samples may indicate a decrease in the content of acrylamide in biscuits per month. Observed by months, the average content of acrylamide in the samples was above reference values only in November and December 2017; 513 µg/kg and 624 µg/kg respectively, when 63 individual samples had an acrylamide content above the reference value of 500 µg/kg defined in Commission Recommendation (2013/647/EU). In February 2019 the average content of acrylamide in biscuits was 195 µg/kg while no individual sample had a value above the reference values of 350 µg/kg according to Commission Regulation (2017/2158).

According to measurements, visibly decrease in the content of acrylamide is evident since the adoption of a new regulation in November 2017 and its application in April 2018. Based on the obtained results it can be concluded that producers are able to adapt to European requirements for acrylamide content, all in order to protect the health of consumers.

MATRIX EFFECT IN QUANTITATIVE ANALYSIS OF PESTICIDE RESIDUES IN FOOD BY GC-MS/MS

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In modern time, it is difficult to imagine agricultural production without the use of pesticides which benefit increased food production and prevent crops infestations. However, after application they do not necessarily stay on the plant but can migrate through the environment by water, air and soil, bioaccumulate and have a harmful effect. Nowadays there are several thousands of available pesticide products and as many active substances, hence, the resulting question is how to control their use.

The methods of choice are the chromatographic methods, both liquid and gas chromatography coupled with mass spectrometry. For that control to be successful, several requirements must be met by the analytical method applied, such as analysis of high number of active substances in one run, high sensitivity, short run time, but overall, the reliability of the obtained results. In gas chromatography, the injected sample's first contact with the instrument is within the liner and the start of the capillary column. When injecting calibration standards dissolved in neat solvent, the analytes can disintegrate and bind to the active sites on the liner and column. However, when injecting the sample, the matrix components are in competition with the pesticide active substances for those active sites preventing thermal degradation/adsorption of analytes. Consequently, less analytes are bound, and more are reaching the detector enhancing the signal. Hence, if solvent calibration is used, the signal enhancing effect might lead to overestimation of the results. The opposite effect is signal suppression due to the instrument contamination where non-volatile sample compounds are deposited on the liner and column increasing the overall number of active sites binding analytes. The stated phenomena are known as "matrix effect" (ME). There are several approaches for overcoming matrix effects, such as use of internal standards, standard addition or protectants, but most commonly used approach is to prepare standard solutions using the same matrix as the sample in question, the approach we apply as well within our laboratory.

In order to examine the ME for method validation and our everyday work, we have conducted both in-solvent and matrix matched calibration for several matrices, such as carrot, wheat flour, vegetable oil, tea, and compared the obtained data looking into changes in peaks. As well, matrix effect was compared between different sample sizes. Overall data show changes in peak shapes, shifting of retention times, and decrease of peak areas for the in-solvent calibration. ME was not expressed in the same manner for all active substances, some showed enhanced ME in matrix matched calibration, some signal suppression while other showed no difference in regression slope between the two types of calibration.

Matrix matched calibration is not the perfect solution as it implies several downsides such as time-consuming calibration preparation, instrument pollution, finding blank samples and taking into account the right commodities group according to SANTE guidance document for pesticide residue analysis. Despite the disadvantages, it is a powerful tool for ensuring the quality and reliability of our results.

MULTIRESIDUE PESTICIDE ANALYSIS USING GC-MS/MS

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Multiresidual analysis of pesticides is very challenging area because of the complex matrix in which it is necessary to analyze as much pesticide as possible in very low concentrations. Gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) is a powerful technique for determination of pesticide residues. Analysis on GC-MS/MS Agilent 7010 GC / MS Triple Quad requires a special approach to each matrix and analyte. The setup requires special instrument configuration and chromatography conditions. The instrument is equipped with a multi-mode inlet (MMI). Two columns HP-5ms 15 m 0.25×0.25 are interconnected by an electronic pneumatic control (EPC) configuration that allows the backflushing system to purge and eliminate unwanted heavy components from the column, shorten the time of analysis and reduce the need for system maintenance. Retention time lock (RTL) allowed adjusting the time segments after each column cut. GC-MS/MS multiple reaction monitoring has been developed for analysis of more than 200 pesticides. Two transitions were determined for each compound and collision energy was optimized for each. Qualitative and quantitative data analyses using MassHunter software were performed. Sample preparation for fruits, vegetables and cereals was performed using QUECHERS method according to HR EN 15662: 2008 on QUECHERS method-dispersive SPE column. Validation parameters such as limit of quantification (LOQ), trueness, precision, specificity, robustness and linearity were evaluated, and validation procedures were carried out according to the requirements of SANTE 11813/2017 Document. During the validation of the method the minimum LOQ 0.01 mg kg^{-1} was achieved meeting criteria of Regulation 396/2005 covering analytes from Directive 2017/660. For baby food, meeting criteria of Directive 2006/141 were achieved for Ethoprophos 0.008 mg kg^{-1}, Propineb, Cadusaphos, Demeton S Methyl 0,006 mg kg^{-1} and Fipronil 0,004 mg kg^{-1}, respectively.

BISPHENOL A ANALYSIS DURING THE ANAEROBIC DEGRADATION OF PAPERS WITH THERMOCHROMIC PRINTS IN SOIL

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Bisphenol A (BPA) has received a great deal of attention since 1996, when it was classified by the European Commission as a substance of external origin with a harmful effect on human health. Numerous toxicological and biochemical studies have confirmed that BPA has estrogenic properties through its agonistic effect toward the estrogenic receptor, and it was classified as endocrine disruptor. The effects of exposure to BPA can be particularly harmful to the fetus, infants and young children. Contact with BPA at that time may lead to irreversible changes appearing even after much delay. Exposure to BPA increases the risk of miscarriages, genetic defects (*e.g.* Down's syndrome), breast and prostate cancer, diabetes, obesity and cardiovascular disease

Thermochromic printing inks, which are widely used in security printing, in thermal printing paper, as temperature indicators, and as a source of various effects in packaging and design often contain BPA as one of the main compounds present in mass fractions of up to several percent.

This study presents the BPA determination during anaerobic degradation of thermochromic prints in soil, which simulated the conditions at a landfill site. Thermochromic ink that contains bisphenol A was printed on seven types of papers. The papers were subjected to anaerobic degradation in soil at time intervals from 14 to 150 days. Reversed-phase liquid chromatographic methods with UV-detection were developed to determine the mass fraction of bisphenol A in paper and in soil, and the rate of BPA degradation was estimated. During the anaerobic degradation, mass fraction of BPA in papers decreased from the initial values of 0.126–0.788 mg g^{-1} to value close to detection limit of the method of 0.0025 mg g^{-1}. Mass fraction of BPA in soil increased during the anaerobic degradation, and after 150 days ranged from 3.21 ng g^{-1} to 35.1 ng g^{-1}.

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