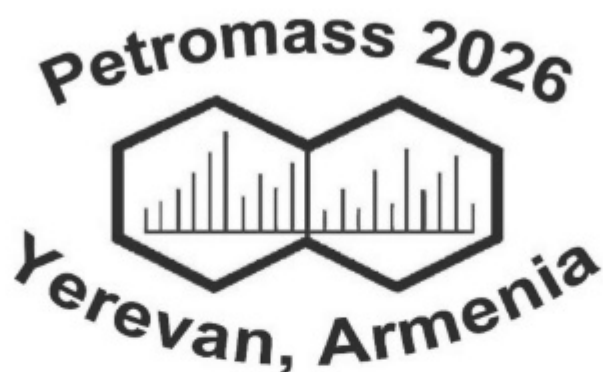


National Academy of Sciences of the Republic of Armenia

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BOOK OF ABSTRACTS

**XIII INTERNATIONAL MASS SPECTROMETRY
CONFERENCE ON PETROCHEMISTRY,
ENVIRONMENTAL AND FOOD CHEMISTRY
PETROMASS 2026**

04-09 OCTOBER 2026

YEREVAN, ARMENIA

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**XIII International Mass Spectrometry Conference on Petrochemistry,
Environmental and Food Chemistry
Petromass 2026**

Programme

October 04-09 2026

Yerevan, Armenia

**October 4th, Sunday
Arrival of participants**

October 5th, Monday

15.00-17.00	Registration
17.00-19.00	Opening ceremony
19.00-21.00	Get-together party

October 6th, Tuesday

10.00-10.40	Plenary session
10.00-10.40	<i>Gross M.L.</i> Mass Spectrometry-based Structural Proteomics for Amyloid and Membrane Protein Footprinting: A Tutorial
10.40-11.40	Oral session
10.40-11.00	<i>Arkhangelskaya P.S.</i> Development of a Tar-geted Mass Spectrometry-Based Method for Profiling Cys-Loop and Ionotropic Glutamate Receptors in Cell Cultures and Tissues Using Stable Isotope-Labeled Standards
11.00-11.20	<i>Dmitrieva E.V.</i> Differentiation of leucine and isoleucine isomeric residues by ExD in <i>de novo</i> peptide sequencing. The case of temporins.
11.20-11.40	<i>Kazakova E.M.</i> MetaDirectMS1: Expanding the Metaproteomics Toolbox with Rapid MS1-Based Analysis
11.40-12.10	Coffee break Poster session
12.10-14.30	Oral session
12.10-12.30	<i>Vasil'ev Yu.V.</i> Interrogation of Ion Mobility Selected Proteins via Electron Capture Dissociation: Does Protein Native-like Structure Survive in the Gas Phase?
12.30-12.50	<i>Pushkareva T.I.</i> Comparison of methods for the analysis of the glycosylation profile of therapeutic proteins: glycopeptide analysis by LC-MS and released glycan analysis by HPLC-FLD
12.50-13.10	<i>Vyatkina K.V.</i> <i>De novo</i> sequencing of nanobodies with the Twister algorithm
13.10-13.30	<i>Kukaev E.</i> First-Trimester Preeclampsia Prediction by Integrated DIA-PASEF and Targeted LC-MRM-MS Serum Proteomics
13.30-13.50	<i>Gorshkov M.V.</i> Mass spectrometry-based degradomics: semi-tryptic peptide profiling of proteomes for mapping disease-specific proteolytic signatures
13.50-14.10	<i>Baygildiev T.M.</i> LC-HRMS Determination of Per- and Polyfluoroalkyl Substances in Water and Food Matrices
14.10-14.30	<i>Ryskina A.M.</i> Accuracy Problems in Bottom-Up Protein Quantification

14.30-15.40

Lunch

Poster session

15.40-17.20

Plenary session

15.40-16.20

Makarov A. Exploring New Heights on the Orbitrap Journey

16.20-16.40

Skorniyakov Yu.V. Aspects of the Use of Mass Spectrometry Methods in Pharmaceutical Manufacturing

16.40-17.20

Shvartsburg A.A. Novel Ion Mobility Separations Leveraging the Macromolecular Dipole Alignment

October 7th, Wednesday

9.30-10.10

Plenary session

9.30-11.30

Heath E. Treated Wastewater Reuse in Agriculture: Uncovering Potential Risks of Emerging Contaminants with Mass Spectrometry

10.00-13.20

Oral session

10.00-10.30

Trebse P. High-Resolution Mass Spectrometric Identification of Semi-Volatile Constituents Released from Oral Nicotine Pouches into Artificial Saliva

10.30-10.50

Koluntaev D. Application of GC-MS to Analyze the Aroma Profile of Dry-Cured Sausage with Different Flavouring Levels in the Formulation

10.50-11.10

Pikovskoi I.I. Molecular-structural analysis of lignin oligomers by Orbitrap High-Resolution Mass Spectrometry

11.10-11.30

Bavcon Kralj M. Ultra-Sensitive micro-QuEChERS-UPLC-MS/MS Method for Determination of Azoxystrobin in *Dolomedes fimbriatus*

11.30-12.00

Coffee break

Poster session

12.00-13.20

Oral session

12.00-12.20

Knudsen T.Š. Occurrence of polybrominated diphenyl ethers in human milk from Serbia

12.20-12.40

Popov M.S. Thermal desorption gas chromatography - high resolution mass spectrometry: a new approach to quantification and screening of toxic rocket fuel transformation products in peat bog soils

12.40-13.00

Koluntaev D. Application of Gas Chromatography - Mass Spectrometry for the Identification of Organic Materials in Cultural Heritage Objects

13.00-13.20

Shaidullina G.M. LECO's Comprehensive GCxGC-TOFMS for Alternative Petrochemistry

13.20-14.20

Lunch

Poster session

14.20-15.20

Plenary session

14.20-15.00

Hakansson K. Hybrid Orbitrap-FT-ICR Mass Spectrometry, Trapped Ion Mobility Spectrometry-FT-ICR MS, and Novel Data Processing Approaches Shed Light on Chemical and Biochemical Dark Matter

15.00-15.20

Melikyan L. Thermo Fisher Scientific Mass Spectrometry Innovations: Expanding High Resolution and Targeted Workflows Across Research, Biopharma, and Environmental Testing

Session discussion

Tour to Hellenistic Temple of Garni

October 8th, Thursday

10.00-10.40

Plenary session

10.00-10.40

Zimmerman R. 100 Years of Photoionization: From History and Fundamental Principles of Photoionization Mass Spectrometry to Current Analytical Applications in Environmental Research and Industrial Monitoring

10.40-11.40

Oral session

10.40-11.00

Tsitsishvili V.G. Steranes and hopanes in Georgian petroleum

11.00-11.20

Pavlovskaya A.A. Determination of Sulfur-Containing Components in Diesel Fuel by Gas Chromatography

11.20-11.40

Kulikova L.N. Kerch Strait disaster: one year later - what happens to fuel oil on the shore?

11.40-12.10

Coffee break

Poster session

12.10-13.30

Oral session

12.10-12.30

Lukina M.V. Application of Hydrogen-Deuterium Exchange Mass Spectrometry (HDXMS) to Study Structural Features of DNA Repair Enzymes and Their Complexes with Nucleic Acids

12.30-12.50

Alekseeva I.V. Metabolomic profiling of aqueous humor for differential diagnosis of uveal melanoma and uveal nevi

12.50-13.10

Frolova A.V. Untargeted LC-HRMS metabolomics for urinary profiling and biomarker discovery in urological cancers

13.10-13.30

Poteshin S.S. Some Recent Concepts for ESI-TOF Mass Spectrometers in Medicine

13.30-14.40

Lunch

Poster session

14.40-15.20

Plenary session

14.40-15.20

Chu I.K. Protein Tyrosine Nitration: From Radical-Mediated Reactions to Clinical Therapy

Session discussion

October 9th, Friday

10.00-10.40

Plenary session

10.00-10.40

Lebedev A.T. What do we drink? Formation of disinfection by-products in water treatment

10.40-12.00

Round Table and Closing ceremony

Poster session

Polyakova O.V. Cosmetic powder of the Great Silk Road based on archaeological data

Polyakova O.V. GC×GC/HRMS and chemometric analysis of volatile and semi volatile compounds in Chinese mushrooms

Baranov N.A. GC/MS analysis of hydroconversion products of rapeseed oil on zeolite catalysts

Gorbova N.S. On-line electrochemistry – high-resolution mass spectrometry in the study of redox metabolism of polyphenolic compounds

Peregudova E.Yu. Determination of Per- and Polyfluoroalkyl Substances in Chicken Eggs Using a Dispersive Solid-Phase Extraction Method Coupled with LC-HRMS

Oprunenko A.Yu. Study of the effect of electron beam irradiation on antibiotics in aqueous solutions

Kondratyeva D.V. Study of the aqueous humor proteome in patients with uveal melanoma

Chernonosov A.A. LC–HRMS-based intracellular and extracellular metabolomic profiling of uveal melanoma cells

Khetsuriani N.T. Study of the shelf life of biodiesel derived from biomass

Ebralidze K.G. Photochemical oxidation of crude petroleum in simulated conditions and study of photoproducts

Tsitsishvili V. Application of GCMS in the study of catalytic transformations on micro-mesoporous zeolites

Poliakova A.N. Synthesis of proteotypic peptide standards for quantitative next-generation MS-based proteomics medical diagnostic assays

Minasyan E. Regional patterns of inorganic anions in Armenian drinking waters revealed by validated ion chromatography

Kirakosyan V. High-Resolution UPLC-QToF-MS Profiling of Phenolic Bioactives in *Vitis vinifera* cv. Mxali Stem Residues: Valorization Potential and Comparative Analysis with Rkatsiteli Cultivar

Davinyan A. FastDDA versus Data-Independent Acquisition (MS^E) for Plant Extract Profiling: Comparison of MS/MS Coverage, Spectral Specificity, and Metabolite Annotation

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Mass spectrometry-based structural proteomics for amyloid and membrane protein footprinting: A Tutorial

M.L. Gross

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Two challenging areas for structure and dynamics of proteins are amyloids and membrane proteins. These two areas will be used to construct a tutorial for "structural proteomics", a subdivision of proteomics used to follow protein structural change. Membrane proteins were chosen because they are difficult to characterize by traditional structural methods X-ray and cryoEM. They readily lose structural information when removed from a membrane or cell. Nevertheless, membrane proteins are the targets for ~ 60% of small-molecule drugs despite that only few solid-state structures are available. To fill this void, we are developing structural proteomics footprinting methods for these proteins in a membrane mimic or in a cell. We have two approaches: (1) create fast and reactive footprinters outside the cell and transport them within, and 2) develop lipid or membrane-soluble precursors to a footprinter, partition them to the membrane, and activate photochemically to produce an in-membrane reactive reagent that footprints a protein.

The second challenging area that motivates structural proteomics is the structure and dynamics of amyloid aggregates like Abeta, a putative cause for Alzheimer's disease. We can determine the structure, in a coarse way, of the starting monomer and, in a refined way, the structure of the end fibril or plaque product. The hundreds, maybe thousands, of intermediate oligomers, however, are highly dynamic and heterogeneous, and they may be toxic and the cause of the disease. We are exploring fast and slow footprinting as a means to determine dynamics and follow structural changes in aggregation.

Recent studies illustrating these two problems, the progress we're making in obtaining structural information, and the role of mass spectrometry in this enterprise will be presented in the lecture.

Exploring new heights on the Orbitrap journey

A. Makarov

Thermo Fisher Scientific, Bremen, Germany

This talk focuses on high-resolution accurate-mass (HR/AM) analysis using Orbitrap™ mass spectrometry. The fundamental principles of the technology are presented against the backdrop of its brief but eventful history.

Since its commercial introduction in 2005, the capabilities of the Orbitrap analyzer have been continuously expanded through integration with quantitative workflows, new fragmentation methods, a wide range of vacuum and ambient ion sources, imaging, and ion mobility. These capabilities have been implemented across six major families of Orbitrap-based instruments, with numerous new modes of operation enabled by parallel detection and ion processing, together with increasingly sophisticated coordination of multiple ion-optical devices.

Fascinating leaps of evolution and cross-pollination between different branches of mass spectrometry technology are highlighted. As a recent example of such evolution, the ASymmetric Track Lossless (Astral™) analyzer combines concepts from Orbitrap and time-of-flight mass spectrometry to dramatically improve the sensitivity and throughput of *-omic* analysis. The principles, performance, and operation of this newest analyzer are discussed in detail.

An overview of the major trends in mass spectrometry instrumentation reveals substantial remaining potential for further expansion of analytical capabilities, particularly through the combination of advanced analyzers with improved ion scheduling, processing, and utilization.

The talk concludes with the perspective that the rapid progress now occurring across mass spectrometry instrumentation and analytical methods will allow the field not only to remain competitive with alternative technologies, but also to address the growing demand for unprecedented throughput and analytical depth in the characterization of very complex samples!

Novel ion mobility separations leveraging the macromolecular dipole alignment

A.A. Shvartsburg

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Ion mobility spectrometry (IMS) has become a powerful separation approach prior to MS. However, linear IMS capturing the ion mobility (K) at moderate electric field (E) is tightly correlated with MS as the K and ion mass (m) are connected via the cross section. The high-field mobility increment captured in differential IMS is much less tied to m and provides superior isomer resolution at equal resolving power. Most differential or field asymmetric waveform IMS (FAIMS) methods rely on the field-dependent ion heating. However, many macromolecules are strong dipoles, aligned by sufficient field without significant collisional heating. The resulting low-field differential (LOD) IMS can characterize the dipole magnitude and ion aspect ratio, and distinguish the conformers by those properties largely orthogonal to absolute mobility.

Although the optimum FAIMS waveforms are rectangular, most systems adopted the bisinusoidal profile to meet the engineering constraints of high-voltage electronics. The much lower voltages needed for LODIMS (~1 kV) permit arbitrary rectangular waveforms, e.g., synthesized by digital key switching. The near-ideal waveforms with two field points double the resolution vs the bisinusoidal analogs (with planar-gap FAIMS cells) and enable more accurate and robust dynamic modeling of measurements to extract the dipole properties.

The inhomogeneous fields in curved FAIMS cells focus the ions to (or defocus them away from) the gap median, depending on the waveform polarity and super- or sub- linearity of $K(E)$ function defining the A/B/C ion types. This allows fractionation of the protein conformers by that metric complementary to the $K(E)$ derivative that broadly governs FAIMS analyses.

Locking dipoles in the N_2 gas at ambient conditions fundamentally requires the dipole moments over ~400 Debye. Those for proteins trend up with the size and charge state, for the denatured conformers (produced by ESI) crossing that level at ~25 kDa. The alignment of larger proteins such as Alcohol Dehydrogenase (ADH, 37 kDa) and Bovine Serum Albumin (BSA, 66 kDa) in differential IMS is manifested by intense bands at compensation voltages (U_C) below zero, often extending to extreme negative values. With increasing waveform amplitude (dispersion voltage, U_D), those scale linearly - rather than cubically in typical FAIMS separations.

The new LODIMS stage employing rectangular waveforms with flexible duty cycle (f) and U_D up to 2 kV is coupled to the Velos ion trap and Orbitrap MS instruments and evaluated for ADH, BSA, and further proteins including Concanavalin A. As with the bisinusoidal waveforms, the aligned conformers emerge at $U_C < 0$ with rotary species near bias. The onset threshold at negative U_C remains linear versus U_D , but the slope about doubles expectedly in proportion to the $\langle F_3 \rangle$ factors for two profiles. The experiments reveal the true ratio of directional to orientationally averaged cross section as the asymptotic limit of measured quantity at high f up to 100.

Same proteins are examined using the dome-geometry cell (comprising the cylindrical and hemispherical regions) in FAIMS and LODIMS regimes. We see the ion transmission with both negative and (not previously observed) positive waveform polarities. The selected conformer subpopulations differ, establishing a new technique of Divergent Field Ion Mobility Focusing (DIFIMOF) based on the local curvature of $K(E)$ function rather than its increment controlling the U_C values. Here we demonstrate the DIFIMOF selection of rare protein conformers/protomers and proteoforms with unusual dipole properties, hidden in regular FAIMS spectra.

These interlocking capabilities, which can be joined into multidimensional platforms, furnish a novel suit of approaches to integrative structural biology and top-down proteomics.

Treated wastewater reuse in agriculture: uncovering potential risks of emerging contaminants with mass spectrometry

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Growing interest in reusing treated wastewater (TWW) stems from the increasing scarcity of water resources, efforts toward a circular economy, and the need to protect the environment. In response, many researchers and practitioners are shifting from the traditional linear approach—in which wastewater is treated as waste at the end of the process—to a circular model in which TWW is recognised as a valuable resource in agriculture. However, such practices raise concerns, particularly about the potential introduction of hazardous substances into the food chain.

The presentation will outline the current state of wastewater reuse in agriculture in Europe, focusing on the regulatory landscape, minimum water reuse requirements, risks associated with emerging contaminants, and the effects on the quality attributes of crops produced using urban wastewater. It will highlight advances in treatment technologies and the regulatory frameworks needed to safely integrate wastewater reuse into agricultural practices. To advance urban wastewater reuse, stronger regulations, enhanced treatment methods, and collaborative efforts are crucial to overcoming both technical and social barriers, ultimately promoting sustainable agriculture and improving food security.

Hybrid Orbitrap-FT-ICR mass spectrometry, trapped ion mobility spectrometry-FT-ICR MS, and novel data processing approaches shed light on chemical and biochemical dark matter

K. Hakansson^{1,2*}, M.L. Aguilera¹, L. Babcock-Adams¹, S.A. DeFiglia^{1,2}, N. Heburn^{1,2}, N.K. Kaiser¹, J.R. Norton^{1,2}, R.P. Rodgers¹, C.R. Weisbrod¹

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The FT-ICR mass analyzer remains unrivaled in providing the highest mass accuracy and resolving power for the most comprehensive analyses of complex mixtures at high dynamic range. However, the Orbitrap excels in rapid tandem mass spectrometry (MS²) workflows, in part due to high ion frequencies and the $1/\sqrt{m/z}$ resolving power dependence. Thus, integrating these two mass analyzers within a single system unlocks unique experimental sequences, e.g., parallelized fast acquisition of medium-resolution, high-accuracy MS² spectra while the FT-ICR acquires longer transients for ultrahigh MS¹ resolution in hyphenated liquid chromatography (LC)-MS. Orbitrap Eclipse-ICR hybrid instrument configurations at the MagLab also enable direct, side-by-side comparison of analyzer performance under tightly controlled experimental conditions.

The 21 T FT-ICR analyzer demonstrates an ~order-of-magnitude improvement in mass measurement error (42 ppb) compared with the Orbitrap (~500 ppb) for Arabian asphaltenes. Maximum resolving powers under these conditions were 6.6 million for FT-ICR MS and ~0.5 million for the Orbitrap. Ion coalescence threshold behavior also revealed strikingly different analyzer responses to increasing ion populations with the FT-ICR exhibiting a “digital-like” response, with relatively constant peak spacing maintained towards a sharp coalescence threshold. In contrast, the Orbitrap displays an “analog-like” behavior, in which peak spacing continuously decreases as function of ion number. However, the Orbitrap demonstrates a lower detection limit for low charge states, achieving an S/N ratio of ~4–5 for ~10 charges, whereas the FT-ICR exhibits S/N ~4–5 for ~20 charges under comparable conditions.

These instrumental advantages along with mass difference calibration, which eliminates the need for internal calibrants,¹ allows greatly improved analytical depth for complex mixtures. For example, produced water from U.S. oil and gas-producing regions showed ~350 heteroatom classes in a single sample from ~22,000 assigned molecular formulas, an order of magnitude more classes than typically observed in asphaltenes. For phytoplankton extracts containing lipids, degradation products, and pigments, hybrid Orbitrap–21 T FT-ICR LC-MS showed mass measurement errors $< \pm 0.2$ ppm with external calibration alone, allowing neutral molecular mass determination from multiple adduct ions across thousands of detected features with MS² structural information. Trapped ion mobility spectrometry on a Bruker prototype FT-ICR instrument enabled isobar separation and further structural analysis with electron-induced dissociation.

1. RP Rodgers, CL Hendrickson, CA Holder Montenegro, AJ Tello-Rodriguez, T Potu, B Shung, M Hagan, CR Weisbrod, P Giusti, CP Rüger, ML Aguilera, G Vallverdu Salvato. *Anal. Chem.* **2025**, 97, 18543–18552.

100 Years of photoionization: from history and fundamental principles of photoionization mass spectrometry to current analytical applications in environmental research and industrial monitoring

R. Zimmermann

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Theoretical and physical descriptions of the nature of light and matter in the late 19th and early 20th centuries were rapidly followed by the rise of mass spectrometry (MS) as a powerful analytical technology. The early decades of mass spectrometry were largely dedicated to studying gases and atomic species. Expanding upon studies of the photoelectric effect in the 1920s, F. L. Mohler developed a photoionization (PI) source in 1926 (Mohler, PNAS 12 (1926) 494–496), which was later integrated into photoionization mass spectrometry (PIMS). With the advent of lasers in the 1960s, further light-based ionization techniques emerged, including resonance-enhanced laser ionization, laser desorption/ionization (LDI), and ultimately matrix-assisted laser desorption/ionization (MALDI).

On the occasion of this 100th anniversary, this lecture begins with a brief history of photoionization processes, specifically single-photon ionization (SPI) and laser-based resonance-enhanced multiphoton ionization (REMPI), followed by an introduction to the fundamental principles of photoionization.

The main part of the lecture will focus on the concepts, technical developments, and practical implementations of analytical PIMS (classical SPI and REMPI) for real-world applications. These range from online threat detection (e.g., traces of explosives or fuels in seawater) at World War II wrecks or ammunition dumpsites using ship-based or submersible membrane-inlet PIMS systems, to industry-relevant monitoring applications (e.g., coffee roasting and catalytic processes)-

Finally recent developments in atmospheric science and aerosol research are addressed. In this context the integration of resonance-enhanced photoionization processes into single-particle mass spectrometry (SPMS) will be highlighted. This approach enables the rapid online detection of trace metals and the molecular profiling of polycyclic aromatic hydrocarbons within individual airborne aerosol nanoparticles. Recent applications of this resonance-enhanced PIMS methodology in atmospheric science will be presented and discussed.

Protein tyrosine nitration: from radical-mediated reactions to clinical therapy

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Protein function can be altered by radical-mediated post-translational modifications that regulate cellular processes and contribute to disease progression. Protein tyrosine nitration (PTN), a hallmark of nitrative stress, is implicated in numerous neurodegenerative disorders. Because PTN occurs at low abundance and exhibits remarkable site selectivity, advanced analytical approaches are required to identify nitrated proteins and elucidate their biological significance.

Our research spans fundamental radical chemistry to clinical applications in neurodegenerative disease. We have developed integrated bioanalytical and bioinformatics platforms for proteome-wide mapping of PTN, generating the largest dataset of endogenous PTN sites reported without targeted pre-enrichment using a *Macaca fascicularis* model of cerebral ischemia [1,2]. We have also investigated the interactions among metal cofactors, ligands, and peptides, revealing diverse metalloprotein structures and radical-mediated reaction pathways through tandem mass spectrometry, isotopic labeling, density functional theory, and infrared multiple-photon dissociation spectroscopy [3,4].

More recently, we have expanded these efforts to cerebrospinal fluid (CSF) omics in Alzheimer's disease (AD). We identified a panel of CSF protein biomarkers that distinguish responders from non-responders to lymphatic-venous anastomosis therapy, enabling improved patient selection for surgical intervention [5]. Our ultimate goal is to define the role of protein tyrosine nitration in AD pathogenesis and translate fundamental discoveries in radical chemistry into clinically actionable diagnostics and therapeutic strategies.

1. Anal. Chem. (2015) 87 (19): 10015
2. Anal. Chem. 2020, 92, 10768
3. PLoS ONE 19(8): e0308164
4. ACS Phys. Chem Au, 2025, 5 (5): 508
5. Anal. Chem. 2026, 98, 20189

What do we drink? Formation of disinfection by-products in water treatment

A.T Lebedev.

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Although being successfully applied all over the world water disinfection brings to formation of hazardous disinfection by-products (DBP) from natural and anthropogenic compounds always present in water. Over 800 DBP are known so far, being formed from natural humic matter. Every novel anthropogenic compound shows its unique scheme of transformation with formation of dozens and even hundreds DBP. Reactions of electrophilic substitution, electrophilic addition, nucleophilic substitution, oxidation, single electron transfer, and even radical substitution result in a consecutive array of DBPs, finishing with haloforms. Identification of novel DBPs and study of the mechanisms of their formation represents a challenging scientific task aiming improvement of human health and prevention of ecosystems pollution.

Aqueous chlorination and bromination of humic matter and numerous emerging contaminants (UV-filters, pharmaceuticals, cosmetic products) was studied with GC-HRMS and LC-HRMS. The reactions were carried out in laboratory in conditions close to that used at the water treatment stations. Besides, drinking water from several Russian cities and from swimming pools was analyzed to find DBPs. The studies involved various disinfecting agents, variation of ratios, pH values, reaction time, addition of inorganic salts. GC-MS experiments were performed using high resolution TOF mass-spectrometers Pegasus GCxGC-HRT (LECO Corporation) and Orbitrap Exactive (Thermo Scientific). LC-MS analyses were carried out with high-resolution TripleTOF 5600+ quadrupole time-of-flight (Q-TOF) (AB Sciex) and Orbitrap Tribrid (Thermo Scientific) mass spectrometers. Besides several low-resolution GC-MS and LC-MS systems were used.

Numerous novel DBPs were identified in the drinking water in the current study. However, the most serious efforts were applied to elucidate the mechanisms of aqueous chlorination. Conjugated double bond appears to be more reactive in aqueous chlorination even than activated aromatic ring. Primary reactions of active chlorine with resveratrol and avobenzone involve double bond, rather than aromatic ring. The presence of two double bonds in limonene allowed studying consecutive reactions of nucleophilic addition and elimination with formation of various chlorinated and hydroxylated products. Radical reactions may also take place as aqueous halogenation of benzalkonium chloride resulted in formation of numerous products with halogens in the aliphatic chain. Pharmaceutical products (doxazosin, umifenovir) demonstrated rather complex transformation including substitution, elimination, and oxidation. Interesting reaction involves dealkylation of alkylamines. It proceeds by single electron transfer with formation of the corresponding aldehyde as the second product. Bromides and iodides in natural water form more toxic organohalogens than the corresponding chlorinated ones. Long-term monitoring of DBPs in drinking water demonstrated fast penetration of bromine into the organic compounds at the initial stages of aqueous chlorination. Final DBPs contain more chlorine than bromine due to higher content of chlorine in the reaction mixture and substitution of bromine for chlorine. The latter reaction was studied on haloaromatic standards. The results show fast substitution of iodine for chlorine in the aromatic substrates due to ipso-substitution. Bromine cation being substituted with chlorine may attack another substrate forming polybrominated species.

A wide range of DBPs containing up to five chlorine atoms, hydroxyl and carbonyl groups were revealed in aqueous chlorination experiments with cocamidopropyl betaine. The proposed mechanism of their formation involves nucleophilic substitution of the secondary amide hydrogen atom followed by subsequent free radical and electrophilic addition reactions resulting in non-selective introduction of halogen atoms and hydroxyl groups into the alkyl chain.

Development of a targeted mass spectrometry-based method for profiling Cys-loop and ionotropic glutamate receptors in cell cultures and tissues using stable isotope-labeled standards

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Cys-loop receptors are membrane ligand-gated ion channels that play a key role in mediating rapid excitatory and inhibitory neurotransmission in the central and peripheral nervous systems. Owing to their structural features and broad distribution, these receptors have become important targets for the development and application of pharmacological agents [1].

Accurate determination of the absolute abundance of these receptors in biological samples, as well as assessment of changes in their expression levels under various physiological and pathological conditions, is essential for drug development and disease diagnostics. However, conventional techniques such as immunohistochemistry and flow cytometry are limited by several challenges, including the lack of highly specific antibodies for individual receptor subunits.

A panel of 54 synthetic peptide standards was developed to enable absolute quantification of human Cys-loop and ionotropic glutamate receptors following tryptic digestion. Proteotypic tryptic peptides for each receptor family were selected using bioinformatic approaches.

Each peptide standard was synthesized in two forms. The unlabeled peptide mixture was used for calibration curve construction, whereas the isotope-labeled peptides served as internal standards for absolute quantification. Peptide purity was assessed by LC-MS/MS, while peptide concentrations were determined by amino acid analysis coupled with mass spectrometric detection.

To improve the accuracy of peptide quantification, two amino acid analysis methods employing stable isotope-labeled amino acids as internal standards were optimized. The second method achieved a limit of quantification (LOQ) of 0.01 nM and was selected for the quantitative characterization of peptide standards.

To ensure high specificity and sensitivity of the PRM assay, optimal fragment ion transitions were selected for each peptide standard, and the instrumental parameters were optimized.

The developed method was validated using the SH-SY5Y neuroblastoma cell line and a primary glioblastoma cell line. Membrane protein fractions were isolated and subjected to tryptic digestion, with the isotope-labeled peptide standard mixture added prior to proteolysis. Fetal bovine serum was employed as a complex biological matrix to evaluate matrix effects on peptide identification and quantification. Peptide losses during sample preparation were assessed by calculating the recovery factor, which was determined to be 66.7%.

The developed targeted mass spectrometry method enabled quantitative determination of the majority of Cys-loop and ionotropic glutamate receptor subunits over a broad linear dynamic range of 50–500,000 fg/μL, with a coefficient of determination of $R^2 = 0.996$. These results demonstrate the applicability of the proposed workflow for the absolute quantification of membrane receptors. In future studies, the method will be applied to generate a quantitative atlas of Cys-loop and ionotropic glutamate receptors in the human brain.

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Differentiation of leucine and isoleucine isomeric residues by ExD in *de novo* peptide sequencing. The case of temporins.

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Temporins are rather short frog peptides (11–13 aa) with 4–6 Leu/Ile residues in the chain possessing antimicrobial and fungicidal properties. Certain peculiarity of these peptides involves mirror position of Leu/Ile isomeric residues in some of the peptides. Reliably distinguishing between these isomeric residues remains a challenging task in peptidomics. To differentiate isomeric Leu/Ile, several approaches exist, i.e., Edman degradation and cDNA cloning, however, they are rather complicated and cumbersome, while MS/MS is much faster, more sensitive, and even cheaper. Among the proposed approaches, the most promising involves detection of characteristic *d*- and *w*-ions. While *w*-ions may be successfully obtained with EThcD, that method does not provide the required series of *d*-ions. Formation of *w*- and *d*-fragments is more often observed when the cleavage site produces high *m/z* fragments. Therefore N-terminal residues are best identified with a *z*-*w* pair of ions. Similarly, the *a*-*d* pair of ions is more efficient for identifying C-terminal residues. Combined use of *w*- and *d*-ions allowed for close to 100% identification of Leu and Ile residues.

To perform the experiment, two female frogs were caught in Arkhangelsk, Russia in May 2024. Secretion was obtained by mild electrostimulation in the field. The peptide fraction of the secretions was analyzed by mass spectrometry. Experiments were performed on an Agilent 6545XT AdvanceBio LC/Q-TOF equipped with an Agilent ExD cell. Peptides (1 µg/mL) in methanol/water solution were run through the Agilent AdvanceBio peptide mapping column with a linear gradient of 5–60% acetonitrile in 60 minutes. In MS1 mode, the ExD cell was tuned for the ion transmission, whereas ECD was used in MS2 mode for ion fragmentation. The DDA approach was applied for precursor identification and selection. ExD Viewer (Agilent) and manual spectra interpretation were used for the determination of the peptide sequence.

In the present study, twelve temporins from in the skin secretion of the frogs were detected in the secretion of the studied frog. The levels of nine of them, namely, temporins B, C, D, G, F, H, K, M, N, were high enough to produce rich ExD spectra, and their sequence was determined by ExD tool. The experiments were carried out with collision energies of 0, 15, and 20 V. ExD mode (without additional collisional activation) revealed 17 *d*-ions and 30 *w*-ions. Application of ExciD (15 or 20 V collisional activation) revealed 21 *d*-ions and 34 *w*-ions. Overall, 96% (51 out of 53) of isomeric Leu/Ile were reliably identified with *w*- and *d*-ions. The absence of both *d*- and *w*-ions in the case of ⁸Ile in temporin G may be rationalized by its close vicinity to ⁷Arg residue, which is highly basic.

It is worth mentioning that CID sometimes triggered radical site migration. Migration was observed for temporins M and F even in the absence of collision energy. To avoid radical site migration, additional collision energy should be as low as possible, or better increased stepwise till the threshold of the migration energy.

Due to the complexity of the task, the literature data on Leu/Ile is controversial. In the current study, the results obtained for temporins M, G, F were corrected in comparison with those described in the literature, as ExciD enabled obtaining more reliable data.

MetaDirectMS1: expanding the metaproteomics toolbox with rapid MS1-based analysis

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Microbial communities play essential roles across diverse biological and environmental systems, from the human microbiome to soil and aquatic ecosystems, where microorganisms also contribute to pollutant biodegradation. Metaproteomics enables characterization of their taxonomic composition and functional activity through analysis of expressed proteins, supporting both environmental and biomedical research [1]. Despite its potential, metaproteomics lacks standardized experimental and computational workflows, with most current approaches relying on data-dependent (DDA) or data-independent (DIA) tandem mass spectrometry. The complexity and dynamic range of metaproteomic samples often require extended chromatographic separation and MS/MS acquisition, limiting analytical throughput. Moreover, extensive peptide coelution increases precursor cofragmentation, potentially biasing peptide identification and subsequent taxonomic and functional interpretation [2]. Here, we present MetaDirectMS1, a bioinformatics framework based on the MS/MS-free DirectMS1 approach [3]. By relying on high-resolution MS1 data for protein identification, MetaDirectMS1 reduces experimental time and integrates taxonomic identification, quantitative taxon profiling, and protein-level differential analysis for functional interpretation. Building on our previous application of DirectMS1 to rapid microbial proteomics [4], the framework was evaluated using controlled protein mixtures, bacterial cultures, benchmark datasets, model microbiomes, and gut microbiome samples. Expected taxa were consistently recovered as dominant identifications in defined communities, while quantitative analysis reproduced expected protein- and taxon-level abundance changes. In complex microbiomes, MetaDirectMS1 further detected biologically relevant shifts in community composition and associated protein-level functional responses. By combining rapid MS1 acquisition with quantitative taxonomic and functional analysis, MetaDirectMS1 broadens the metaproteomics toolbox and provides a scalable strategy for large microbiome studies where analytical throughput is a major limitation.

MetaDirectMS1 development was supported by the Russian Science Foundation (grant no. 26-15-00131)

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Interrogation of ion mobility selected proteins via electron capture dissociation: does protein native-like structure survive in the gas phase?

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Ion mobility (IM) spectrometry combined with mass spectrometry (MS) is widely used in native mass spectrometry to study the structures of proteins and protein complexes. One of the important questions is to understand to what extent the solution-phase protein structure is retained after its injection into a mass spectrometer. The determination of protein collision cross sections (CCS) with drift gases after passing IM and comparing with theoretical CCS predicted from X-ray or NMR experiments is the main tool to determine if the solution structure survives or changes in the gas phase. The quality and precision of this estimation depends on the accuracy of the theoretical approaches and IM calibration methods. There is a quite large discrepancy in IM-determined and theoretical CCS for native protein structures in literature, even in the case of small proteins. The application of a new approach with the ability to work with protein structural data is therefore in great demand. Tandem MS with the application of a soft fragmentation method in addition to IM could be another tool to solve the question.

Electron capture dissociation (ECD) MS might be the most perspective approach here. Working with several differently sized proteins from ubiquitin to monoclonal antibodies, we found experimental conditions that meet the requirement of retaining native protein structures intact during the MS experimental time domain. The experiments were conducted on the Agilent Q-TOF 6560 mass spectrometer equipped with a drift tube for IM and an Agilent ExD cell to conduct the ECD. The ionization of proteins was performed with static nanospray ionization. To understand the stability and dynamics of structural changes, an opposite technique, charge stripping in the atmosphere (namely proton transfer with a base prior to introducing to MS), was also applied. The folding/unfolding processes were found to be protein-charge and size dependent. Structural differences between ions originating from the native solution and ions of the same charge state originating from denatured solutions with proton stripping were examined by both IM and ECD mass spectrometry. The complementary activation of proteins prior to IM and ECD via collision induced unfolding (CIU) was applied to study dynamics of the protein structure change after cleavage of the noncovalent bonding in the proteins.

Comparison of methods for the analysis of the glycosylation profile of therapeutic proteins: glycopeptide analysis by LC-MS and released glycan analysis by HPLC-FLD

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Protein N-glycosylation is a post-translational modification that occurs via the addition of an oligosaccharide residue to the amino acid, asparagine (Asn), in protein. The glycosylation profile is a critical quality attribute of therapeutic monoclonal antibodies. Therefore, rapid methods for glycosylation profiling are required, particularly in upstream process development. There are three main approaches to glycosylation profiling: released glycan analysis, glycopeptide analysis and analysis of subunits.

For released glycan analysis by HPLC-FLD using a commercial dye-labelling kit, a relatively long chromatographic run (55 min) is required to achieve separation of more than 30 glycan species. However, even with such a long gradient, some glycans cannot be completely resolved. For example, Man5, G1 and G1F-N co-elute, although they belong to different glycan groups: high-mannose, galactosylated, fucosylated species. This complicates data interpretation, since the relative abundance of individual glycans within this peak may differ between samples. Released glycan analysis using a labeling kit is associated with relatively high reagent costs. This method provides non-site-specific glycan profiling. Correct peak integration requires mass spectrometry (MS)-based annotation, which is time-consuming and labor-intensive.

In this work we investigate the possibilities of glycopeptide analysis by LC-MS. Glycopeptide analysis can provide site-specific glycan profiling. In general, the glycopeptide analysis workflow involves enzymatic digestion, glycopeptide enrichment and MS detection. Glycopeptide enrichment was performed using silica-coated magnetic particles.

Despite comparable sample preparation times, a reduction in chromatographic elution time was achieved. MS-based annotation is no longer required, and the results are not affected by co-eluting glycans. In addition, analysis costs were reduced by approximately 20-fold. The only limitation was a decrease in the number of detected glycans.

De novo sequencing of nanobodies with the Twister algorithm

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Antibodies play an essential role in protecting the organism from pathogens and harmful foreign substances. Of particular interest are camelid single-chain antibodies, called VHH antibodies or nanobodies [1]. Their single-domain structure allows them to access epitopes hidden from conventional antibodies, making them an attractive choice as the base for novel medicines.

The purpose of this work is to adapt the Twister *de novo* sequencing algorithm [2,3] to elucidation of the primary structure of camelid nanobodies. The proposed approach was tested on a synthetic camelid antibody.

To assure the full coverage of the sequence, we applied several enzymes, including trypsin, chymotrypsin, GluC, LysC, and proteinase K (ProtK). The resulting peptide hydrolysate from VHH was then analyzed by UHPLC-HRMS. The raw data was obtained on a HRAM mass-spectrometer Q Exactive with an Orbitrap mass analyzer (Thermo Scientific) at a resolution 70000 and 17500 at m/z 400 for MS1 and MS/MS, respectively. In distinct experiments, the normalized collision energy (NCE) was set to 28, 30, and 35 eV for tryptic peptides, and always to 30 eV for the other enzymes. The final dataset comprised 31715, 22110, 20969, 10156, and 21315 MS/MS spectra acquired from tryptic, chemotryptic, GluC-, LysC-, and ProtK-peptides, respectively.

When analyzing mass spectra, we profited from the ability of Twister to process the data making no assumptions on the specificity of the enzymes employed, and to consider any post-translational modifications that might be expected. Amino acid modifications taken into account included S-pyridylethylation [C] (used to stabilize the cysteine residues), oxidation [M], carbamylation [K, R] and deamidation [N, Q].

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We successfully retrieved the full nanobody sequence, with a follow-up of an experimental protocol for the next studies. Our future research will be devoted to further automatization of retrieval of the camelid nanobody sequences with the Twister algorithm, along with optimization of the sample preparation and mass spectrometry protocols.

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First-trimester preeclampsia prediction by integrated DIA-PASEF and targeted LC-MRM-MS serum proteomics

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Preeclampsia is a heterogeneous pregnancy disorder that usually becomes clinically evident after 20 weeks of gestation, whereas the underlying molecular changes may start much earlier. Current first-trimester screening algorithms are useful but do not fully capture the systemic biological alterations preceding disease onset. Therefore, reproducible mass-spectrometry-based serum proteomics may provide an additional layer for early risk stratification and molecular characterization of preeclampsia.

We developed and applied an integrated serum proteomics workflow for first-trimester pregnancy samples. The analytical strategy combined targeted LC-MRM-MS quantification with stable isotope-labeled peptide standards, MoM-based normalization, machine-learning classification, and, in a discovery-validation design, untargeted DIA-PASEF-MS followed by targeted MRM-MS verification. A healthy pregnancy reference study established pregnancy-specific quantitative profiles for first-trimester serum proteins and provided a methodological basis for further comparison with pathological pregnancies¹.

Across the project, first-trimester serum proteomic profiling revealed reproducible protein signatures associated with subsequent development of preeclampsia and related hypertensive pregnancy disorders. In targeted LC-MRM-MS analysis, the preeclampsia-associated signature was mainly linked to complement and coagulation pathways, including F10, C8A, C1QA, SERPING1, and VTN. An 18-protein SVM model demonstrated high predictive performance and outperformed routine first-trimester screening². In an integrated DIA-PASEF-MS/MRM-MS study, 33 candidate protein markers were identified, and four proteins, AFM, AHSG, C8A, and IGHG1, were confirmed across platforms. The resulting SVM model achieved an AUC of 0.95, with 95% sensitivity and 97% specificity³.

These findings show that mass spectrometry can move beyond single-marker measurement and become a platform for early pregnancy risk stratification. Integrated DIA-PASEF and targeted LC-MRM-MS serum proteomics provide reproducible quantitative data, reveal biologically meaningful pathways, and support machine-learning-based prediction of preeclampsia before clinical manifestation.

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Mass spectrometry-based degradomics: semi-tryptic peptide profiling of proteomes for mapping disease-specific proteolytic signatures

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Mass spectrometry (MS) and positional proteomics (degradomics) open new avenues for mapping proteolytic events in cells and tissues. The native peptidome and pools of protease-specific peptides serve as a direct source of in vivo information regarding the activity of intra- and extracellular proteases associated with the development of pathological processes. In trypsin-based HPLC-MS/MS bottom-up approaches, degradome analysis focuses on identifying semi-tryptic peptides and determining the cleavage motifs of their corresponding proteins. However, the proteome-wide identification of these peptides—and particularly the measurement of changes in their relative abundances—remains one of the most challenging tasks in proteomics. These challenges include search sensitivity, as only a limited number of semi-tryptic peptides are generated, subjected to fragmentation, and potentially identified against a vast whole-proteome pool of tryptic peptides. Another obstacle is decoupling changes in protease activity from confounding shifts in general protein expression. In this work, we developed a semi-tryptic peptide search workflow using the IdentiPy search engine operated in semi-tryptic mode, and re-analyzed proteomic data from multiple consortium studies spanning various pathologies. The analyzed data include the largest collection of Alzheimer's disease proteomic studies, the NCI-60 proteome-exome dataset combining whole-exome and quantitative MS profiles across 60 human tumor cell lines, and deep, discovery-based MS datasets from the Clinical Proteomic Tumor Analysis Consortium (CPTAC).

One of the key highlights of this work is the identification of neo-N-terminal fragments of the tubulin isoform TBB2B_HUMAN, featuring a cleavage at the DEHGID↓P motif. The corresponding N-terminal semi-tryptic peptide, PTGSYHGSDQLER, was identified in post-mortem brain tissue samples from hundreds of patients, balanced and stratified into three clinical groups: cognitively healthy controls, asymptomatic AD (AsymAD) patients, and clinically verified AD patients. The presence of a proline residue at the P1' position sterically hinders the catalytic pockets of apoptotic caspases, excluding their involvement. Conversely, this motif points toward granzyme B, which is known to possess a rare affinity for D↓P-type bonds, making this semi-tryptic peptide a direct molecular footprint of its activity. Further quantitative dynamics analysis revealed a stage-specific correlation. The baseline level of tubulin cleavage observed in the control group increased during the AsymAD stage and peaked during the terminal phase of clinical AD. This demonstrates that granzyme B-mediated destruction of the neuronal cytoskeleton manifests long before the onset of the first cognitive symptoms. To our knowledge, this finding represents the first mass spectrometry-based proteomic demonstration of brain parenchyma infiltration by CD8+ T lymphocytes secreting the serine protease granzyme B, a phenomenon that has recently entered the focus of AD mechanism research [Gate, D. et al. (2020). *Nature*, 577(7790), 399-404].

Additionally, this study mapped the protease-specific landscape of cellular degradomes across diverse cancer cell lines, focusing on identifying protein fragments arising from mutagenesis. In summary, mass spectrometry analysis of semi-tryptic peptides enabled the verification of early, specific protein degradation events that may drive or be driven by disease. These protease-specific fragments represent promising biomarkers for molecular diagnostics and novel targets for therapeutic intervention.

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LC-HRMS determination of per- and polyfluoroalkyl substances in water and food matrices

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In recent decades, the rapid expansion of the chemical industry has led to the widespread release of numerous synthetic compounds into the environment. Among these, per- and polyfluoroalkyl substances (PFAS) are of particular concern due to their exceptional resistance to thermal, chemical, and biological degradation. Their desirable industrial properties have resulted in their incorporation into a vast array of consumer products, while their persistence and high mobility have facilitated global distribution. PFAS can migrate through water systems and food webs, ultimately entering the human body, where they tend to accumulate. Given the growing body of evidence on their toxicological effects and the fact that the biological half-life of certain PFAS congeners may extend to several years, there is a sustained health risk even under chronic exposure to low concentrations.

The aim of this study was to develop simple and effective analytical approaches for the determination of PFAS in water and food matrices using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). For the analysis of chicken eggs, a sample preparation method based on dispersive solid-phase extraction (d-SPE) was proposed. Unlike other techniques, the developed procedure does not require additional clean-up steps, thereby reducing overall preparation time, minimizing the risk of contamination, and decreasing potential analyte losses. The average recovery was 82%, and matrix effects were negligible for the majority of target compounds, confirming the suitability of the method for real-sample analysis. When applied to egg samples from major Russian producers, the average total concentration of the investigated PFAS was 30 µg/kg of egg (12.9 ng/mL), with concentrations ranging from below the limit of detection to 144 µg/kg of egg (63 ng/mL).

For aqueous matrices, a solid-phase extraction (SPE) procedure utilizing weak anion-exchange cartridges was developed, achieving an average recovery of 81%, which meets the criteria for quantitative analysis. Compared to standardized protocols, the proposed scheme is simpler, relying exclusively on SPE without supplementary clean-up steps. The method was successfully applied to river and natural water samples collected from several Russian regions, including Moscow, Saint Petersburg, and Yaroslavl. In the analyzed samples, the total PFAS content was generally low; the highest contamination was observed in water from the Gulf of Finland, where perfluorononanoic acid (PFNA) reached a maximum concentration of 7.3 pg/mL.

This study presents the first reported data on PFAS levels in Russian environmental and food samples, highlighting the need for more extensive and in-depth investigations in this area. In the absence of national regulatory standards, these findings may serve as a valuable reference for establishing appropriate PFAS thresholds tailored to the specific conditions in Russia.

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Accuracy Problems in Bottom-Up Protein Quantification

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Modern mass-spectrometry (MS)-based proteomics enables the simultaneous quantification of thousands of peptide and protein species. Data analysis relies on sophisticated mathematics that are implemented slightly differently in each software solution. Thus, its accuracy must be evaluated using standard benchmark datasets with proteins or proteomes mixed at predefined ratios. Such models are generally based on chemically identical proteins at different concentrations across compared samples and primarily assess the accuracy of recovering known abundance ratios. This does not fully reflect real biological experiments, where changes in proteoform composition and disappearance of peptide species upon concentration changes can alter peptide profiles and assignment between protein groups and affect protein-level quantification. Here, we propose an alternative benchmark model designed to evaluate the reliability of protein quantification in biologically distinct samples. Unlike conventional LFQ benchmarks, our approach focuses on the consistency of statistical significance of protein-level changes between biologically distinct samples. This criterion more closely reflects the goals of typical bottom-up proteomic studies, in which biological conclusions are generally based on the statistically significant protein sets.

To achieve this goal, we used two human cell lines, HEK293T and THP-1, as distinct human proteomes, and mixed them separately with an *A. thaliana* background proteome (roughly the same size as the human proteome) at gradually increasing dilution levels. Direct comparison of the two human proteomes without the *A. thaliana* matrix provides a reference set of differentially expressed proteins (DEPs), while addition of the background proteome progressively reduces peptide detectability and thereby models the loss of quantitative information at the peptide level. This design allows the consistency of differential expression results to be assessed under conditions that more closely resemble biological proteomic experiments. The dataset produced contains DDA and DIA experiments performed on the human samples without the matrix, followed by 1:1, 1:10 and 1:100 dilutions.

Using the non-diluted samples, we established a reference set of statistically significant DEPs, hereafter referred to as the True DEP (TDep) set. We then assessed the consistency of DEPs after addition of the background proteome. Proteins that became significant only after dilution were classified as False DEPs (FDep). The false quantification rate (FQR), defined here as $FDep/(TDep+FDep)$ in each dilution, was 22%, 26%, and 32% across the three dilution levels, while the conventional LFQ benchmark based on the agreement between observed and expected protein abundance ratios yielded an FQR of 3%, 15%, and 41%, respectively. We also examined factors affecting the probability of incorrect protein quantification. The overall proportion of identified DEPs decreased with dilution, indicating that protein-level statistical significance is progressively impaired at lower intensities. The number of quantified peptides was also important: proteins quantified with only 2-3 peptides were approximately twice as likely to be incorrectly quantified as proteins quantified with >4 peptides. This effect is particularly relevant for low-intensity proteins with few quantified peptides, where a change in the intensity of a single peptide may substantially affect the inferred protein-level change, while the limited number of peptides provides insufficient information to distinguish a true protein-level trend from peptide-specific variation. Our results demonstrate that proteoform heterogeneity and the resulting variability of peptide-level measurements can affect protein-level statistical inference in a manner not captured by conventional LFQ benchmarks. The partial overlap between proteins identified as unreliable by the two benchmark approaches suggests that this effect represents a distinct source of quantification uncertainty rather than merely general measurement error.

Aspects of the use of mass spectrometry methods in pharmaceutical manufacturing

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Unlike most other manufacturing sectors—including the chemical industry—pharmaceutical manufacturing is subject to strict licensing and statutory regulation (for example, Article 45 of Federal Law No. 61-FZ dated April 12, 2010, "On the Circulation of Medicines"). The State Pharmacopoeia of the Russian Federation also constitutes a legislative act that is mandatory for compliance in pharmaceutical production.

Intensifying competition in the pharmaceutical market and lobbying by major manufacturers have led to a significant tightening of legislative requirements regarding the quality of active pharmaceutical ingredients (APIs) and finished dosage forms (FDFs). Consequently, the General Pharmacopoeial Monograph OFS.1.2.1.1.0008 "Mass Spectrometry" was introduced—spanning the transition from the 13th to the current 15th edition of the State Pharmacopoeia of the Russian Federation—and came into effect on September 1, 2023.

Fields of application:

1. Verification of the authenticity of drug substances.
2. Quantitative determination of pharmaceutical substances and impurities in dosage forms.
3. Identification of impurities and elucidation of unknown structures.
4. Quantitative determination of trace amounts of substances in pharmacokinetics and metabolomics.
5. Quantitative determination of over 70 elements with detection limits ranging from 10 to 0.1 ppt (parts per trillion) using inductively coupled plasma mass spectrometry.

This presentation will examine examples of using mass spectrometric methods in the development, validation, and production quality control of active pharmaceutical ingredients (APIs) and finished dosage forms (FDFs).

High-Resolution mass spectrometric identification of semi-volatile constituents released from oral nicotine pouches into artificial saliva

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The increasing popularity of tobacco-free nicotine pouches has highlighted the need for detailed chemical characterization of compounds released during use. In this study, high-resolution gas chromatography mass spectrometry (GC-HRMS) was applied as a non-target analytical tool to identify semi-volatile organic compounds extracted from three commercially available nicotine pouch products into artificial saliva under simulated consumption conditions. The main objective was to characterize the released chemical constituents and demonstrate the capability of high-resolution mass spectrometry for reliable compound identification.

Samples were analyzed using GC coupled to a high-resolution time-of-flight mass spectrometer. Compound identification relied on accurate-mass measurements (<1 ppm), NIST23 spectral library matching, retention-index confirmation, and interpretation of characteristic fragmentation pathways. This comprehensive workflow enabled the identification of approximately 200 compounds belonging to diverse chemical classes, including terpenes and terpenoids, alkaloids, alcohols, ethers, amides, heterocycles, phenols, and esters.

The terpene fraction was dominated by limonene, caryophyllene, germacrene D, menthol, menthone, and carvone, while nicotine-related alkaloids such as myosmine, cotinine, and nicotine were also detected. High mass resolution was particularly valuable for distinguishing structurally related compounds and confirming elemental compositions of molecular and fragment ions. Several identified compounds are recognized fragrance allergens, whereas pulegone and β -myrcene are classified as possible human carcinogens.

Particular emphasis was placed on structural elucidation of unknown compounds. Accurate-mass fragment analysis enabled tentative identification of two trace chlorinated compounds, 2-(1-chloroethoxy)acetaldehyde and ((chloromethoxy)methyl)benzene. Furthermore, a series of previously unreported nitrogen-containing constituents exhibiting a characteristic fragment ion at m/z 161.1072 suggested the presence of nicotine-derived transformation products. These findings demonstrate the strength of GC-HRMS for discovering and characterizing unknown constituents in complex nicotine product matrices.

Overall, the study confirms that nicotine pouches contain a chemically diverse mixture extending beyond nicotine itself and highlights high-resolution mass spectrometry as an indispensable tool for comprehensive chemical profiling, structural identification, and future toxicological assessment of emerging nicotine products.

Application of GC-MS to analyze the aroma profile of dry-cured sausage with different flavoring levels in the formulation

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Dry-cured and raw-smoked sausages occupy a distinctive position in the meat product market. Their high energy value and consumer demand are driven by specific physicochemical characteristics, including a dense texture, elevated protein and lipid content, and low moisture mass fraction.

Undoubtedly, consumer preferences heavily rely on the organoleptic properties of sausages, which are determined by their aroma, taste, and texture. To modify the aroma profile, manufacturers frequently incorporate flavorings into meat products, thereby introducing new product variations to the market.

Modeling and optimizing new flavor and aroma characteristics are complex technological process. It typically involves an expensive stage of engaging focus-groups for sensory evaluation at various production phases. Evidently, the results of such assessments are inherently dependent on the subjective opinions, personal preferences, and dietary habits of the panelists. This issue is particularly relevant in the post-COVID period, given that a significant portion of the population (including expert tasters) may experience long-term alterations in taste and olfactory perception.

The application of gas chromatography-mass spectrometry (GC-MS) to analyze the aroma profile of food products allows for the elimination of such subjective testing stages, enabling the use of this method as an independent analytical test at any production stage.

The complexity of investigating dry-cured sausages stems from the specific nature of their multicomponent and dense matrix, which forms during the salting, fermentation, and drying processes. This structure effectively retains a wide range of volatile substances. The analyzed aroma profile includes both endogenous compounds (products of lipolysis, proteolysis, and microbiological meat ripening) and exogenous highly volatile molecules introduced into the formulation as complex food flavorings.

During the study, three types of dry-cured sausage samples with varying flavoring content in the formulation were analyzed under different storage conditions. A total of 40 volatile organic compounds, possessing 58 olfactory descriptors and forming the overall flavor of the sausages, were identified. Since the concentrations of volatile components were not opportunity to analyze, the relative odor activity value (rOAV) calculation method was used to assess the contribution of individual molecules to the overall sensory profile, and sensory profiles were constructed. All analyses were performed using a static headspace sampler and Maestro- α MS gas chromatography-mass spectrometry system (*Interlab, Russia*).

Molecular-structural analysis of lignin oligomers by Orbitrap High-Resolution Mass Spectrometry

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Lignin, an aromatic polymer accounting for up to 40% of plant cell wall content, represents a valuable renewable source of aromatic compounds, sorbents, antioxidants, and composite materials. For the molecular characterization, Orbitrap high-resolution mass spectrometry has proven to be one of the most powerful analytical tools, successfully applied to lignins of diverse origins and their depolymerization products. We propose an integrated analytical approach for the comprehensive molecular characterization of lignin.

The most promising ionization method is photoionization. Under APPI conditions, lignin degradation primarily involves cleavage of β -O-4 ether bonds between phenylpropane units, along with demethylation (in negative ion mode) and loss of water or formaldehyde (in positive ion mode). The negative ion mode offers higher ionization efficiency for high molecular weight species but induces greater fragmentation of β -O-4 linkages, leading to partial depolymerization within the ion source. Coupling APPI with Orbitrap MS enables acquisition of mass spectra with resolved fine structure for lignins from various plant feedstocks, containing signals from up to 3000 oligomers in the m/z range 300–1800 Da. This capability supports comprehensive molecular-level characterization and monitoring of compositional changes during various processing steps.

To overcome ionization suppression caused by impurities, we employed gel permeation chromatography coupled with Orbitrap MS, using a styrene–divinylbenzene column and atmospheric pressure photoionization with tetrahydrofuran as a dopant. Unlike direct photoionization, prior chromatographic separation introduces lignin oligomers of different molecular weights into the ionization region sequentially, allowing more detailed analysis of individual polymer fractions. An additional benefit is the removal of co-eluting low-molecular-weight impurities, which otherwise severely suppress ionization of the target analytes.

Tandem mass spectrometry is a promising approach for structural analysis of lignin. The combination of dopant assisted APPI with broadband collision induced dissociation Orbitrap MS (bbCID-HRMS) allows detection of a series of negatively charged lignin fragment ions in the m/z range 90–210, resembling pyrolytic degradation products. Tentative identification based on accurate mass elemental compositions led to the selection of 20 target structures, assigned to S-, G-, and H-type units within the lignin macromolecule. Since increasing collision energies in bbCID promotes demethoxylation, leading to the formation of H-type structures, reliable S/G/H ratios are obtained at a minimum normalized collision energy of 10 eV, which ensures sufficient cleavage of the predominant ether bonds. On this basis, we propose a rapid APPI(–)-bbCID-HRMS method for assessing S/G/H ratios in native (low modified) lignin preparations, using flow injection of 5 μ L of a 50 μ g/mL sample, co-injected with the solvent/dopant stream into the ion source.

These findings contribute to the emerging field of lignomics, one of the fastest growing areas in bioanalytical chemistry. They provide deeper insight into lignin structure and biosynthesis, enable the tracking of biopolymer transformations in biorefining processes, and support detailed characterization of lignin as a promising renewable feedstock for diverse chemical products and environmentally friendly materials.

This study was performed using instrumentation of the Core Facility Center “Arktika” of the Northern (Arctic) Federal University and supported by Russian Science Foundation (grant 26-73-10072).

Ultra-Sensitive micro-QuEChERS-UPLC-MS/MS Method for Determination of Azoxystrobin in *Dolomedes fimbriatus*

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This research investigates the effects of environmentally relevant pesticide concentrations occurring in Slovenia's surface waters on the reproductive fitness of the semiaquatic spider *Dolomedes fimbriatus*, which links aquatic and terrestrial ecosystems. The spider is exposed to pesticides through the consumption of emerging aquatic insects and through its habitat at the water's edge. Azoxystrobin (AZX) was selected as the model pesticide because it has been detected in Slovenian rivers at environmentally relevant concentrations (up to 400 ng L⁻¹). Before evaluating the biological implications of sublethal exposure, it is essential to confirm the presence/absence and quantify the concentration of AZX in spider tissues.

To achieve this, an ultra-sensitive micro-QuEChERS extraction method coupled with UPLC-MS/MS was developed for the determination of trace concentrations of AZX in *D. fimbriatus*. Due to the limited biomass available from field-collected spiders, the house cricket (*Acheta domesticus*) was used as a surrogate validation matrix, while critical validation parameters were confirmed directly in spider tissue. The extraction procedure was optimized for 100 mg samples and included tissue rehydration, acetonitrile extraction, QuEChERS salting-out partitioning, lipid freeze-out at -20°C, and dispersive solid-phase extraction using MgSO₄, PSA, and C18 sorbents. Quantification was performed using UPLC-MS/MS on a Waters Xevo TQ Absolute tandem quadrupole mass spectrometer operating in positive electrospray ionization (ESI+) mode for AZX and its isotopically labelled internal standard (AZX-D4).

The method enabled excellent linearity over the concentration range of 0.00 - 10.00 ng g⁻¹. Calibration curves showed coefficients of determination (R²) of 0.9995 for *D. fimbriatus*, 0.9982 for the surrogate matrix (*A. domesticus*), and 0.9985 for solvent-based standards. The validated limit of quantification (LOQ) was established at 0.20 ng g⁻¹ (n = 5). At this concentration, recoveries were 113.5% (RSD = 4.06%) for *D. fimbriatus* and 93.6% (RSD = 5.52%) for *A. domesticus*, meeting all SANTE validation criteria. The method also achieved a low expanded measurement uncertainty of 12.8%. Overall, the method represents a valuable tool for assessing pesticide exposure in ecotoxicological studies and supports further investigations into the sublethal effects of pesticides in insects populations.

Occurrence of polybrominated diphenyl ethers in human milk from Serbia

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Polybrominated diphenyl ethers (PBDEs) are a class of industrial chemicals characterized by their persistence, long-range transport, biomagnification in food chains, and bioaccumulation in both, humans and wildlife.

The objective of this study was to analyze concentrations of PBDEs in human milk samples collected in Serbia. In the period between 2014 and 2016, a total of 33 samples were collected from healthy women within 30 days after delivery. The study protocol received approval from the Ethical Committee, and all participants voluntarily took part in this study after being informed.

The total fat in human milk samples was determined using the Weibull-Berntrop gravimetric method (ISO 8262-1|IDF 124-1:2005). Extracted lipids were cleaned using multi-layer columns designed for PBDEs analyses. The samples were analyzed by high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS). The individual congeners were quantified against ¹³C₁₂-labeled PBDE standards and their content was expressed as ng/g fat.

The total PBDEs (the sum of 26 PBDE congeners) in the human milk samples analyzed in this study ranged from 0.32 to 17.15 ng/g fat, with a mean of 4.17 ± 4.55 ng/g fat. These levels are comparable to those reported for European countries and belong to the lower tier.¹

DecaBDE (BDE-209) was the most prevalent congener in the samples, accounting for 76 % of all present PBDEs, and ranging from 0.18 to 14.82 ng/g fat, with a mean concentration of 3.17 ± 4.01 ng/g fat. Additionally, HeptaBDE and OctaBDE were found in 10 and 8 human milk samples, respectively.

Some studies have suggested higher DecaBDE concentrations in urban environments than in rural environments, which may explain the elevated levels of DecaBDE observed among mothers from urban areas, which is most probably due to the endogenic pollution processes.²

To achieve more representative results, future studies should include donors from various regions across Serbia.

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Thermal desorption gas chromatography – high resolution mass spectrometry: a new approach to quantification and screening of toxic rocket fuel transformation products in peat bog soils

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One of the key aspects of the functioning of ground-based space infrastructure is the environmental support of space-rocket activity, an important place in which is given to solving the problems of controlling the pollution of environmental objects with highly toxic rocket fuel based on 1,1-dimethylhydrazine and its transformation products. Such objects primarily include peat bog soils, characteristic of most areas where spent parts of launch vehicles fall on the territory of the Russian Federation. In this regard, there is a need to create a set of methods for the rapid and highly sensitive determination of transformation products of 1,1-dimethylhydrazine in this type of soil. The determination of these transformation products in peat soils is challenging because of the high moisture content and strong interactions of analytes with soil organic matter. Conventional approaches for the determination of hydrazines and their transformation products in soils are typically based on extraction techniques. While these methods can provide satisfactory sensitivity, they are often labor-intensive, time-consuming, and require large volumes of organic solvents, limiting their applicability for rapid and environmentally friendly analysis.

In this study, a direct thermal desorption gas chromatography–high-resolution mass spectrometry (TD-GC-HRMS) method was developed for the targeted determination and non-target screening of UDMH transformation products in peat soil. Alkaline matrix modification with 5% NaOH was introduced to improve analyte desorption and chromatographic performance. Thermal desorption was optimized over the temperature range of 100–200 °C and desorption times of 30–90 min, with 200 °C for 30 min selected as the optimal conditions. The developed method enabled the simultaneous determination of 13 nitrogen-containing UDMH transformation products. Limits of detection were below 1 µg kg⁻¹ for most analytes, while higher values of 11–31 µg kg⁻¹ were obtained for compounds with lower extraction efficiencies. The method demonstrated good linearity ($R^2 > 0.99$), accuracy with recoveries of 80–100%, and precision with relative standard deviations of 7–20%. Application to peat samples from rocket launch-affected areas demonstrated the presence of multiple UDMH transformation products, with 1-methyl-1H-1,2,4-triazole reaching concentrations of 196 mg kg⁻¹. In addition to targeted analysis, non-target screening revealed 96 and 17 tentative transformation products in two peat samples, respectively, including nitrogen-containing heterocycles, amides, amines, nitriles, and oxygen-containing heterocycles. Compared with pressurized liquid extraction coupled with GC-MS/MS, the proposed approach required a substantially smaller sample amount, avoided the use of toxic organic solvents, and generated less waste. The developed method demonstrated improved environmental performance, with AGREE and AGREEPrep scores of 0.66 and 0.79, respectively, compared with 0.36 and 0.12 for the PLE-GC-MS/MS approach. The developed TD-GC-HRMS method provides a simple, resource-efficient, and environmentally preferable approach for targeted determination and exploratory characterization of UDMH transformation products in complex peat matrices.

This study was performed using instrumentation of the Core Facility Center “Arktika” of the Northern (Arctic) Federal University and supported by Russian Science Foundation (grant 26-73-00141).

Application of gas chromatography-mass spectrometry for the identification of organic materials in cultural heritage objects

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The study of culturally and historically significant museum values using mass spectrometry is a rapidly evolving field in technical and technological examination. Numerous studies have demonstrated the high reliability of this method, which successfully detects markers of natural organic materials. It provides detailed information on the composition of lipid and protein binders, varnishes, glues, organic dyes, and solvent residues used during both the creation and restoration of artworks. Due to their physicochemical properties, most of these markers are low-polarity, volatile, or semi-volatile compounds with a molecular weight below 500 Da, making them ideal targets for gas chromatography-mass spectrometry (GC-MS).

This paper presents the results of a comprehensive application of GC-MS obtained during the technical and technological examination and restoration of cultural heritage sites. The study was conducted in several key areas and covers both the non-target profiling of surface contaminants on objects collected during historical expeditions, and target analysis. The latter involved determining the type of oil binder in the paint layer, as well as identifying the components of coating varnishes, which is crucial both for authenticating artworks and for conducting restoration work. Furthermore, a new methodological approach based on mass spectrometry of microbial markers is proposed to reconstruct the environmental and storage conditions of historical items.

All analyses were performed using a Maestro- α MS single-quadrupole gas chromatography-mass spectrometry system (*Interlab, Russia*).

The objects of study included paint layer samples from paintings, as well as coating varnish samples from a bookbinding cover and a casket lid from the Qajar painting collection of the State Museum of Oriental Art (Russia). The study also examined fragments of 17th-century mica window panes from the collection of the Pereslavl-Zalessky Museum-Reserve. In addition, dust particles collected from the tombstone of Metropolitan Triphyllius (dated 1701 and located in the Church of the Assumption at the Krutitsy Patriarchal Metochion). Surface contaminant fragments in the form of oily secretions on the scoop of a pitch-boiling ladle, discovered in 1973 in the Tyumen Region (Russia).

LECO's comprehensive GCxGC-TOFMS for alternative petrochemistry

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The ongoing transition from fossil fuel processing to a circular carbon economy involves the use of new, chemically heterogeneous feedstocks, including pyrolysis oils from plastic and biomass waste, hydroprocessed esters, and Fischer-Tropsch synthetic crudes. These matrices exhibit compositional heterogeneity often exceeding that of crude oil, containing a broad distribution of oxygen-, nitrogen-, and sulfur-containing compounds along with extensive assemblies of isomeric hydrocarbons. This complexity makes one-dimensional gas chromatography insufficient for comprehensive molecular profiling, especially when process optimization and end-product certification are required. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS) addresses these limitations through an orthogonal separation mechanism that substantially increases effective peak capacity relative to single-column systems.

LECO's latest Pegasus BTX 4D platform provides spectral acquisition rates up to 500 spectra per second, sufficient for accurate reconstruction of narrow modulated peaks without spectra skewing. The configuration equipped with Paradigm Reverse Fill-Flush (RFF) modulator and a Shift flow splitter for parallel TOFMS and FID detection enables simultaneous acquisition of qualitative and quantitative data within a single injection. TOFMS data provides the base for compound identification, while FID detection supports robust group-type area percentage classification. Data processing by ChromaTOF[®] software includes automated peak finding, spectral deconvolution algorithms, classification by mass filters and chromatographic regions. The recently released ChromaTOF-SYNC2D software enables statistical analysis of datasets.

Hydrogen has emerged as a viable alternative to helium as a carrier gas, providing faster separations with comparable efficiency while reducing supply chain constraints. All LECO's GC-TOFMS and GCxGC-TOFMS systems support hydrogen as a carrier gas. The StayClean[®] electron ionization source, used in open-geometry direct extraction platforms, maintains performance over long analytical runs without the need for source cleanup, even after prolonged exposure to heavy petrochemical matrices.

For more detailed molecular composition studies, the Pegasus GC-HRT 4D high-resolution configuration delivers mass accuracy down to 1 ppm, enabling confident identification of unknowns and effective use of ChromaTOF software capabilities, including accurate mass library searching, IGS result ranking, analyte classification, Spectral Analysis Tool, mass defect analysis, Van Krevelen and RDBE diagrams, and more. The newly introduced Multi-Mode Source[™] (MMS2) enables seamless switching between electron ionization, positive chemical ionization, and negative electron capture ionization without changing hardware configuration.

By integrating high-resolution separation, rapid mass acquisition, and advanced data processing algorithms, LECO's GCxGC-TOFMS platforms transform alternative petrochemical processing from a black box into a molecular-based engineering design — improving resource efficiency, streamlining workflows, preserving data integrity, and delivering measurable progress toward a sustainable, circular carbon economy.

Thermo Fisher Scientific mass spectrometry innovations: expanding high-resolution and targeted workflows across research, biopharma, and environmental testing

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Thermo Fisher Scientific's recent mass spectrometry portfolio introduces major advancements across high-resolution Orbitrap systems, applied GC-HRMS platforms, and targeted triple-quadrupole technologies. Three new Orbitrap instruments address distinct analytical needs: the Orbitrap Tribrid Apex MS delivers up to five-fold higher sensitivity and near-complete sequence coverage for complex multiomics; the Orbitrap Excedion MS enhances biopharmaceutical workflows such as drug metabolism, peptide, and oligonucleotide analysis through improved dynamic range and upgradeable capability; and the Orbitrap Exploris GC S MS provides twice the resolution of many existing systems for confident identification of dioxins, POPs, and emerging contaminants. Complementary instruments—including the Orbitrap Exploris EFOX Mass Detector and TSQ Certis Triple Quadrupole MS—extend high-resolution and targeted quantitation to PFAS, pesticides, and regulated bioanalysis. End-to-end biopharma solutions such as Vanquish Amplify UHPLC systems and SurePac RP MDi columns further strengthen sensitive therapeutic and oligonucleotide workflows.

Steranes and hopanes in Georgian petroleum

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The molecular composition of biological markers in Georgian petroleum was investigated using GC, MS, and GC/MS methods. Any regularity in the molecular weight distribution of these compounds in samples serve as geochemical criteria and are used to perform various correlations, genetic and chemical typification of petroleum, to solve various problems in petroleum geochemistry, and to identify the source of spilled oil. The most important biomarkers are saturated cyclic hydrocarbons with one to five rings (steranes and triterpanes), normal and isoprenoid alkanes (pristane, phytane), steroids, porphyrins, and others.

The 50-200°C, 200-350°C, 200-420°C and >420°C fractions of various types of Georgian petroleum have been studied using the methods of distillation, adsorption and gas-liquid chromatography, deparaffinization, thermal diffusion, extraction, MS and GC-MS spectrometry.

Based on the identified relict hydrocarbons in all fractions the main geochemical criteria (related to biodegradation— K_i , with catagenesis— K , and the genetic type of petroleum— iC_{19}/iC_{20} , Σ hopanes/ Σ steranes, etc.) were calculated, and the chemical types of petroleum were determined: Norio - B^1 , Teleti - B^1 ; Mirzaani - B^2 , Satskhenisi - B^2 ; Samgori- A^1 , Ninotsminda- A^1 , Taribani - A^1 ; Shromis-Ubani - A^2 . A distinctive feature of Georgian petroleum of all types is the high concentrations of isoprenoid alkanes such as pristane (iC_{19}) and phytane (iC_{20}), with $iC_{19}/iC_{20} > 1$ (one of the main the so-called genetic characteristics of petroleum). It is assumed that chemical composition of petroleum is determined by different conditions of accumulation and biological oxidation in geological layers in the environment of catalytic interaction with natural aluminosilicates.

In the fractions boiling above 420°C, large molecules of tetra- and pentacycloalkanes (C_{27} – C_{33}) – steranes and triterpanes were identified – the primary biomarkers of petroleum that are characterized by the greatest geochemical informative value. The studied petroleum belong to the 17 α H-hopane series ($C_{30}H_{52}$); 17 α H-hopane itself is present in the highest concentration. Steranes (C_{27} – C_{29}) are presented by α - and iso-steranes, the main mass of them being thermodynamically more stable iso-steranes. Despite differences in chemical composition, the studied petroleum belong to a single genetic type based on geochemical characteristics – ΣnC_{13} – $nC_{15}/\Sigma nC_{25}$ – nC_{27} , $\Sigma isoC_{19}$ – $isoC_{20}/\Sigma nC_{17}$ – nC_{18} , α -iso-steranes, “naphthenic profile”, etc. Certain geochemical correlations have been established, suggesting (when determining the degree of catagenesis) that the Samgori and Mirzaani petroleum exhibit the highest degree of transformation, the Shromis-Ubani petroleum – the lowest, and the remaining petroleum an intermediate degree of transformation. The formation of B^1 , B^2 , and A^2 -type petroleum is based on bacterial degradation, while the primary-generation A^1 petroleum were not subjected to biooxidation; the main material in the source rock of this basin most likely was the fatty fraction of marine plants—fatty acid esters, steroids, terpenoids, etc. In Mirzaani petroleum sterane structures predominate.

The presence of known biomarkers in Georgian petroleum has been established, the characteristics of their chemical composition have been determined, genetic criteria have been calculated, and various geochemical correlations have been performed.

Determination of sulfur-containing components in diesel fuel by gas chromatography

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Developing suitable analytical approaches for the determination of sulfur-containing compounds using gas chromatography with a flame ionization detector (GC-FID) is a pressing task. However, such a detector lacks selectivity toward sulfur-containing components, and the hydrocarbon matrix of the fuel masks the signals from sulfur. Therefore, it is also necessary to develop a sample preparation procedure aimed at the selective extraction of sulfur-containing components from a model fuel solution into a solvent suitable for chromatography.

A new GC-FID analysis method employing an internal standard has been proposed and naphthalene was used as the internal standard. A sample preparation procedure aimed at the selective extraction of sulfur-containing components was also developed, with acetonitrile selected as the most suitable extractant for this purpose. A model diesel fuel—cetane spiked with sulfur-containing compounds (benzothiophene, 3-methylbenzothiophene, and diphenyl sulfide)—was chosen as the analytical subject; these compounds were selected to represent the most common classes of sulfur compounds found in diesel fuel.

The conditions for the gas-chromatographic separation of the system components (naphthalene, benzothiophene, 3-methylbenzothiophene, and diphenyl sulfide) in acetonitrile were optimized (Fig. 1), and calibration curves were constructed for a total sulfur concentration range (the sum of the concentrations of all sulfur compounds) of 0.06 to 2.4 mmol/L. The calibration relationships are characterized by linear regression with a high coefficient of determination ($R^2 > 0.999$).

Thus a method was developed for the determination of a range of sulfur-containing components in model diesel fuel using gas chromatography with a flame ionization detector, involving preliminary sample preparation based on the extraction of sulfur-containing substances with acetonitrile.

Kerch Strait disaster: one year later – what happens to fuel oil on the shore?

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The accidental fuel oil spill in the Kerch Strait in December 2024, caused by the wreck of the tankers Volgoneft-212 and Volgoneft-239, resulted in large-scale contamination of the coastal ecosystems of the Krasnodar Territory and Crimea. Several thousand tons of petroleum products were released into the environment, making the monitoring of their behavior and transformation highly relevant. One year after the accident, fuel oil residues are still detected both on beach surfaces and within coastal sediments. The aim of this study is to analyze the composition and properties of fuel oil sampled 12 months after the spill in order to assess the degree of its degradation and its potential hazard to human health and biota.

In this work, 14 samples of fuel oil and contaminated sand/sediments collected from the tankers, beach surfaces, and coastal deposits in Anapa, Crimea, and the Krasnodar Territory in March 2026 were analyzed by gas chromatography–mass spectrometry (GC–MS). The oil products recovered from both tankers were found to be chemically similar, confirming a common source and serving as reference samples for spill fingerprinting. All environmental samples were screened against these reference profiles using diagnostic biomarker ratios. As a result of this comparison, 3 of the 14 samples were identified as unrelated to the spilled fuel oil, indicating the presence of extraneous petroleum contamination in the study area.

GC–MS data show that the relative abundances of low-molecular-weight n-alkanes and low-molecular-weight aromatic hydrocarbons decreased markedly over the one-year period due to evaporation and biodegradation. However, the composition of the high-molecular-weight PAH fraction remained largely preserved, indicating that the heavier components of the spilled fuel oil are resistant to weathering under the environmental conditions of the study area. To further characterize the high-molecular-weight fraction, selected samples were analyzed by high-resolution mass spectrometry with atmospheric pressure photoionization (APPI HRMS) and electrospray ionization (ESI HRMS). The APPI HRMS data confirm the GC–MS findings, demonstrating that the distributions of high-molecular-weight aromatic hydrocarbons and their alkylated homologues show no significant alteration relative to the original fuel oil. ESI HRMS analysis reveals no substantial increase in the abundance of oxygen-containing classes, indicating that oxidative transformation of the aromatic fraction proceeds slowly over the one-year period. Furthermore, no significant formation of potentially toxic naphthenic acids was detected.

Thus, one year after the spill, the fuel oil retains its chemical identity and remains environmentally persistent. The high-molecular-weight PAH fraction, which includes compounds with well-documented carcinogenic, mutagenic, and teratogenic properties, has undergone only minor weathering. Oxidative transformation proceeds slowly, and no significant formation of toxic naphthenic acids is observed, indicating that the aromatic core of the fuel oil remains largely intact and bioavailable. The presence of extraneous petroleum contamination in three samples further suggests that the study area is subject to additional anthropogenic inputs. Overall, the residual fuel oil should be regarded as a continuing source of long-term environmental risk, particularly in the event of sediment remobilization or chronic exposure of benthic organisms.

Application of hydrogen–deuterium exchange mass spectrometry (HDX-MS) to study structural features of DNA repair enzymes and their complexes with nucleic acids

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Structural features and subtle conformational details of proteins and protein–nucleic acid complexes play a crucial role in the proper functioning of biological systems, and their detailed investigation is essential for understanding the molecular mechanisms of cellular processes. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) provides a unique opportunity to study dynamic and structural changes in these complexes under native conditions.

The HDX-MS approach measures the exchange rate of protein amide protons for deuterium in solution by mass spectrometric analysis of the resulting mass shifts of peptides. A standard HDX-MS experiment involves incubating the protein in a heavy water-based buffer, followed by stopping the reaction, then UPLC-MS analysis coupled with proteolytic digestion of the protein with immobilized pepsin is performed. Processing the resulting mass spectra at various time points provides information on the rate of peptide mass change. This method gives information on protein conformational dynamics, reveals structural changes, and identifies regions with different solvent accessibility and flexibility. In the present work, HDX-MS was applied to study the structural and dynamic features of the human DNA glycosylase NEIL2, which removes oxidized DNA bases. This enzyme exhibits high conformational mobility of its domains and contains extended disordered regions, complicating determination of its 3D structure by X-ray crystallography. In this work, we studied the catalytically inactive hNEIL2 K50A mutant as well as a deletion variant of hNEIL2 in which the proline-rich loop (residues 58–128) was replaced by short glycine–serine linkers. To achieve higher sequence resolution, we developed HDX-MS workflows employing various proline-specific proteases in addition to pepsin (which is traditionally used in HDX-MS). This allowed us to increase the depth of peptide sequence coverage, particularly in previously unresolved disordered regions.

Deuterium incorporation kinetics were obtained for hNEIL2 peptides, revealing region-specific variability in exchange rates that correlated with secondary and tertiary structural features typical of NEIL family glycosylases. HDX-MS comparison of free hNEIL2 K50A with its complex with single-stranded DNA revealed that complex formation is accompanied by decreased deuterium incorporation in multiple protein regions, reflecting conformational rearrangements within the protein globule. The most pronounced reduction in deuteration was observed in the proline-rich disordered loop of the N-terminal domain and in the DNA-binding motifs of the C-terminal domain. Correlating the HDX-MS data with available crystal and computational structures of NEIL2 family proteins allowed us to rationalize the decreased deuteration for each structural motif upon transition from the free protein to the protein–DNA complex. HDX-MS directly detected the involvement of the proline-rich extended disordered motif (Leu58–Arg128) in DNA binding. Analysis of the deletion mutants revealed that the enzyme is able to form a wild-type-like structure during DNA binding, despite removal of an extended disordered region from the initial structure.

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Metabolomic profiling of aqueous humor for differential diagnosis of uveal melanoma and uveal nevi

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Differential diagnosis of uveal melanoma (UM) and uveal nevi (UN) remains a major challenge in modern ophthalmic oncology. In the early stages, these lesions often exhibit similar clinical features, leading to diagnostic uncertainty. UM is the most common primary intraocular malignancy in adults, while UN are benign melanocytic neoplasms. Aqueous humor (AH) represents a valuable biological matrix for metabolomic analysis, as its composition reflects metabolic alterations associated with intraocular tumor development. The use of high-resolution liquid chromatography–mass spectrometry (LC–HRMS) in combination with modern bioinformatics approaches allows for the identification of metabolic markers in AH, potentially suitable for noninvasive diagnostics.

In this study, we compared the metabolomic profiles of AH obtained from patients in three groups: those with confirmed UM, those with UN, and those without tumors (controls). Principal component analysis of the metabolomic data revealed that the metabolomic clusters for each group were well separated, with the profiles from UM and controls being the most distant from each other. Patients with UM exhibited increased levels of metabolites associated with enhanced catabolism and altered redox metabolism, consistent with the increased metabolic demands of malignant cells. The metabolomic profile of UN was intermediate between those of healthy controls and UM, but lacked the extensive metabolic alterations observed in UM. Nevertheless, in both groups with melanocytic lesions—both UM and UN—an increase in amino acid metabolism was observed, which likely reflects the presence of an inflammatory component common to both types of lesions.

The identified metabolite panel enabled discrimination between patients with UM and UN, supporting the potential of this approach for the development of diagnostic assays. However, validation in larger patient cohorts is required.

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Untargeted LC-HRMS metabolomics for urinary profiling and biomarker discovery in urological cancers

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Untargeted high-resolution liquid chromatography-mass spectrometry (LC-HRMS) of urine is a powerful non-invasive approach for evaluating systemic metabolic alterations in oncology. This study aimed to identify urinary biomarker signatures associated with urogenital cancers using an untargeted LC-HRMS workflow combined with multivariate statistical modeling and biological pathway mapping.

Urinary metabolomic profiling was performed on an LC-HRMS system across a total cohort of 100 participants, comprising patients with urogenital malignancies and healthy control individuals. Raw dataset processing yielded thousands of detected features with corresponding tandem mass spectra (MS²). Feature selection was conducted using univariate statistical tests (moderated t-tests, fold-change analysis) and multivariate classification algorithms (such as PLS-DA and Random Forest) to isolate significant differential signals. Candidate biomarkers were structurally curated through spectral analysis to ensure reliable metabolite annotation.

Comparative statistical analysis revealed clear metabolic differentiation between urogenital cancer patients and control subjects. Multivariate modeling refined the dataset into compact panels of highly discriminatory features that achieved clear cohort separation.

Biological interpretation of the identified metabolites demonstrated significant dysregulation across several key metabolic pathways. Major alterations were observed in fatty acid β -oxidation and lipid membrane remodeling (including acylcarnitines, sphingolipids, and ether phospholipids), purine and amino acid turnover (such as adenosine and kynurenine pathway intermediates), and peptide catabolism. These metabolic shifts reflect tumor-associated energy adaptation, alteration of the inflammatory microenvironment, and systemic metabolic rewiring in urological malignancies.

This untargeted LC-HRMS urinary profiling workflow revealed characteristic metabolic patterns associated with urogenital cancers. The identified panel of discriminatory metabolites provides valuable insights into tumor-related physiological shifts and highlights promising candidate markers for non-invasive diagnostic applications.

Some Recent Concepts for ESI-TOF Mass Spectrometers in Medicine

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Our primary goal is to make mass spectrometers more accessible for widespread clinical practice.

Addressing a broad range of analytical tasks in medicine requires high-performance instruments that combine high m/z resolution with rapid spectral scanning and high sensitivity, while also being highly robust against contamination. Given this combination of requirements, time-of-flight mass analyzers (ToF-MA) are well-suited for this role.

The main limitation of ToF-MA is low sensitivity resulting from ion losses due to a high duty cycle. To overcome this, ions must be accumulated prior to being injected into the orthogonal accelerator. However, this induces space-charge effects, leading to a loss of resolution and peak mass shifts.

Another challenge is increasing analytical sensitivity by improving ion transmission efficiency through differential pumping interfaces without increasing the gas load on the vacuum pumps. Developing an efficient and low cost-to-implement ion transport system will reduce the overall manufacturing cost of the mass spectrometer.

Over recent years, our team has been developing a tandem time-of-flight mass spectrometer designed to address these challenges.

To overcome the first group of limitations, we propose utilizing a multi-turn mass analyzer based on sector electrostatic fields with a figure-eight trajectory.

To achieve an efficient interface, a novel approach utilizing ion transport channels with a traveling wave field is proposed. To date, an ion transmission efficiency of 80%–90% per interface stage has been experimentally achieved.

This report discusses the successes and challenges encountered in solving these problems.

Cosmetic powder of the Great Silk Road based on archaeological data

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The Northern Silk Road was a vital branch of the ancient trade network, starting in China and going to Central Asia and eventually the Roman Empire, facilitating trade and cultural exchange. The Eastern Aral region served as a critical hub on the Northern Silk Road. The Jetyasar was an archaeological culture in the Eastern Aral Sea region, flourishing from the 1st millennium BC to the 8th century AD. Khorezm expedition in 1991 discovered numerous findings, kept in the Institute of archeology RAS. Complex cosmetics used by this early medieval population were found in two female burials. Two samples of blocks of chalk of Jetyasar culture (museum numbers GMV 53951/2742 and GMV 53380/257) were subjected to GC/MS, SEM, and RFS analyses.

Samples in powdery state were analyzed using SPME (DVB/C-WR/PDMS, Restek, USA) with LECO L-PAL3 autosampler. Agilent 8890 gas chromatograph (Santa Clara, CA) coupled with LECO Pegasus BT mass spectrometer (St. Joseph, MI) was used to analyze organic compounds. 30m column - Rxi-5ms (Restek). Oven temperature: 40 °C (2 min) - (10°C/min) - 270 °C (5 min). Acquisition rate - 10 spectra/second, mass range 29–500 Da. Electron energy - 70 eV. Automatic peak finding, deconvolution, and library search were performed using ChromaTOF LECO software version 5.56. NIST 23 library score, mass measurements with two decimal points, retention indices, and manual spectra interpretation were used for the identification. M4 Tornado (Bruker, Germany) was used for RFA, Phenom (Thermo, Netherlands, Eindhoven) for SEM.

RFA showed that the major constituent of the samples was Ca (>45 mass percents), while the main inorganic compound was chalk. Alternative possibility of using powdered pearls was rejected on the basis of SEM analysis. GC/MS study allowed identifying over 300 organic compounds. Terpenes constituted the most representative group of the discovered compounds. These natural substances synthesized by plants show protective functions, while each of them has peculiar smell. Nowadays they are actively used as ingredients in cosmetics, body-care products and household preparations. Definitely, they could be used many years ago, at least to provide nice odor of the cosmetic products. The array of the detected terpenes allowed making some hypotheses about the plants used to produce the studied cosmetics. Usually, same terpenes may be extracted from several plants. Anyway, ten of them are the constituents of wormwood (*Artemisia absinthium*). Moreover, sabina ketone is treated as biomarker of wormwood. Therefore, wormwood is the most probable source of the terpenes in the samples. Six detected terpenes belong to lavender oils, six to Rosmarin, and five to eucalyptus. Besides, cinnamaldehyde is known to present only in the cinnamon oil, trans-3-carene-2-ol only in cumin, isomenthol only in mint. The oils of these plants also could be used in preparation of the studied cosmetics.

Other constituents involved hydrocarbons, alcohols, aldehydes, ketones, acids, and esters. The majority of them has a plant origin. Aldehydes possess characteristic aromas with low odor thresholds. Esters are usually treated as compounds responsible for sweet and fruity flavors. Glycerol was one of the major ingredients in both samples. Most probably it was formed during complete hydrolysis of plant oils. Vanillin, detected in both samples, may be considered as natural ingredient, added to provide pleasant smell of the product. Therefore, rather complex cosmetic mixtures were composed even about 2000 years ago.

GC×GC/HRMS and chemometric analysis of volatile and semi volatile compounds in Chinese mushrooms

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Mushrooms, being one of the oldest food products in the human history, are well known for their nutrition and medicinal properties. Although composition and properties of useful components of mushrooms are intensively studied, the main efforts are applied to rather large bioactive molecules including vitamins, polysaccharides, polyphenols, while lighter molecules were not studied properly so far. Thus, in the current study SPME GC×GC-HRMS analysis allowed identifying over 500 volatile and semi volatile compounds using NIST-23 library search, manual spectra interpretation, and two-dimensional retention indices in the samples of six popular edible mushrooms popular in China: snow fungus (*Tremella fuciformis*), chestnut mushroom (*Pholiota adiposa*), matsutake (*Tricholoma matsutake*), shiitake (*Lentinula edodes*), stinkhorn fungus (*Dictyophora indusiate*), and wood ear fungus (*Auricularia spp.*).

SPME (fiber - DVB/PDMS/Carbon) was conducted in Gerstel autosampler. Agilent 8890 gas chromatograph (Agilent, Santa Clara, CA) coupled with LECO Pegasus HRT 4D⁺ high resolution mass spectrometer (LECO, St. Joseph, MI) was applied for comprehensive GC×GC operation. Primary 30m column - Rxi-5ms, secondary 1,5m column - Rxi-17Sil MS (Restek). Modulation time - 4s. Oven temperature: 40 °C (2 min) - (6°C/min) - 250 °C (5 min). Acquisition rate - 200 spectra/second, mass range 29–500 Da. Electron energy - 70 eV. Automatic peak finding, deconvolution, and library search were performed with ChromaTOF software version 5.56. NIST 23 library, accurate masses, retention indices, manual interpretation, and ChromaTOF. Tile software followed by PCA were used for the identification and species' differentiation.

Comprehensive 2D gas chromatography and high-resolution mass spectrometry was applied for the analysis of mushrooms' volatile and semi volatile organic components. On the basis of SPME with GC×GC-HRMS analysis over 500 volatile and semi volatile compounds were detected and identified in the samples of six popular edible mushrooms. The identified compounds belong to the following classes: terpenes, pyrazines, alcohols, carbonyl compounds, acids and esters, organosulfur compounds, organochlorine compounds, amides/amines, pyrroles, phenols, hydrocarbons, etc. Contaminants composed a separate group. The differences in the composition of ingredients between the studied mushroom species are quite pronounced both qualitatively and quantitatively. The analysis allowed proposing certain biomarkers unique for each of the studied mushroom species. Linear and cyclic sulfur containing compounds, certain pyrroles and aliphatic amides may be treated as biomarkers of *Lentinus edodes*. Alkylpyrazines, especially with long alkyl groups (isoamyl and isobutyl), as well as certain esters - as biomarkers of *Pholiota adiposa*. Over 80 unique sesquiterpenes and sesquiterpenoids were found in the samples of *Dictyophora indusiate*. They may be treated as biomarkers of this mushroom. Besides, this specie contains quite a number of unique carbonyl compounds, esters, alcohols. These molecules may be also considered biomarkers of *Dictyophora indusiate*. 2-Bornanone was detected only in *T. matsutake* at high levels (position 4 in the Top-20 list). This terpene definitely contributes to the smell of this mushroom and may be treated as its biomarker. 3-Methoxy-1-butanol acetate, ethyl 3-hydroxybutanoate, 3-methyldecene-2, hexahydro-8a-methyl-cis-8(2H,5H)-naphthalenedione, may be tentatively selected as biomarkers of *Tremella fuciformis*. Only 6,10-dimethyl-2-undecanone could be proposed as a biomarker of *Auricularia spp.* Application of ChromaTOF.Tile software followed by PCA analysis enabled reliable distinguishing between all studied mushrooms. The first two components described 83.00 and 7.25% of the variance, respectively.

GC/MS analysis of hydroconversion products of rapeseed oil on zeolite catalysts

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Currently, one of the priority areas in petrochemistry is the processing of renewable feedstocks to produce ‘green’ components for motor fuels and petrochemical products. Vegetable oil triglycerides are being investigated as such feedstocks. The processes of hydroconversion or catalytic cracking of such oils on zeolite catalysts lead to the formation of highly complex multicomponent mixtures, including gaseous olefins (C₂–C₄), liquid hydrocarbons of various classes (paraffins, isoparaffins, naphthenes, aromatic compounds), as well as oxygen-containing products of incomplete dehydration.

An important task in the development of catalysts and the optimisation of process parameters is the qualitative and quantitative analysis of the composition of the products obtained. Given the complexity of the mixture, conventional methods (gas chromatography or elemental analysis) often fail to provide a complete picture due to the overlap of chromatographic peaks or insufficient reliability of identification. This study demonstrates that gas chromatography–mass spectrometry (GC/MS) is the most informative tool for addressing this challenge, providing high resolution and enabling the identification of individual components across a wide range of boiling points.

During the study, the hydroconversion products of rapeseed oil, obtained at 450 °C and 70 atm on the «nano»/ZSM-5, 1%Pt/«nano»/ZSM-5/SiC and 2%Pt/«nano»/ZSM-5 composites, were analysed by GC/MS. The gas chromatography–mass spectrometry analysis was carried out on a Chromatec-Kristall 5000 gas chromatograph equipped with an MSD Chromatec mass-selective detector (Chromatec, Russia). Separation was carried out on a CR-5MS capillary column (30 m, 0.25 mm, 0.25 µm). GC-analysis conditions: the injection port temperature was 250 °C; the column thermostat temperature varied from 40 °C to 300 °C at a rate of 15 °C × min⁻¹. Thanks to the high resolution of the column and the capabilities of mass-selective detection, it was possible to separate an extremely complex mixture comprising more than 50 individual compounds.

Of particular significance for this work is mass spectrometric detection, which allows identification not only by retention time but also by electron ionisation mass spectra. This is important for distinguishing between xylene isomers or branched olefins, which are difficult to identify reliably using a flame ionisation detector. During the analysis of the liquid products of C₅+, not only were the target aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes) were identified, but also impurities of oxygen-containing compounds — phenols, carboxylic acids and esters — present in trace quantities (less than 0.1 % by mass), which attests to the high sensitivity of the method.

Thus, this study demonstrates that GC/MS possesses high resolution, sufficient to separate a multicomponent hydrocarbon product obtained from rapeseed oil into its individual components. Detailed analysis of the spectral data allows the precise composition of the mixture to be determined and the selectivity of the catalyst to be calculated for each target product.

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On-line electrochemistry – high-resolution mass spectrometry in the study of redox metabolism of polyphenolic compounds

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Due to high antioxidant and biological activity, polyphenolic compounds (lignans, flavonoids, stilbenes, etc.) represent the most important classes of plant secondary metabolites. Their effects on the human and animal body are inextricably linked to redox transformations initiated by single-electron transfer mediated by liver cytochrome, leading to the formation of a set of transformation products. Elucidating the mechanisms and pathways of polyphenol transformation processes is of great importance for understanding the mechanisms of their biological activity and represents a challenging analytical task. To address this problem, the present study proposes using electrochemical oxidation under controlled conditions hyphenated to online electrospray ionization and high-resolution mass spectrometry detection (EC-ESI-HRMS) as an alternative to common labor-intensive and time-consuming *in vivo* or *in vitro* experiments involving liver microsome incubation.

The experiments were performed on a Roxy Exceed potentiostat (Antec Scientific, Netherlands) with a three-electrode thin-film electrochemical flow cell (0.7 μ L internal volume) coupled with an Orbitrap ID-X mass spectrometer (Thermo Scientific, Germany). The objects of the study were: flavonoids (aromadendrin, naringenin, and their glycosides) and lignans isolated from aspen and spruce knotwood, respectively [1,2]; and polyphenolic metabolites of the moss *Polytrichum commune* represented by Communin A and recently discovered oligomeric dihydrochalcones [3].

The effects of the EC conditions (electrode material, electrode potential, concentration of the studied compounds, flow rate, pH) on the resulting mass spectra were studied and optimal EC-ESI-MS conditions allowing reliable detection and identification of the reaction products were proposed. The best results were achieved using boron-doped diamond working electrode that provides the widest electrochemical window even at low pH and is less prone to the surface contamination. To eliminate the interferences from impurities, the use of on-line HPLC separation (HPLC-EC-ESI-HRMS) was proposed.

The developed and optimized methodology was successfully implemented in the identification of a wide range of polyphenols oxidation products and revealing the main transformation pathways. The latter include dehydrogenation, decarbonylation, elimination of phenolic fragments and carbohydrate residues (in the case of glycosylated compounds), incorporation of additional oxygen atoms into the structure, and dimerization/oligomerization.

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Determination of Per- and Polyfluoroalkyl Substances in Chicken Eggs Using a Dispersive Solid-Phase Extraction Method Coupled with LC-HRMS

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Per- and polyfluoroalkyl substances (PFAS) are persistent anthropogenic contaminants known as "forever chemicals" due to their extreme resistance to thermal, chemical, and biological degradation. Their widespread use in industrial and consumer products has led to global environmental distribution and accumulation in food chains. Chicken eggs, as a widely consumed food with high lipid and protein content, can accumulate PFAS from contaminated feed, water, and soil. Human exposure to PFAS is associated with hepatotoxicity, immunotoxicity, endocrine disruption, and increased cancer risk, with biological half-lives extending to several years for long-chain congeners such as PFOA and PFOS. In the European Union, strict maximum levels have been established for PFAS in food (sum of PFOA, PFNA, PFHxS, PFOS ≤ 1.7 $\mu\text{g/kg}$). However, in Russia, no national regulatory limits exist, and systematic data on PFAS contamination in food products are virtually absent.

The aim of the work was to develop a simple and effective sample preparation method for the determination of PFAS in chicken eggs using LC-HRMS, and to apply it to commercial egg samples from major Russian producers, generating the first baseline data on PFAS contamination in this matrix in Russia.

A dispersive solid-phase extraction (d-SPE) method was developed, eliminating additional clean-up steps to reduce preparation time, minimize contamination risk, and prevent analyte losses. The target list included 9 PFAS compounds (PFCAs: PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFSA; TFMSA, PFBS, PFHxS). The method was validated for linearity, recovery, precision, and matrix effects, and applied to commercial egg samples from major Russian poultry producers.

The developed d-SPE method achieved an average recovery of 82% with RSDs below 12%. Matrix effects were negligible for the vast majority of target compounds, confirming method robustness. In Russian egg samples, the average total concentration of investigated PFAS was 30 $\mu\text{g/kg}$ of egg (12.9 ng/mL), with concentrations ranging from below the LOD to 144 $\mu\text{g/kg}$ of egg (63 ng/mL). Notably, some samples exceeded the EU maximum levels for individual PFAS and the tolerable weekly intake established by EFSA (4.4 ng/kg body weight per week for the sum of four PFAS).

This study presents the first systematic data on PFAS contamination in chicken eggs from the Russian Federation. The proposed method is simple, rapid, and reliable, making it suitable for routine monitoring. The detected PFAS levels, with maximum concentrations reaching 144 $\mu\text{g/kg}$ and several samples exceeding EU safety thresholds, highlight the need for comprehensive monitoring programs and the urgent establishment of national regulatory limits in Russia. Future regulations should consider both long-chain and short-chain PFAS, given the increasing prevalence of short-chain alternatives in the environment.

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Study of the effect of electron beam irradiation on antibiotics in aqueous solutions

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The widespread use of antibiotics in medicine, agriculture, and livestock farming has led to their widespread use in aquatic environments, raising serious concerns among the scientific community regarding human health and the environment. Antibiotic residues entering water bodies can contribute to the development of drug-resistant bacteria, posing a serious threat to human health.

Antibiotic residues can persist in the environment for long periods of time because they are difficult to biodegrade. Therefore, in this study, we examined the effect of ionizing radiation (electron beam) on antibiotics in aqueous model solutions and food matrices. Liquid chromatography combined with mass spectrometric detection appears to be the most promising method for identifying antibiotics and their degradation products.

The developed HPLC-HRMS approach allows us to study the degradation of the parent antibiotic depending on the electron beam irradiation dose and identify antibiotic degradation products in model aqueous solutions and then in food matrices.

Study of the aqueous humor proteome in patients with uveal melanoma

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Uveal melanoma (UM) is the most prevalent malignant ocular tumor in adults, and is distinguished by a high rate of metastasis. Conventional methods of evaluating metastatic risk necessitate the invasive procedure of a tumor biopsy, a process that is inherently associated with the potential for adverse local complications. Consequently, the aqueous humor (AH) of the anterior chamber of the eye is a promising source of biomarkers reflecting tumor microenvironmental characteristics and the molecular mechanisms of tumor progression. The potential of an AH liquid biopsy as a minimally invasive approach for identifying specific proteins associated with AH is considered promising. Such an approach could be used to predict metastasis and identify targets for targeted therapy. The objective of this study was to ascertain a distinctive protein profile within the aqueous humor of patients diagnosed with uveal melanoma.

The present study made use of aqueous humor (AH) samples obtained from a total of nine patients diagnosed with UM, as well as 16 healthy donors and 14 patients with benign (non-cancerous) ocular tumors. The sample preparation protocol encompassed sample concentration, trypsin digestion, and subsequent LC-MS/MS analysis on an Orbitrap Q Exactive HF instrument. The resulting spectra were processed and protein identification was performed using Proteome Discoverer 3.1, with the Uniprot database being searched. Functional annotation was performed using the Gene Ontology (GO, <https://geneontology.org/>) resource for *Homo sapiens*.

Proteomic analysis of aqueous humor samples from patients with uveal melanoma revealed a statistically significantly altered protein profile when compared to healthy donors and patients with benign ocular tumors. Increased levels of regulatory proteins associated with angiogenesis, cell proliferation, and the immune response, as well as inflammatory cytokines, were detected. These alterations may be indicative of characteristics of the tumor microenvironment and signal the activation of molecular mechanisms that regulate tumor growth and progression.

Comprehensive proteomic profiling of aqueous humor provides a promising approach for investigating the molecular mechanisms underlying uveal melanoma. The identified protein signatures may serve as candidate biomarkers of disease progression and potential targets for targeted therapy, thereby advancing our understanding of uveal melanoma pathogenesis and facilitating the development of molecular diagnostic approaches.

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LC–HRMS-Based Intracellular and Extracellular Metabolomic Profiling of Uveal Melanoma Cells

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Uveal melanoma (UM) is the most common primary intraocular malignancy. Despite its relatively low incidence, it is associated with high mortality due to its strong propensity for metastatic dissemination. Current therapeutic strategies are primarily focused on local tumor control and removal of the primary lesion and are largely ineffective once metastatic disease has developed. Moreover, effective systemic treatment options remain limited, with currently available targeted therapies benefiting only a small subset of patients. Therefore, identifying metabolites associated with the metabolic phenotype of UM cells and involved in the establishment of the tumor microenvironment remains an important objective. High-resolution liquid chromatography–mass spectrometry (LC–HRMS) enables comprehensive characterization of intracellular and extracellular metabolic alterations in uveal melanoma cells.

To explore these metabolic alterations, human UM cells established from a patient's primary tumor were analyzed by LC–HRMS. A total of 417 and 463 metabolites were annotated in cell lysates and conditioned culture medium, respectively, with approximately 201 compounds detected in both fractions. UM cells exhibited elevated levels of saturated fatty acids, their hydroxylated derivatives, and oxylipins. Compared with conditioned medium from normal melanocytes, that from UM cells contained elevated levels of free amino acids and their metabolites. Furthermore, nucleosides and nucleobases accumulated in the extracellular medium despite their relatively low intracellular abundance. This observation may indicate enhanced export of nucleic acid degradation products into the extracellular space as a consequence of the increased nucleotide turnover characteristic of rapidly proliferating tumor cells. In normal melanocytes, the levels of these metabolites were substantially lower. Extracellular accumulation of purine and pyrimidine bases may contribute to the maintenance of a tumor-supportive microenvironment through modulation of intercellular signaling and inflammatory responses.

In conclusion, the findings of this study provide a basis for the identification of potential therapeutic targets and prognostic biomarkers for uveal melanoma.

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Study of the shelf life of biodiesel derived from biomass

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Biodiesel is considered one of the most effective, environmentally friendly, and carbon-neutral alternative biofuels. Produced from renewable bioresources, biodiesel is a clean, renewable, eco-friendly replacement for common petroleum fuel, and the production of biodiesel has been steadily developing and gaining more share in the fuel industry worldwide.

The presented work is aimed at analyzing the quality, stability, and shelf life period of biodiesel fuel received from Used Cooking Oil (UCO), the waste product from the food industry, through the process of esterification. We have promoted an improved composition of biodiesel – the nano-biodiesel, prepared based on commercially produced B100. The nano-biodiesel, while meeting the EN 14105, ASTM D 6751, and EN 590 standards, significantly improved the long-term storage and quality stability of biodiesel fuel.

The possibility of increasing the shelf life and maintaining top-quality parameters is very important for the further development of biodiesel production and its integration into the energy sector of any economy, for further diversification and stability of energy supply.

The individual hydrocarbons in biodiesel fuel were studied and identified by liquid chromatography, and the functional groups of the fuel were analyzed using a Fourier IR spectrometer. Fig.1.

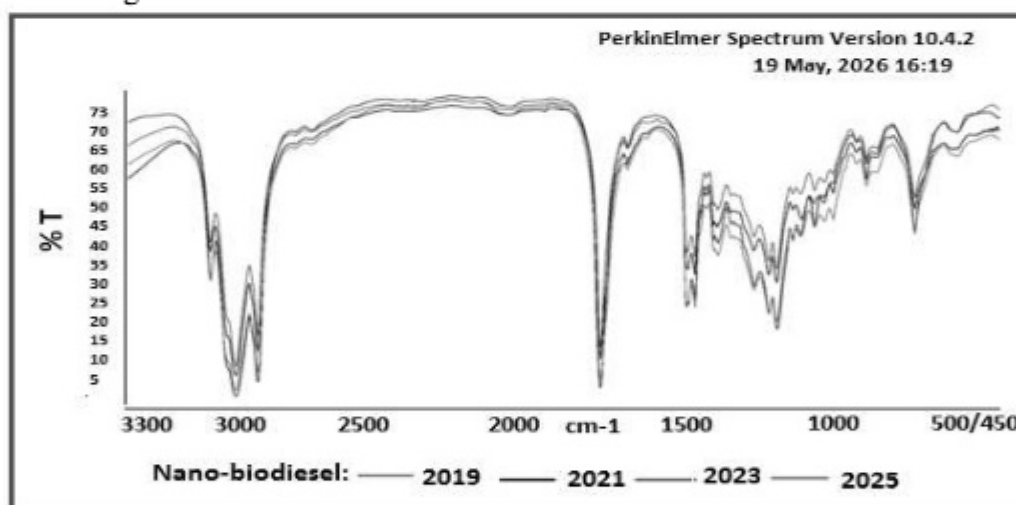


Fig.1. Nano-biodiesel quality stability 2019 - 2025

The results have shown that the pure biofuel i.e. B100, produced from bioresources by the plant Biodiesel Georgia LLC, as well as its blends with conventional diesel fuel, B5, B10, B20, maintained the highest quality for 4 + years, fully meeting the demands of international standards, therefore they can be used in almost all kinds of internal combustion diesel-type engines serving as an alternative, renewable, and reliable fuel.

Photochemical oxidation of crude petroleum in simulated conditions and study of photoproducts

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The degree of crude petroleum use is determined by content of high-molecular compounds, a significant part of which are substances that represent the largest group of the most high-molecular heteroatomic compounds of crude petroleum. Chemical and thermal research methods are based on the destructive transformation of these compounds into simple fragments and their identification by various methods of analysis. The mentioned compounds are exposed to erosion and destruction under environmental conditions, having an impact on asphalt roads in mountainous areas. For investigation the developed in our laboratory photochemical method was used that was different from general chemical methods by its softness of the provided conditions.

The objective of the present work is to clarify the process of photochemical transformation and the structure and properties of the most high-molecular heteroatomic compounds from Georgian crude petroleum of various chemical types (Ninotsminda - paraffinic, Mirzaani - aromatic, Tchaladidi - tarry).

Photolysis of the fractions under investigation was carried out in simulation conditions by way of irradiation with a mercury quartz lamp. The products of photochemical transformation (PPCT) were investigated every 20 hours. Methods of analysis were elemental analysis, adsorption chromatography, gas-liquid chromatography and spectrometry (IR, UV and MS). The content of the investigated fractions was studied and high content of hybrid compounds was determined. The results of the analyses showed that during irradiation of the said substances in air, there can be seen photochemical destruction of molecules with emission of gaseous products, and intensive photo-oxidative processes with formation of higher melting insoluble in organic solvents compounds, as well as liquid biologically active products.

The same results were obtained when investigated fractions were irradiated in environmental conditions during 30 days.

Analysis of gaseous products showed the presence of C₁-C₄ hydrocarbons, obtained by splitting-off the alkyl substituents and cleavage of naphthenic rings. In liquid products the OH-functional groups of phenolic and alcoholic character were found in large quantities. It should be noted that 15-50% of PPCT can be dissolved in water. These mainly include hydroxyl-containing compounds. It was also determined that in the process of photochemical oxidation the saturated fragments of molecules were destroyed predominantly and the aromatic structures stayed practically unaffected.

Destructive transformation of high-molecular heteroatomic hybrid polycycloaromatic fractions during photochemical oxidation and formation of water-soluble forms explains the destruction and erosion of mountainous asphalted roads, and formation of biologically active substances that stimulate plant growth, as well as self-purification of water basins polluted with petroleum and petroleum products.

Application of GCMS in the study of catalytic transformations on micro-mesoporous zeolites

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Zeolite catalysts are fundamental materials in chemical synthesis, largely due to their role in petrochemistry and their growing use in fine chemicals. They function as solid acid catalysts, replacing traditional sulfuric and other acids in more environmentally friendly processes. Zeolites play a crucial role in oil refining, especially in fluid catalytic conversion (FCC), isomerization and alkylation processes, particularly in the production of ethylbenzene and cumene. Zeolite catalysts are used for selective synthesis in pharmaceuticals and agrochemicals, including Friedel-Crafts alkylation and acylation. The popularity of zeolite catalysts stems from their large surface area, adjustable acidity and, most importantly, “shape selectivity” at the molecular level due to the presence of uniform micropores with diameters less than 2 nm. No less important is the presence of mesopores with a diameter of over 2 nm, which facilitate the transport of large molecules to active centers.

Despite the increased selectivity of zeolite catalysts, the spectrum of compounds obtained is in many cases very broad, and the GCMS method is indispensable for the analysis of catalytic conversion products. Previously, at the 12th Petromass conference, we reported on the use of GC-MS in studying the catalytic transformations of terpene alcohols linalool and geraniol on micro-mesoporous BEA-type zeolite with the “one-pot” production of long-chain (farnesol, geranylgeraniol) and macrocyclic (thunbergol, thunbergene) compounds. This report discusses the esterification of 4-nitrobenzoic acid with ethanol into ethyl 4-nitrobenzoate [1] and isonicotinic acid into ethyl isonicotinate [2] in the presence of hydrogen forms of natural zeolites (heulandite-clinoptilolite, mordenite and phillipsite), as well as the oxidation of trans-anethole on a vanadium-containing BEA zeolite catalyst [3]; the analysis of products of catalytic transformations was carried out by the GCMS (Agilent Technologies, 5890B/5977A, capillary column HP-5ms, Ultra Inert, 30 m x 0.32 mm x 0.25 µm)..

It has been shown that the use of microwaves, ultrasound, or catalysts made from ultrafine natural zeolites allows for the successful synthesis of ethyl 4-nitrobenzoate in an argon atmosphere at a temperature of approximately 80°C without the use of solvents, aggressive substances, or multi-stage processes; this ensures a conversion rate of approximately 60-70%, a product yield of 55-67%, and an ester selectivity of over 90%. Compared to the thermocatalytic ethylation of isonicotinic acid, on ultrafine crystallites of hydrogen forms of zeolites, the conversion and selectivity for ethyl isonicotinate increase to 35 and 98%, respectively, synergistically under the action of a catalyst with microwaves or ultrasound. The thermocatalytic oxidation of trans-anethole leads to non-selective conversion mainly into the products of isomerization and other compounds, while by catalytic oxidation on highly dealuminated V-BEA zeolites the trans-anethole is oxidized mainly to the desired 4-methoxybenzaldehyde with a selectivity of 80% at a conversion of 79/2%.

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Synthesis of proteotypic peptide standards for quantitative next-generation MS-based proteomics medical diagnostic assays

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Targeted mass spectrometry (MS)-based proteomics is increasingly used for quantitative analysis of protein biomarkers and development of minimally invasive diagnostic assays [1]. The analytical performance of targeted assays depends critically on the availability of well-characterized proteotypic peptide standards, including stable isotope-labeled internal standards (SIS). The present work was performed as part of an industrial immersion at MS-Technologies LLC, a Moscow-based company specializing in mass-spectrometry technologies and development of peptide standards and kits for quantitative proteomics. The study combined direct participation in the company's production workflow with an independent bioinformatic and literature-based task aimed at expanding the application of targeted proteomics to hepatocellular carcinoma (HCC) diagnostics.

During the industrial immersion, the author was integrated into the routine production process for a commercial peptide kit rather than performing a separate training project. The workflow included solid-phase peptide synthesis, resin cleavage, high-performance liquid chromatography (HPLC) purification, capillary zone electrophoresis (CZE), lyophilization, and mass-spectrometric quality control by matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) and liquid chromatography-mass spectrometry (LC-MS). In total, 52 samples were analyzed by CZE, 32 peptides were lyophilized, 51 samples were purified by HPLC, and 52 and 34 samples were subjected to quality control by MALDI-TOF MS and LC-MS, respectively. Ten newly synthesized peptides completed the full post-synthetic processing cycle. One tetranectin-derived proteotypic peptide was subsequently selected for incorporation into the peptide kit under development.

A complementary objective was to establish a candidate targeted-MS panel for early HCC detection. Published proteomic data were systematically reviewed, and candidate proteins and proteotypic peptides were evaluated using bioinformatic analysis and sequence-level verification [2]. This resulted in a reference panel of 17 proteotypic peptides corresponding to PPBP, SERPINC1, MCAM, CFL1, C4A, AMBP, THBS1, UCHL3, LCAT, C1QC, CNDP1, C2, CA2, C6, SAA4, SERPINA10, and APOH. Several candidates were absent from the company's existing expanded kit, providing a basis for a dedicated HCC-oriented mini-panel.

The industrial immersion therefore linked two complementary stages of translational targeted proteomics: experimental production and analytical quality control of peptide standards, and rational selection of biomarker-derived peptides for a clinically oriented application. The resulting HCC panel provides a molecular basis for subsequent development and validation of a multiplex targeted-MS assay. At the time of the study, MS-Technologies LLC was continuing development of its commercial peptide kit containing 214 peptide standards; the work described here was integrated into this broader production and assay-development context.

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Regional patterns of inorganic anions in Armenian drinking waters revealed by validated ion chromatography

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The ionic composition of drinking water reflects both natural hydrogeochemical processes and anthropogenic influences and represents an important component of environmental water-quality assessment. Ion chromatography (IC) with suppressed conductivity detection provides a sensitive and robust approach for simultaneous determination of inorganic anions in complex water matrices. In the present study, a validated IC method was applied to characterize regional patterns of seven inorganic anions-fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulfate-in drinking waters collected from different geographical regions of Armenia.

Fourteen drinking-water samples, comprising nine tap-water and five spring-water samples from Yerevan and the Aragatsotn, Syunik, Tavush, and Vayots Dzor regions, were investigated. Separation was performed on a Dionex IonPac AS22 (4 x 250 mm) column using 4.5 mM Na₂CO₃/1.4 mM NaHCO₃ as eluent and suppressed conductivity detection. All seven target anions were separated within 15 min. The method demonstrated good linearity ($R^2 > 0.998$), low detection limits (0.04-0.30 mg/L), recoveries of 92.74-114.67%, and satisfactory precision and stability, supporting its suitability for environmental water analysis.

Distinct regional variations in anion profiles were observed. Nitrate was detected in all drinkingwater samples and ranged from 0.607 to 8.920 mg/L, while fluoride concentrations ranged from 0.099 to 0.407 mg/L. Chloride and sulfate also showed substantial geographical variability. Bromide concentrations ranged from <LOQ to 1.029 mg/L, with comparatively elevated levels occurring in several sampling locations. Nitrite was below the quantification limit in all drinkingwater samples. Overall, most measured anion concentrations were consistent with drinking-water guideline values, while the observed spatial differences demonstrate the value of multi-anion profiling for identifying region-specific characteristics of water composition.

The results demonstrate that validated suppressed-conductivity IC provides a practical analytical platform for simultaneous inorganic-anion profiling and comparative assessment of drinking waters across geographically diverse regions. Such approaches can support systematic environmental surveillance and evidence-based evaluation of regional water-quality patterns.

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High-Resolution UPLC-QToF-MS Profiling of Phenolic Bioactives in *Vitis vinifera* cv. Msxali Stem Residues: Valorization Potential and Comparative Analysis with Rkatsiteli Cultivar

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This study presents a comparative phytochemical evaluation of stem extracts from the endemic Armenian grape cultivar *Vitis vinifera* cv. Msxali and the Georgian cultivar Rkatsiteli using high-resolution UPLC-QToF-MS. Extraction was performed via cold maceration with 70% ethanol at 38–40 °C for 5 hours. Quantitative analysis demonstrated that Msxali stems contain significantly higher levels of gallic acid (10.25 mg/kg) compared to Rkatsiteli (2.49 mg/kg). Conversely, Rkatsiteli exhibited higher concentrations of flavan-3-ols, specifically catechin (107.43 mg/kg) and epicatechin (16.68 mg/kg), compared to Msxali (7.52 mg/kg for both). Both cultivars accumulate valuable bioactives such as ϵ -viniferin, resveratrol, polydatin, and oleanolic acid. These findings highlight the strong potential of Msxali winemaking by-products as natural raw materials for therapeutic, nutraceutical, and pharmaceutical applications.

FastDDA versus Data-Independent Acquisition (MS^E) for Plant Extract Profiling: Comparison of MS/MS Coverage, Spectral Specificity, and Metabolite Annotation

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Untargeted LC-HRMS/MS profiling of plant extracts requires a balance between broad metabolome coverage and precursor-specific fragmentation suitable for reliable structural annotation. Data-dependent acquisition (DDA) isolates selected precursors before fragmentation, whereas data-independent acquisition (DIA) collects fragment information more comprehensively but requires computational precursor-fragment association^{1,2}.

Here, quadrupole-isolated FastDDA and data-independent MS^E were compared using three chemically complex botanical extracts: *Juglans regia* L. leaves, *Syringa vulgaris* L. leaves, and *Artemisia absinthium* herb. Analyses were performed by UPLC coupled to a Waters Xevo G3 QToF mass spectrometer in positive- and negative-ESI modes. FastDDA used precursor-triggered MS/MS with a +/-0.5 Da Q1 isolation window, up to seven MS/MS events per cycle, 4 s dynamic exclusion, and a 15-45 eV collision-energy ramp. MS^E used alternating low-energy (6 eV) and elevated-energy (15-45 eV) functions without precursor-specific Q1 isolation. Data were processed in MZmine, and GNPS was used for spectral-library interrogation^{3,4}.

Across all three matrices, a consistent coverage-versus-specificity trade-off was observed. Based on normalized comparative indices, MS^E provided 4.56-fold greater LC-MS feature coverage and 1.92-fold greater MS2 spectrum quantity than FastDDA. In contrast, FastDDA generated less complex, more directly precursor-associated spectra, with a 1.79-fold higher MS2 spectral-quality index and a 36.9-fold greater normalized GNPS database-matching index. These values are comparative normalized indices and do not represent absolute feature, spectrum, or confirmed compound-identification counts.

The results demonstrate that the two acquisition modes emphasize different analytical outcomes. Under the evaluated conditions, MS^E was advantageous for comprehensive feature and fragmentation acquisition, whereas quadrupole-isolated FastDDA generated more precursor-specific, structurally interpretable, and library-searchable MS/MS information. FastDDA and MS^E should therefore be regarded as complementary strategies, with their relative analytical value determined by whether broad coverage or MS/MS-based metabolite annotation is the primary objective.

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