20th International Chromatography School

BOOK OF ABSTRACTS

FKITMCMXIX

University of Zagreb
Faculty of Chemical Engineering and Technology

18th – 19th September 2025

ZAGREB, CROATIA

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Sample delivery for the 19th Proficiency Test

The Certificate of Participation will be sent to you by email!

The 20th ICS Book of Abstracts will soon be available on the school's website.

PREFACE

Welcome to 20th ICS 2025

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 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.

In the end, I would just like to point out: Twenty years of the International School of Chromatography, which emerged from the Department of Analytical Chemistry thanks to our professors, is proof of the need for education of this kind. We dedicate this jubilee School of Chromatography to Professor Štefica Cerjan-Stefanović (born September 19, 1939 - died August 10, 2024), who initiated the entire story in a very visionary way with colleagues from Slovenia. Now we are part of a tradition that I hope we will be able to maintain for at least another twenty years.

Therefore, welcome to the 20th International Chromatography School, collect the provided knowledge, meet new colleagues and make new friends!

Prof. Danijela Ašperger

President of the Organizing Committee

We would like to thank sincerely all the lecturers and sponsors donors for their contributions at 20th ICS 2025.

LECTURES

IMPLEMENTATION AND COMPARISON OF LC-MS/MS AND IC-MS/MS APPROACHES FOR POLAR PESTICIDE ANALYSIS IN FOOD SAMPLES OF PLANT ORIGIN

S. Đekić

Analysis doo, Application Support, Japanska 4, Belgrade, Serbia
sanja.djekic@analysis.rs

The analysis of highly polar pesticides represents a major analytical challenge due to their high polarity, and poor retention on conventional reversed-phase columns. In line with the current QuPPe EU guidelines, our team has recently implemented two analytical approaches in two accredited laboratories in Serbia. The first laboratory adopted a method based on LC-MS/MS using HILIC and Hypercarb columns, while the second laboratory implemented an IC-MS/MS method – a technology exclusively offered by Thermo Scientific.

The LC-MS/MS method development and validation were performed using the Vanquish CORE LC system coupled to TSQ Quantis Plus™ MS/MS, targeting 13 polar analytes in plant-based food matrices. Significant method optimization efforts were needed to overcome the limitations of column stability, matrix effects, and peak shape issues—especially for glyphosate. Multiple validation protocols had to be developed due to fosetyl degradation and overlapping analytes, while the method still met the stringent LOQ requirements set by the EU.

In contrast, the IC-MS/MS approach enabled the separation of all 13 targeted analytes in a single method, with improved retention time stability, no need for column preconditioning, and better chromatographic behavior of key analytes such as glyphosate and phosphonic acid.

This presentation provides a comparative overview of the analytical performance, instrument sensitivity, practical considerations, and regulatory implications of both methods. Based on our experience, IC-MS/MS proved to be more robust and future-proof, though it requires a higher initial investment. Both laboratories successfully validated and accredited the implemented methods.

This case study highlights the importance of selecting the appropriate chromatographic technique for the analysis of polar pesticides and demonstrates the power of strong application support during implementation in routine laboratories.

Keywords: polar pesticides, LC-MS/MS, IC-MS/MS, method validation, food safety

PFAS ANALYSIS IN FOOD AND ENVIRONMENTAL SAMPLES

V. Stankov[1], P. Ožegović Haramina[1], B. Skenderović[1]

[1] *Sample Control d.o.o., Instrumental analysis Department, Puškarićeva ulica 18, 10250 Zagreb, Croatia*

vladimir.stankov@sample-control.hr

Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic chemicals (CnF2n+1COOH) that are used in many commercial products and have diverse applications such as polymers, fire-retardant polyurethane foam, cookware, food packaging, and textiles. PFAS have been used since the 1950s. All PFAS contain a chain of carbon atoms linked to fluorine atoms. Some also have a functional group at the end of the chain. Due to the stability of the C-F bond, many PFAS are resistant to biological, chemical, and physical transformation.

PFAS cannot be broken down into carbon dioxide or methane by bacteria, enzymes or sunlight, which is why they are called ‘forever chemicals’. Thousands of PFAS have been described. Hundreds of PFAS are produced industrially. It is estimated that around 100,000 sites in Europe emit PFAS into the environment. The non-fluorinated parts of emitted PFAS degrade. The PFAS found in the environment are not necessarily identical to those produced by companies. From the time they are emitted to the time they are in the environment, these PFAS can undergo changes that effectively make them ‘new PFAS’, different from their original form. It is a major challenge for regulators to regulate all PFAS.

Sample Control d.o.o. analyzes 24 PFAS by liquid chromatography-tandem mass spectrometry with analytical column Synergy 4 µm Hydro-RP 250 x 2 mm, mobile phase 0,9 % HCOOH in water (A), 0,9 % HCOOH in acetonitrile (B) with gradient elution. The analysis time is 21 minutes. The flow rate is 0,3 mL/min and the column temperature is 40 °C. Injection volume is 10 µL. The method is validated and accredited by Croatian Accreditation Agency.

The Ordinance on compliance parameters, methods of analysis and monitoring of water intended for human consumption (Official Gazette 64/2023) defines the maximum permissible concentration of PFAS for assessing the quality of water intended for human consumption as well as the measurement uncertainty of methods for chemical analysis and monitoring of water status. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain

contaminants in food and repealing Regulation (EC) No 1881/2006 regulates four PFAS - PFOS, PFOA, PFHxS and PFNA.

Keywords: PFAS, food safety, environmental analysis, LC-MS/MS, regulatory limits

MICRO-FLOW LC-MS/MS: A SCALABLE AND ROBUST PLATFORM FOR HIGH-THROUGHPUT PROTEOMICS

V. Petrušić, I. Tomaz

University of Zagreb, Faculty of Agriculture, Department of viticulture and enology
INFODOM d.o.o, Andrije Žaje 61, Zagreb , Croatia
vidapetrusic196@gmail.com

Mass spectrometry-based proteomics has become an essential tool in modern life sciences, enabling comprehensive analysis of protein expression, modification, and interaction across a wide array of biological contexts. Traditionally, nano-flow liquid chromatography coupled with tandem mass spectrometry (nano-LC-MS/MS) has been the method of choice, due to its exceptional sensitivity required for detecting low-abundance peptides and post-translational modifications. However, nano-flow configurations are often constrained by low robustness, complex maintenance, limited throughput, and issues with reproducibility, particularly in large-scale or routine applications. The emergence of micro-flow LC-MS/MS as a compelling and versatile alternative, offering a promising balance between sensitivity, robustness, and throughput. Operating at flow rates between 10–200 µL/min, micro-flow systems provide increased chromatographic stability and lower maintenance demands, enabling consistent performance across thousands of injections. While this approach typically requires a higher input of sample material compared to nano-flow systems, it compensates with improved retention time stability, enhanced quantitative reproducibility, and higher sample throughput—often exceeding 70–100 samples per day. Recent technological advances in micro-flow column design, mass spectrometer acquisition speed, and ionization efficiency (e.g., multi-nozzle emitters and post-column solvent addition) have significantly improved the sensitivity and depth of micro-flow-based proteome analyses. Studies demonstrate that more than 9,000 proteins can be quantified in single-shot runs using label-free or isobaric labeling strategies, with excellent consistency over large sample cohorts. These features render micro-flow LC-MS/MS especially suited for applications such as clinical proteomics, drug target deconvolution, systems biology, plant proteomics and biomarker discovery, where robustness and scalability are critical. While nano-flow LC-MS/MS will remain indispensable for applications requiring maximal sensitivity—such as single-cell proteomics or the detection of rare post-translational modifications, the adoption of micro-flow systems is rapidly expanding.

Notably, their ability to integrate into high-throughput workflows makes them highly attractive for future diagnostic and translational research pipelines.

In conclusion, micro-flow LC-MS/MS is evolving into a standard platform for proteomic research beyond niche applications. It offers a compelling solution to the growing need for scalable, reproducible, and cost-efficient proteome analysis across biology and medicine. As instrumentation and informatics continue to mature, micro-flow strategies are likely to play a central role in enabling the next generation of large-scale proteomic investigations.

MOLECULAR MAPPING OF PROTEINS AND PEPTIDES TO ENHANCE CLINICAL DIAGNOSTIC TOOLS

I. Đapić

Laboratory for Synthetic methodologies in Organic Chemistry, Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute

Despite significant advances in analytical technologies over the past decade, extracting detailed molecular information from brain tumours remains a major challenge. This is largely due to the nervous system's complexity, the tumour's location within the brain, and its close involvement in essential physiological functions. Today, mass spectrometry has become an indispensable tool for assessing tumour (tissue) complexity enabling the detection of proteins and their degradation products in the context of disease diagnosis and treatment. Proteomics enables direct assessment of proteins and can reveal molecular-level changes that often remain undetectable through other analytical approaches. Moreover, proteomics has the ability to capture adaptive changes in the tumours and detect their adaption under treatment or stress which ultimately may lead to novel insights and more effective therapies.

In our recent study of glioblastoma (GBM), primary brain tumour, we have used high-resolution mass spectrometry to identified over 1400 proteins from biopsy-sized tissue. We developed LC-MS method for analysis of small tissue amounts and showed increased levels of some proteins such as alpha-1-antichymotrypsin, osteopontin, transthyretin, haptoglobin, vitronectin, complement factor H and different classes of immunoglobulins in GBM patients. Further, we have investigated effect of the tissue type on the protein detection and method sensitivity and evaluated several different methods for protein extraction. Currently we investigate how MDM2 inhibition affects protein and peptides presentation in GBM cells. For this we use novel fluorophore-peptide probes for inhibition of MDM2 and investigate the effect of MDM2 inhibition on MHC peptides presentation. In this way we hope to elucidate the metabolic processes that drive GBM resistance to standard therapies, and to better understand mechanisms of tumour to escape standard treatments.

IC7000 ION CHROMATOGRAPHY ON-LINE ANALYSIS SYSTEM HOT SPRING WATER SEISMIC FORECAST MONITORING SOLUTION

Y. Liu, D. Delgalo

Anhui Wanyi Technology Co., Ltd. (Wayeal)
KemoLab d.o.o., Nadinska 11, 10010 Veliko Polje, Croatia
nklyang@gmail.com

Research has shown that variations in stress levels before and after an earthquake can lead to significant alterations in the ionic composition of hot spring water, well water, and groundwater in the vicinity of the incident. Numerous research findings consistently demonstrate a strong correlation between seismic fluid geochemical anomalies and seismic activity.

SAY HELLO TO THE HPLC MADE FOR TOMORROW

B. Ferčec

Altium International d.o.o. (AlphaChrom), Karlovačka cesta 24, 10000, Zagreb
fercec.borna@gmail.com

Agilent's 1260 and 1290 Infinity III LC systems represent the newest generation of HPLC and UHPLC technology, designed to deliver reliability, performance, and user-friendly operation for both routine and advanced laboratories. The 1260 Infinity III is built for dependable, everyday analyses, while the 1290 Infinity III provides maximum flexibility and throughput with pressures up to 1300 bar, enabling true UHPLC performance.

One of the key innovations is the new InfinityLab Assist touchscreen module, which transforms how users interact with their instruments. It offers guided startup and shutdown, automated purging, built-in diagnostics, customizable dashboards, and even remote monitoring. Together with InfinityLab Level Sensing to avoid solvent depletion, the Sample ID Reader for secure sample tracking, barcode automation, CO_2 footprint reporting, and predictive system insights, laboratories benefit from smarter, safer, and more sustainable workflows.

The InfinityLab LC portfolio is fully supported by Agilent OpenLab software, which ensures regulatory compliance (21 CFR Part 11), data integrity, and streamlined digital processes.

This presentation will not only highlight the technical innovations but also provide an application overview, showing how the Agilent Infinity III LC systems enhance productivity across pharmaceuticals, food safety, environmental monitoring, and life science research.

LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY IN ADME SCREENING

M. Čizmić

Selvita, DMPK, Zagreb, Hondlova 2, 10000 Zagreb, Croatia
mirta.cizmic@selvita.com

Drug discovery comprises a lengthy, demanding and complex process that aims to find safe and effective medicines. Assessing a drug's fate and behavior in the body, as well as optimizing its safety and efficacy represents a crucial part of drug discovery process. A drug activity and fate are largely governed by its Absorption, Distribution, Metabolism, and Excretion (ADME) properties. Early ADME characterization of drug properties help identify and select the most promising leads with favorable pharmacokinetic (PK) properties and reduce further progression of compounds with poor and unacceptable profiles.

ADME screening in early drug discovery involves a range of *in vitro* assays that evaluate a compound's solubility, transport through the membrane, distribution in the body, metabolism and potential for any drug-drug interactions. During this stage in the discovery process, a large number of compounds is submitted for testing, resulting in an extensive number of samples generated daily. These samples must be analyzed rapidly while maintaining precision and accuracy, which is where the use of LC-MS/MS demonstrates major benefits. LC-MS/MS is a powerful analytical technique that can be widely used for quantification of test compounds with different properties, with a high level of sensitivity and selectivity. Consequently, it represents the preferred method of analysis in high-throughput ADME screening.

Keywords: drug discovery, ADME, liquid chromatography, mass spectrometry

LC-MS IN FORCED DEGRADATION STUDIES: FROM IMPURITY IDENTIFICATION TO DEGRADATION PATHWAY MAPPING IN NEW DRUG DEVELOPMENT

A. Mornar, J. Kovačić, D. Amidžić Klarić

University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Analysis, A. Kovačića 1, 10000 Zagreb, Croatia
ana.mornar@pharma.unizg.hr

Forced degradation studies elucidate the chemical behavior of active pharmaceutical ingredients, which in turn is crucial for the development of drug formulations and their packaging. Although regulatory guidance is often very general, forced degradation studies are mandatory in the pharmaceutical industry. These studies are essential for determining the degradation mechanisms, quantifying potential degradants during drug substance analysis, and elucidating the composition of degradation products.

Given its exceptional sensitivity and ability to provide detailed structural information, LC-MS has become the preferred analytical tool in forced degradation studies. The chromatographic component (LC) ensures the efficient separation of complex mixtures of active pharmaceutical ingredient, excipients, degradants and impurities, while the mass spectrometry component (MS) allows for the identification of unknown compounds.

Etrasimod is an oral, once-daily small-molecule drug that selectively modulates $S1PR_1$, $S1PR_4$, and $S1PR_5$ receptors. Clinical trials demonstrated that it effectively treats moderate to severe ulcerative colitis in patients who are refractory or intolerant to other therapies. With recent approvals by the FDA and EMA, etrasimod has become a new therapeutic option for ulcerative colitis in clinical practice.

Ritlecitinib, an irreversible JAK3 and TYK2 inhibitor, is the first approved treatment for severe alopecia areata. The drug received rapid market authorizations in the USA, Japan, Europe, and Canada throughout 2023 and 2024. Due to its high selectivity and oral bioavailability, it is also being investigated in clinical trials for the treatment of other autoimmune conditions such as vitiligo and inflammatory bowel diseases (ulcerative colitis and Crohn's disease).

New stability-indicating UHPLC-DAD-MS/MS methods were developed, validated, and applied for forced degradation studies of etrasimod and ritlecitinib under ICH guidelines. The stability of etrasimod and ritlecitinib were evaluated by exposing the substances to various stress conditions.

Monitoring of the degradation products relied on the in-depth study of the mass spectrum of active pharmaceutical ingredients and their degradation products to pinpoint the characteristic fragmentation processes. A highly sensitive Triple Quadrupole mass spectrometer coupled with an electrospray ionization source was used to characterize etrasimod, ritlecitinib, and their degradation products. Furthermore, kinetic studies were conducted, and the rate constant, half-life, and shelf-life for each drug were determined.

Keywords: LC-MS, forced degradation study, ritlecitinib, etrasimod

SUPERCRITICAL FLUID CHROMATOGRAPHY IN PESTICIDE MONITORING: PRINCIPLES, ADVANTAGES AND APPLICATION

L. Furjan[1]

[1] *Shimadzu d.o.o., Zavrtnica 17, 10000 Zagreb, Croatia*
lucija.furjan@shimadzu.hr

Supercritical Fluid Chromatography (SFC) is a modern separation technique that combines features of both gas chromatography (GC) and liquid chromatography (LC). It utilizes a supercritical fluid, most often carbon dioxide, as the mobile phase. In its supercritical state, carbon dioxide possesses intermediate properties between gases and liquids, including low viscosity, high diffusivity, and adjustable solvating power. These characteristics enable rapid, high-resolution separations with significantly reduced solvent consumption. From both an analytical and environmental perspective, SFC is considered a sustainable and efficient alternative to conventional chromatographic approaches.

In pesticide analysis, the advantages of SFC are particularly relevant. Pesticides comprise a chemically diverse group of compounds, often including structural isomers and chiral molecules that present challenges for conventional LC or GC methods. The ability of SFC to exploit a wide variety of stationary phases, including chiral columns, makes it highly effective in resolving such complex mixtures.

The coupling of SFC with mass spectrometry (SFC–MS) has further extended its application in pesticide residue analysis. While LC–MS has long been the dominant platform for pesticide monitoring due to its sensitivity, robustness, and compatibility with a wide range of analytes, SFC–MS offers complementary and, in some cases, superior performance. Compared with LC–MS, SFC–MS typically provides faster separations, improved resolution of structurally similar or chiral compounds, and reduced matrix effects. Moreover, the lower use of organic solvents in SFC–MS enhances method sustainability and decreases operational costs. However, LC–MS remains advantageous in terms of broader availability, established regulatory methods, and compatibility with highly polar analytes that may not be well retained under typical SFC conditions.

Overall, SFC–MS represents a powerful and environmentally conscious alternative or complement to LC–MS in pesticide analysis. Shimadzu provides advanced SFC instruments and solutions that support reliable, high-throughput, and environmentally friendly analyses, making this technique more accessible for routine and specialized applications.

Keywords: supercritical fluid chromatography (SFC), mass spectrometry (MS), pesticide analysis

INNOVATIVE SEC APPROACHES FOR SMALL AND LARGE MOLECULES – BRIDGING SCIENCE AND INDUSTRY

M. Ivić, O. Platiša Cerovski, A. Šporec

BIOCentre Ltd., Central laboratory, Borongajska cesta 83H, 10000 Zagreb, Croatia
marija.ivic@biocentre.hr, olivera.platisa-cerovski@biocentre.hr, anita.sporec@biocentre.hr

Size exclusion chromatography (SEC), also known as gel filtration or gel permeation chromatography, is a widely used, non-destructive technique for the purification and characterization of synthetic and biological polymers, including proteins, polysaccharides, and nucleic acids. It separates molecules based on their hydrodynamic size using a column packed with porous gel beads. This entropy-driven separation mechanism, based purely on physical size, avoids chemical interactions and preserves the native structure of sensitive compounds.

The ability to differentiate molecules based solely on hydrodynamic volume is particularly beneficial in biopharmaceutical and polymer analysis. This technique operates in aqueous (gel filtration) or organic (gel permeation) mobile phases, depending on the analyte's solubility and stability. SEC offers remarkable adaptability, making it suitable for both small molecules, such as oligonucleotides, peptides, and excipients, as well as large biomolecules like monoclonal antibodies (mAbs), proteins, and polysaccharides. One of its main strengths lies in the absence of analyte-stationary phase interactions, which enables flexibility in mobile phase conditions, even in the presence of detergents, urea, or salts. Proper column selection is essential for optimal resolution.

In addition to its analytical use, SEC is commonly employed in preparative workflows, often as a final purification step. For example, it enables the separation of a target protein from its multimeric forms or, when using desalting columns, from smaller molecules such as salts or affinity tags. Furthermore, it detects and quantifies aggregates, fragments, and high molecular weight species, ensuring safety, efficacy, and regulatory compliance.

In polymer science, SEC - HPLC provides detailed molecular weight information, including number average (Mn), weight average (Mw), z-average (Mz), and polydispersity index (PDI). For example, sodium hyaluronate, used in ophthalmology and dermatology, is analyzed by SEC to confirm size distribution that correlates with viscosity and bioactivity.

This lecture will explore SEC principles and applications in both scientific research and industrial settings, with emphasis on innovative approaches for characterizing small and large molecules.

Keywords: Size exclusion chromatography (SEC), protein purification, polymer science

APPLICATION OF GAS CHROMATOGRAPHY IN THE QUANTIFICATION OF REGENERATED SOLVENTS

J. Lasić[1], S. Petković[1], S. Babić[2]

[1] *PREMIFAB d.o.o. Laboratory for Organic Solvents, Poduzetnička ulica 8, Kerestinec, 10431 Sveta Nedelja, Croatia*
[2] *University of Zagreb, Faculty of Chemical Engineering and Technology, Department of Analytical Chemistry, Trg Marka Marulića 19, 10000 Zagreb, Croatia*
silvija.petkovic@premifab.hr

Guided by the principles of the circular economy, solvent recycling industries specialize in the regeneration of waste solvents through batch distillation. Waste solvents from industries such as paints and coatings, flexible packaging, automotive, and pharmaceuticals are recovered and returned to the market as regenerated solvents. To support this process, solvent recyclers usually have in-house laboratories that not only provide quality control during the regeneration process but also ensure that regenerated solvents meet market requirements for safe and effective reuse. For this reason, the fastest and most efficient analytical technique – gas chromatography – is employed as a central tool in quality assessment. Additionally, since industrial solvents often contain a large number of organic components, reliance solely on external standard method can be time-consuming and costly, requiring extensive preparation of calibration standards.

To address these industrial needs for speed and efficiency, relative response factors (RRFs) within internal standard method were determined as an alternative approach, allowing simultaneous quantification of multiple analytes with reduced effort. Therefore, the aim of this study was to validate a method for quantitative determination of solvents using RRFs, and to compare this approach with conventional methods. The validation assessed key performance characteristics: selectivity, linearity, precision, and accuracy. With defined acceptability criteria, RRFs were established for commonly encountered organic solvents across concentration ranges typically observed in aforementioned industries.

Following RRFs determination, a sample originating from the automotive industry was analyzed. Quantification was carried out using four methods: area normalization, corrected area normalization, external standard, and internal standard with RRFs. A comparative evaluation highlighted the strengths and limitations of each approach, demonstrating how methodological choice can influence quantitative results in complex industrial solvents.

STUDYING ENVIRONMENTAL EXPOSURE VIA HUMAN BIOMONITORING

T. Kosjek[1,2], H. Plešnik[1,2], Ž. Rekar[1,2], L. Ropič Bizjak[1,2], Ž. Tkalec[1], N. Kosirnik[3], M. Horvat[1,2]

[1] *Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia*
[2] *Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
[3] *University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*
tina.kosjek@ijs.si

Exposure to numerous chemicals, both known and unknown, is a part of our daily lives. These chemicals enter our bodies through various pathways, including food, water, air, and skin contact, having significant effect on human health. Many of these compounds are recognized as persistent, bioaccumulative, and toxic, and are subject to human biomonitoring programs. However, there exists a vast array of unknown chemicals posing potential risk to human health.

This presentation delves into our strategies for monitoring both established harmful chemicals and those yet to be identified. Our approaches rely on gas or liquid chromatography coupled with tandem and/or high-resolution mass spectrometry. With an increasing demand for more effective preventive measures, our work seeks to trace exposure trends and extend the understanding of the chemical exposome. This entails broadening the spectrum of monitored compounds and establishing links between various biomarkers and various matrices. Suspect and non-targeted screening techniques represent a cutting-edge approach capable of capturing substances from diverse classes, including contaminants of emerging concern. We also highlight orthogonal liquid chromatography separation systems like HILIC, complementing commonly used reverse-phase separation and expanding the chemical space for highly polar biomarkers.

In summary, this presentation sheds light on the ongoing challenge of biomonitoring the myriad exposure biomarkers impacting human health. Our methods, grounded in advanced analytical techniques, pave the way for a more comprehensive assessment of chemical exposure, ultimately supporting proactive health measures.

RECENT ADVANCES IN HRAM BASED -OMICS APPROACHES

L Mihajlovic[1]

[1] *Analysis doo, Japanska 4, lok 49, 11070 Belgrade, Serbia*
luka.mihajlovic@analysis.rs

This lecture aims to cover recent advances in technologies that are relevant for development of high throughout -omics technologies. Progress in mass spectrometry has, for the first time, allowed complete -omics data (proteomics, lipidomics, etc.) to be acquired on a population scale, which also necessitates the need for faster chromatography and better software. Single-cell -omics are becoming mainstream due to increased sensitivity and scan speed across different platforms. Novel concurrent fragmentation technologies are being implemented by various manufacturers. In addition to progress in nanoLC and emitter technology, real time retention alignment algorithms allow for exquisite inter-sample reproducibility. Specialized columns are being developed implementing photolithographic technologies that offer sharper peaks from first principles. Real-time data-dependent scanning is allowing sample multiplexing, with most recent chemistries going up to 35plex. A tremendous increase in data quantity has pushed the development of a new generation of software with AI-enabled searches and cloud computing, with a simultaneous push to software compliance with GxP requirements**.** Due to wider use of HRAM in pharmaceutical industry, translational medicine and commercial laboratories, further advances in the field are expected in the near future.

Keywords: HRAM(high resolution accurate mass), mass spectrometry, proteomics, -omics, nanoLC

Acknowledgements: Analysis doo wishes to thank the organizers for the chance to present this lecture

CHROMATOGRAPHY AS A PROCESS ANALYZER

M. Cvetnić, D. Lastovčić, M. Markić, Š. Ukić, T. Bolanča

Faculty of Chemical Engineering and Technology University of Zagreb
Matija.cvetnic@fkit.unizg.hr

Chromatography has evolved beyond its traditional role as a laboratory separation technique to become a powerful tool for real-time process analysis and control. Its ability to provide high-resolution, quantitative, and selective measurements of complex mixtures makes it particularly valuable in industries such as pharmaceuticals, biotechnology, petrochemicals, and food processing and in environmental issues such as drinking and wastewater systems ensuring good water quality. Recent advances in instrumentation, miniaturization, and automation have enabled chromatography to be integrated directly into process streams, allowing continuous monitoring of critical quality attributes and process parameters.

This lecture discusses the application of chromatographic methods primarily gas chromatography (GC) and liquid chromatography (LC) as process analyzers, with emphasis on their advantages, limitations, and implementation challenges. Case studies highlight how online and at-line chromatographic systems can improve process efficiency, reduce off-specification production, and support regulatory compliance in the framework of Process Analytical Technology (PAT).

Future perspectives include the development of faster separation technologies, robust sampling systems, and integration with multivariate data analysis for enhanced process understanding. For this purpose, Wayeal ion chromatography system ICS 6600 has been tested as process analyzer.

THIN-LAYER AND GAS CHROMATOGRAPHY: KEY TOOLS FOR THE RESEARCH AND QUALITY CONTROL OF ESSENTIAL OILS

S. Vladimir-Knežević

University of Zagreb Faculty of Pharmacy and Biochemistry, Department of Pharmacognosy, Trg Marka Marulića 20, 10000 Zagreb, Croatia
sanda.vladimir@pharma.unizg.hr

Essential oils are chemically diverse natural products consisting mainly of volatile plant metabolites dominated by monoterpenes, sesquiterpenes and phenylpropanoids. Their chemical composition is strongly influenced by botanical origin, geographical location, seasonal variations and extraction conditions, often resulting in different chemotypes even within a single plant species. Due to their complex composition, essential oils exhibit a broad spectrum of bioactivities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, analgesic, anxiolytic and insecticidal effects. Although distillation is still the conventional approach for their isolation, advances in environmentally friendly and efficient extraction technologies are improving the recovery of thermolabile compounds and expanding the range of applications in the pharmaceutical, food, cosmetics and agricultural sectors. The biological properties, therapeutic potential and commercial value of essential oils are directly determined by their chemical composition, emphasising the importance of robust analytical methods for research and quality control. Thin-layer chromatography (TLC) and gas chromatography (GC) remain among the most widely used and complementary techniques in this field. TLC offers a fast, cost-effective and visually accessible fingerprinting tool for qualitative assessment and detection of adulterants. GC, especially in combination with mass spectrometry (MS), enables high-resolution separation as well as precise identification and quantification of volatile components. The resulting phytochemical profiles not only underpin the therapeutic and sensory properties of essential oils, but also form the basis for their standardisation. Quality control of essential oils is necessary to verify their authenticity and compliance with quality standards, to ensure their safety and regulatory compliance, and to monitor their consistency. In this context, examples of TLC and GC-MS analyses of essential oils isolated from selected Croatian aromatic medicinal plants will be presented to illustrate the key role of chromatography in their evaluation and application.

Keywords: essential oils, phytochemical profile, quality control, thin-layer chromatography, gas chromatography

NEW APPROACHES OF SAMPLE PREPARATION IN CHROMATOGRAPHIC ANALYSIS: A STEP TOWARDS MORE SUSTAINABLE LABORATORY PRACTICE

R. Škvorc Vidović[1]

[1] *Bioinstitut Ltd., R. Steinera 7, Čakovec, Croatia*
rosanda.skvorc@bioinstitut.hr

Sample preparation for chromatographic analysis is a crucial step that significantly affects the overall chemical consumption and the generation of waste solvents. Traditional extraction methods, especially liquid-liquid extraction, rely on large amounts of organic solvents such as n-hexane, dichloromethane, acetone or methanol. These solvents have a negative impact on the environment and require careful handling and disposal. To reduce this burden, more advanced sample preparation techniques are being developed that minimize solvent use while increasing efficiency. Automated solid-phase extraction (SPE) can reduce solvent consumption by up to 50%. An even more efficient approach is µDrop –an innovative microextraction method that requires only 3.2 ml of solvent and 35 ml of water sample, while allowing the simultaneous extraction of multiple groups of analytes. Compared to conventional methods, µDrop stands out as a more environmentally friendly and time-efficient alternative, in line with the principles of green chemistry.

Keywords: chromatography, water, sample preparation, µDrop

APPLICATION OF CHROMATOGRAPHIC TECHNIQUES IN MYCOTOXIN ANALYSIS OF PLANTS

M. Sokolovic[1], M. Berendika[1], N. P. Kalogiouri[2], V. F. Samanidou[2], A. Vulić[3], C. Dorin[4], R. Tripon[4], C. Tulcan[4]

[1] Croatian Veterinary Institute, Poultry Centre, Feed Analysis Laboratory, Heinzelova 55, Zagreb, Croatia
[2] Laboratory of Analytical Chemistry, School of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3] Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska cesta 143, Zagreb, Croatia
[4] Faculty of Engineering and Applied Technologies, King Michael I of Romania Life Sciences University, Timisoara, Romania
sokolm2021@gmail.com

Mycotoxins are low molecular mass secondary metabolites of filamentous fungi that often contaminate Plants at low concentrations. So far, more than 400 different compounds have been described. Some of them have been extensively studied due to their potential harmful effects in humans and animals. A long history of mycotoxin analysis shows successful application of various methods based on thin-layer chromatography and high-performance liquid chromatography coupled with various detectors like fluorescence, diode array, UV and/or mass spectrometry. Chromatographic methods follow sample preparation procedures that include selection of representative sample, sample preparation, extraction and different clean-up procedures. Currently, a number of different detection methods are used (culture based, molecular, antibody or biosensor based) while enzyme-linked immunosorbent Assays and Chromatographic techniques are still the most common and important tool for their analysis. In a scope of the Research of Plant Extracts, determination of their safety includes detection of major mycotoxins. For screening purposes, enzyme-linked Immunosorbent Assays are applied due to their relatively simple and reliable procedure, while chromatography is used for confirmation. This paper will present Methods and Approaches in Mycotoxin Detection of Plants that was performed in the study funded by the European Union NextGenerationEU and supported by the Ministry of Science and Education of the Republic of Croatia through the project No. NPOO 15 of Croatian Veterinary Institute titled "Comprehensive Research on the Impact of Plant Extracts against resistant Staphylococcus (CRISP)"

Keywords: mycotoxins, chromatography, plants

Acknowledgments:

This study was funded by the European Union NextGenerationEU and supported by the Ministry of Science and Education of the Republic of Croatia through the project No. NPOO 15 of Croatian Veterinary Institute titled "*Comprehensive Research on the Impact of Plant Extracts against resistant Staphylococcus (CRISP)*"

GAS CHROMATOGRAPHY WITHOUT FEAR: WHAT GC-MS/MS CAN DO FOR PAH DETERMINATION IN VARIOUS ATMOSPHERIC SAMPLES?

D. Dabić

Meteorological and Hydrological Service, Ravnice 48, 10000 Zagreb
ddabic@dhz.hr

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds known for their persistence, toxicity, and potential carcinogenic effects. They are primarily formed during incomplete combustion processes and are commonly found in air and precipitation. Due to their harmful effects and regulatory importance, accurate and sensitive determination of PAHs in atmospheric samples is essential. Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) has emerged as one of the most powerful techniques for the analysis of trace-level PAHs in complex environmental matrices.

GC-MS/MS enables high selectivity and sensitivity using multiple reaction monitoring (MRM), significantly reducing background noise and interferences from co-eluting compounds. This is especially beneficial when analyzing low concentrations of PAHs in ambient air, particulate matter (PM), or precipitation samples. The method allows for precise quantification, reliable identification, and validation according to internationally recognized standards such as ISO/IEC 17025.

In the development and application of such analytical methods, key steps include sample collection, extraction coupled with purification, and instrumental analysis. GC-MS/MS plays a crucial role in confirming the identity of individual PAH compounds based on their unique mass transitions and retention times. This technique is especially advantageous in routine environmental monitoring programs, research studies on air quality, and source apportionment of pollution. In accredited laboratories, method validation, quality control procedures, and inter-laboratory comparisons ensure the robustness and reproducibility of GC-MS/MS results. Additionally, advances in instrumentation, automation, and software tools have made the technique more accessible and reliable for use in national and regional monitoring networks.

In conclusion, GC-MS/MS represents a gold standard in PAH determination in atmospheric samples, combining analytical power with practical reliability.

Its application supports informed decision-making in environmental protection, air quality regulation, and public health initiatives.

Keywords: method development, GC-MS/MS optimization, polycyclic aromatic hydrocarbons (PAHs), sample preparation, environmental analysis

ADVANCED CHROMATOGRAPHIC AND ELECTROPHORETIC APPROACHES FOR CLINICAL APPLICATION IN BREAST CANCER THERAPEUTIC DRUG MONITORING

M. Sertić, L. Turković, Z. Mlinarić

[1] *University of Zagreb Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Analysis, Ante Kovačića 1, Zagreb, Hrvatska*
miranda.sertic@pharma.unizg.hr

Breast cancer remains the most prevalent cancer worldwide and a major public health challenge. In the era of personalized medicine, therapeutic drug monitoring (TDM) is increasingly recognized as a valuable approach for optimizing therapy, ensuring efficacy and minimizing toxicity. Reliable, sensitive and selective bioanalytical methods are a prerequisite for the successful implementation of TDM in oncology.

We developed and validated multiple analytical methods for the quantification of seven clinically relevant breast cancer drugs (alpelisib, palbociclib, ribociclib, abemaciclib, anastrozole, letrozole, fulvestrant). Chromatographic separation was achieved using both liquid chromatography and capillary electrophoresis, coupled to UV-Vis, fluorescence and mass spectrometric detection.

Considering the complexity of plasma as a biological matrix, special emphasis was placed on sample preparation so several approaches were compared: protein precipitation, solid-phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME) and phospholipid removal (PLR). DLLME enabled simultaneous extraction of all investigated drugs with excellent recoveries (82–96%) from only 50–100 µL of plasma, meeting green chemistry principles. PLR, however, significantly improved robustness of LC-MS analysis by efficient removal of phospholipids.

All methods were fully validated according to ICH guidelines and successfully applied to dozens of patient plasma samples, demonstrating their applicability in real clinical settings. Limits of detection were in the low ng/mL range (0.2–1.5 ng/mL depending on the analyte), fully covering clinically relevant plasma concentrations of all investigated drugs. Accuracy (bias) and precision (RSD) complied with ICH acceptance criteria (±15% for all validation levels, ±20% at LLOQ), with most values well within ±10%, confirming the robustness of the methods.

These results underline the innovative potential of advanced sample preparation strategies in oncology bioanalysis and pave the way for their routine use in clinical TDM of breast cancer drugs.

Keywords: breast cancer, bioanalysis, therapeutic drug monitoring, LC-MS, CE-MS

Acknowledgements:

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GC×GC–TOF MS AS A COMPREHENSIVE SOLUTION FOR CHALLENGING ANALYSES IN MODERN LABORATORIES

T. Kovalczuk

[1] *LECO Instrumente Plzeň, Purkyňova 15, Plzeň, Czech Republic*

Over the past decade, analytical chemistry has advanced toward maximizing the number of analytes determined in a single run while minimizing sample preparation. Supported by modern instrumentation, this approach has enabled significant improvements in sample throughput and analytical sensitivity.

Such strategies are now routine in modern laboratories dealing with practically any type of analyses. Nevertheless, challenges still persist. Even with validated and optimized methods, issues frequently arise during sample preparation, sample introduction, or analyte determination, most often due to the complexity analyzed matrices.

Time-of-flight mass spectrometry (TOF MS) is a valuable tool in addressing these challenges. It provides complete mass spectral information that is available at even trace concentration levels, delivers high-quality, non-skewed spectra for individual analytes, and offers robustness through its open-source ion source design, which reduces maintenance needs and instrument downtime.

Comprehensive two-dimensional gas chromatography (GC×GC) offers substantial advantages over conventional GC in the analysis of complex mixtures. Its significantly enhanced chromatographic resolution and improved detectability make it particularly suitable for applications where analyte separation and identification are otherwise limited.

The coupling of GC-, and/or GC×GC with TOF MS has been recognized for over two decades as an effective solution for complex analytical applications. The combination integrates the strengths of both techniques, providing superior separation capacity, sensitivity, and spectral quality. To fully utilize these benefits, advanced data processing tools are required to manage and interpret the multidimensional datasets generated.

These capabilities will be demonstrated through multiple applications, specifically on determination of mineral oil saturated hydrocarbons (MOSH) & mineral oil aromatic hydrocarbons (MOSH/MOAH) and Food-omic analyses.

Keywords: GC-TOF MS, GCxGC, Food analysis, difficult matrices

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Naša rješenja!

Sustavi za plinsku kromatografiju (GC) osiguravaju pouzdanu i osjetljivu analizu hlapljivih spojeva sadržanih u uzorku.

Masena spektrometrija s induktivno spregnutom plazmom (ICP-MS) je tehnika u kojoj se induktivno spregnuta plazma koristi kao ionizacijski izvor, a detekcija se vrši masenom spektrometrijom.

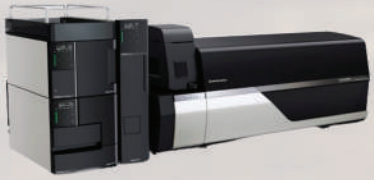
Spektrofotometri mjere intenzitet svjetlosti koju apsorbiraju ili emitiraju kemijske tvari.

Analizatori ukupnog organskog ugljika (TOC) mjere ukupnu količinu organskog ugljika u vodi, plinovima i čvrstim tvarima. Zadovoljavaju raznolik raspon potreba u područjima kao što su istraživanje okoliša, kontrola kvalitete i upravljanje procesima.

Sustavi za tekućinsku kromatografiju (LC) uspješno razdvaja tvari na osnovi razmještanja između čvrste stacionarne faze i tekuće mobilne faze.

Sustavi za plinsku kromatografiju - masenu spektrometriju (GC/MS) omogućuju brzu identifikaciju nepoznatih komponenti i preciznu kvantitativnu analizu, čak i za komponente u tragovima.

Sustavi za visoko učinkovitu tekućinsku kromatografiju (HPLC) podržavaju širok raspon aplikacija, uključujući analizu hrane, lijekova te uzoraka iz okoliša.



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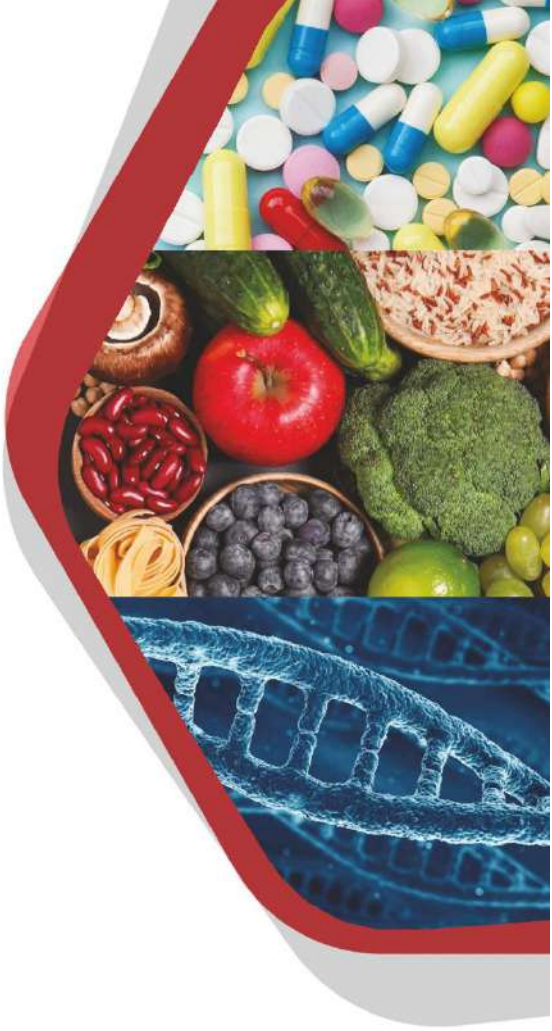

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Široka paleta proizvoda koje nudimo, u kombinaciji s našim znanjem i iskustvom stečenim radom u laboratoriju, omogućuje nam da ponudimo najbolje rješenje za sve vaše analize.

Jedno od naših temeljnih načela jest etikom vođeno poslovanje, jer se tako zadovoljavaju ključne vrijednosti: vjerodostojnost, pouzdanost, kompetentnost i čestitost.

Na zadovoljstvo naših korisnika kontinuirano unaprjeđujemo i prilagođavamo poslovanje njihovim potrebama, ostvarujući time svoj cilj.

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