The 13th International Symposium on Drug Analysis and the 36th International Symposium on Pharmaceutical and Biomedical Analysis

ABSTRACT BOOK

July 6 – 9, 2026
Ghent, Belgium

GHENT UNIVERSITY

Organized by the
Belgian Society of
Pharmaceutical Sciences

SPONSORS

GOLDEN SPONSORS

The power of precision

BRONZE SPONSORS

BGB Analytik Benelux B.V.

ELSEVIER

WITH THE SUPPORT OF

ionBench®

FACULTY OF PHARMACEUTICAL SCIENCES

GHENT UNIVERSITY

DruQuR

LP PAT

ABSTRACTS

PLENARY LECTURES

PL-01

Multi-dimensional liquid chromatography in combination with high-resolution mass spectrometry as a powerful tool to determine the environmental fate of drugs

Deirdre Cabooter[1], Matthias Miertz[1], Toon Verdonck[1], Raf Dewil[2], Marie Pardon[1]

[1]Department of Pharmaceutical and Pharmacological Sciences, University of Leuven (KU Leuven), Herestraat 49, 3000 Leuven, Belgium
[2]Department of Chemical Engineering, University of Leuven (KU Leuven), J. De Nayerlaan 5, Sint-Katelijne-Waver 2860, Belgium

The discharge of pharmaceuticals in the environment is becoming a topic of increasing concern. Pharmaceuticals are emitted into environmental waters through human and animal excretions, agricultural processes and industrial activities. Most water discharges are subjected to treatment in wastewater treatment plants (WWTPs) before being released into the environment. However, the current generation of WWTPs is not equipped to adequately degrade pharmaceuticals. This results in environmental concentrations of pharmaceuticals in the ng/L to µg/L range, that can have negative effects on aquatic organisms. It has for example been demonstrated that ethinylestradiol impairs fish reproduction, while oxazepam alters the behavior and feeding rate of wild perch at environmental concentrations. It has also been shown *in vitro* that at environmental concentrations, antibiotics drive the selection of resistant bacteria.

It is hence imperative that the presence of pharmaceuticals in the environment is carefully monitored and efficient degradation strategies for their removal from wastewater are devised. This requires sensitive, high-resolution analytical techniques, such as liquid chromatography in combination with mass spectrometry (LC-MS). Since pharmaceuticals typically display a variety in physicochemical characteristics and environmental water matrices can be very complex, a single LC-MS method is, however, often insufficient for the full characterization of all compounds of interest. As an alternative, two-dimensional (2D) LC techniques, combining two LC methods by transferring fractions from a first-dimension column to a second-dimension column for further separation, have shown great promise as they provide an enlarged separation space for complex samples. The best results are obtained when columns operating according to orthogonal separation mechanisms are combined, such as reversed-phase liquid chromatography (RPLC) and hydrophilic interaction chromatography (HILIC). However, such separation mechanisms often require highly incompatible mobile phases, making their online combination challenging.

This presentation aims to give an overview of the different two-dimensional (2D-LC) MS solutions that have recently been developed in our lab to analyze pharmaceuticals in the environment. The potential of different 2D-LC modes, such as (single) heart-cutting,

selective comprehensive and full-comprehensive 2D-LC will be compared in terms of achievable peak capacities, sensitivities and matrix effects for real environmental samples. Special emphasis will be put on effective modulation strategies for the online combination of RPLC and HILIC, such as flow splitting, active solvent modulation and total breakthrough as a promising new modulation technique. It will also be discussed how peak capacities can be further enhanced and tailored to the sample at hand by moving from a 2D-LC approach to a truly multi-dimensional configuration, employing multiple different columns and separation methods in the second dimension, tailored to the properties of the compounds eluting from the first dimension.

Finally, as an outlook, it will be discussed how novel hardware and software developments in our team aim to further enhance the performance, robustness, and ease-of-use of multidimensional LC-MS separations for the analysis of pharmaceuticals in the environment in the future.

PL-02

Label-free technologies and data science for pharmaceutical R&D: from single cells to 3D models

Carsten Hopf

Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Technical University Mannheim, Mannheim, Germany;

MALDI-Mass spectrometry imaging (MSI), also referred to as spatial metabolomics, has emerged as a powerful technology for spatially resolved analysis and visualization of lipids and metabolites in pharmaceutical R&D and clinical research. Advancement of MSI requires rapid progress in multiple areas such as instrumentation, experimental workflows and computational strategies to harness big data. MSI technology has recently moved into two new directions: Single-cell metabolomics and 3D-reconstructed metabolomics.

To study single-cell metabolite changes associated with proinflammatory activation of iPSC-derived microglia by bacterial lipopolysaccharide (LPS), we developed the PRISM-MS (PRescan Imaging for Small Molecule - Mass Spectrometry) platform for analysis and on-cell MS2 identification of low mass metabolites (<200 Da) in large cell populations. Itaconate and taurine were identified as markers for “activated” versus “resting” microglia, respectively. Translation of single cell results to endogenous microglia in organotypic rat hippocampal slice cultures indicated that LPS-activation involves changes of the itaconate-to-taurine ratio and alterations in neuron-to-glia glutamine-glutamate shuttling.

To advance spatial omics data analysis, we combined spatial autocorrelation analysis with hyperspectral imaging technologies and rigorous statistical testing – introducing a concept of molecular probabilistic mapping of metabolite ensembles in human tumor tissue sections.

Finally, to investigate fibroblast-colon cancer cell interactions in a simple 3D-culture model and in patient-derived organoids (PDOs), we built a translational 3D MSI platform as an end-to-end solution for 3D-enabling sample preparation, 3D-reconstruction and data processing, 3D-rendering, and immersive user interaction with organoid big data in a mixed reality. When applied to colon cancer PDOs, the methodology revealed that fluid-filled cysts characteristic of these PDOs were rich in purine nucleotides.

References

Abu Sammour D, Cairns JL, Boskamp T, Marsching C, Kessler T, Ramallo Guevara C, Panitz V, Sadik A, Cordes J, Schmidt S, Mohammed SA, Rittel MF, Friedrich M, Platten M, Wolf I, von Deimling A, Opitz CA,

Wick W, Hopf C. Spatial probabilistic mapping of metabolite ensembles in mass spectrometry imaging. Nat Commun. 2023 Apr 1;14(1):1823. doi: 10.1038/s41467-023-37394-z.

Cairns JL, Huber J, Lewen A, Jung J, Maurer SJ, Bausbacher T, Schmidt S, Levkin PA, Sevin D, Göpfrich K, Koch P, Kann O, Hopf C. Mass-Guided Single-Cell MALDI Imaging of Low-Mass Metabolites Reveals Cellular Activation Markers. Adv Sci (Weinh). 2025 Feb;12(5):e2410506. doi: 10.1002/advs.202410506.

Iakab SA, Cordes J, Enzlein T, Keller F, Kastner K, Mulholland T, Fröhlich BC, Gruber L, Cairns JL, Schmidt S, Hafner M, Schneider R, Betge J, Fischer F, Reichwald J, Rudolf R, Hopf C. From Sample to Mixed Reality: A Translational 3D MALDI Imaging Platform for Advanced 3D Spatial Omics Analysis of 3D Cell Culture Disease Models. Adv Sci (Weinh). 2026 Feb;13(12):e16098. doi: 10.1002/advs.202516098.

Rittel MF, Ebert N, Abu Sammour D, Graf S, Fröhlich BC, Birgin E, Mohammed SA, Rahbari NN, Wellmann A, Wasenmüller O, Weis CA, Schmidt S, Hopf C. Cohort-Scale Spatial Autocorrelation for Tumor Prediction in Mid-Infrared Pathology and Spatial Biomarker Discovery Using MALDI Imaging Lipidomics. Adv Sci (Weinh). 2026 Apr;13(24):e16847. doi: 10.1002/advs.202516847.

PL-03

Product and Process Characterisation using Native Mass Spectrometry

Corentin Beaumal[1], Noemí Dorival-García[1], Maikel Gaitkoski[1,2], Sara Carillo[1], Jonathan Bones[1,2]

[1]Characterisation and Comparability Laboratory, National Institute for Bioprocessing Research and Training, Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, A94 X099, Ireland.
[2]School of Chemical and Bioprocess Engineering, University College Dublin, Belfield, Dublin 4, D04 V1W8, Ireland.

The development of native separations coupled to high resolution native mass spectrometry has enabled deep characterisation of protein based biopharmaceuticals. Our initial efforts in this area focused on the creation of a toolbox of different separation chemistries such as size exclusion, affinity and ion exchange operated using mass spectrometry friendly buffers to facilitate direct hyphenation to native Orbitrap mass spectrometry. These methods were applied to various modalities, including monoclonal antibodies, Fc fusion proteins, antibody drug conjugates and other proteins of interest. Key observations included the identification of low level posttranslational modifications present that were often not observed when using peptide centred methods, however verification of these observations often required collection of peaks and offline sample processing followed by peptide mapping to confirm the identity of the modification and its position within the primary sequence.

We have built upon this platform and expanded activities in two different directions. Firstly, to avoid sample collection and offline processing, we have explored the use of native top-down mass spectrometry using various ion activation methods such as collisional, electron and photon based dissociation to increase sequence coverage and confirm the identity and location of a modification from combination of the resulting chromatographic and MS/MS spectral data. While not without its challenges, the method was successfully applied to investigate comparability between trastuzumab and its EMA approved biosimilar counterparts to demonstrate feasibility of the approach. Examples of platform expansion for the characterisation of complex multi-specific antibodies and antibody drug conjugates will also be presented.

Secondly, we have deployed native methods, namely Protein A directly coupled to native Orbitrap mass spectrometry, within an integrated, autonomous platform as a process analytical technology used for monitoring upstream processing within bench scale bioreactors. When combined with other integrated solutions and in situ probe measurements, the ability to measure titre and the distribution of quality attributes present on the molecule provides insights into process dynamics and dependencies on process parameters and media components. Application across different manufacturing

processes will be presented as will our strategy for data management, utilisation and knowledge extraction as we move towards autonomous process control.

PL-04

Bridging Untargeted and Targeted Metabolomics in Biomedical Analysis

Coral Barbas

Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Boadilla del Monte, Spain. cbarbas@ceu.es

The comprehensive characterization of the lipidome remains a major analytical challenge due to its vast structural diversity and the presence of isomeric species that cannot be resolved by conventional workflows. In this plenary lecture, we will present recent advances in structural lipidomics, with a particular focus on the development of high-confidence lipid annotation strategies based on human plasma reference materials.

We will describe our work using the National Institute of Standards and Technology (NIST) plasma, where we have established an extensive lipid database incorporating not only lipid class and fatty acyl composition, but also double bond localization. This effort provides a robust framework for improving annotation accuracy and harmonization across laboratories, addressing a critical bottleneck in lipidomics [1, 2].

Building on this analytical foundation, we will highlight the application of these approaches in human plasma studies, including investigations in rare diseases such as Alport syndrome [3], where lipid remodelling offers novel insights into disease mechanisms. These findings highlight the importance of moving beyond global lipid profiling toward more refined and biologically meaningful molecular resolution.

This transition naturally leads to oxylipins, a class of bioactive lipid mediators derived from polyunsaturated fatty acids, which require highly targeted analytical strategies due to their low abundance and dynamic regulation. We will discuss the development of targeted workflows for oxylipin profiling and their application in translational research, including studies in Niemann–Pick disease [4], where dysregulated lipid signalling pathways play a central role.

Overall, this lecture will illustrate how advances in analytical chemistry—ranging from structural elucidation to targeted semi-quantification—enable a deeper understanding of lipid biology and open new avenues for biomarker discovery and precision medicine.

References

[1] J. Lipid Res. (2024) 65(11) 100671
[2] JPBA (2025), 117081
[3] Kidney Int Rep. (2025) 26;10(8):2805–2820
[4] Prog Neuropsychopharmacol Biol Psychiatry.(2025) 20:139:111387.

PL-05

Three-dimensional LC and two-dimensional LC-MS/MS analysis of chiral amino acids and related compounds for the screening of physiologically active substances and biomarkers

K. Hamase

Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582 – JAPAN

Enantioselective analysis of amino acids and related compounds is an effective approach for the screening of new biologically active substances and/or biomarkers. Most of the chiral compounds have typical homochirality features in living beings, with one of the enantiomers (e.g. L-forms for amino acids) predominantly present in the tissues and physiological fluids. It is long believed that the minor antipodes are absent at least as the physiologically significant molecules. In the latest decades, several D-amino acids were found in higher animals including humans, and their biological functions and diagnostic values have been gradually clarified. However, most of these D-amino acids are trace substances, and highly sensitive and selective analytical methods are essential for their screening in biological matrices. For the purpose, multi-dimensional HPLC (especially, three-dimensional LC and two-dimensional LC-MS/MS) systems are effective. In these multi-dimensional LC systems, the target chiral analytes (amino acids and related compounds) are separated as their scalemic enantiomer mixtures by a reversed-phase column, and only the target fractions were collected into the on-line connected trap loops. These fractions were automatically injected to the next dimension, and the enantiomers were separated and determined in the final dimension. Between the first (reversed-phase mode) and the last (enantioselective mode) dimensions, an anion-exchange mode, a mixed mode or a different reversed-phase mode separation could be inserted to obtain higher selectivity. For the appropriate retention and also for the better detectability, amino acids and related compounds were derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). A key to accomplish comprehensive analysis of chiral amino acids is having the enantioselective columns that enable enantiomer separation of all proteinogenic amino acids (plus *allo*-forms of isoleucine and threonine) as their NBD derivatives. To obtain appropriate enantioselective columns, we designed plenty of Pirkle-type chiral stationary phases (CSPs) having *N*-(3,5-dinitrophenylaminocarbonyl)-amino acids as their selectors. Among these CSPs Singularity CSP-001S (L-leucine type), 016S (L-2-aminobutyric acid) and 017S (L-norvaline) columns showed sufficient enantiomer separations with appropriate elution orders (D-forms eluted faster). Concerning tryptophan, the fluorescence intensity of the NBD-derivative is extremely weak due to the photoinduced electron transfer between the benzofurazan and indole rings. In this regard, a photo-reactor for the online UV irradiation was designed and integrated into the multi-dimensional HPLC system. The designed three-dimensional LC and two-dimensional LC-MS/MS systems could be successfully applied to the determination of chiral amino acids and related compounds in a

variety of real-world samples and further studies for the screening of physiologically active substances and biomarkers are ongoing.

References

1. M. Oyaide, K. Hamase et al., *J. Pharm. Biomed. Anal.*, 273, 117348 (2026).
2. C. Ishii, K. Hamase et al., *Chromatography*, 46, 71–75 (2025).
3. C. Ishii, K. Hamase et al., *Anal. Chem.*, 96, 4876–4883 (2024).
4. H. Naraoka, K. Hamase et al., *Science*, 379, eabn9033 (2023).
5. Y. Nagata, K. Hamase et al., *J. Pharm. Biomed. Anal. Open*, 2, 100013 (2023).
6. C. Ishii, K. Hamase et al., *Chromatography*, 41, 1–17 (2020).
7. A. Furusho, K. Hamase et al., *Anal. Chem.*, 91, 11569–11575 (2019).

PL-06

Paper-based electrochemical (bio)sensors as smart devices for biomedical applications

Fabiana Arduini

Department of Chemical Science and Technologies, University of Rome Tor Vergata, Rome, Italy
(fabiana.arduini@uniroma2.it)

As reported in my recent perspective entitled "Paper as a Sustainable Material for Smart Electrochemical (Bio) sensors with Unprecedented Features: A Perspective" published in Analytical Chemistry [1] "*The use of paper in electrochemical devices not only provides additional features to the electrochemical devices such as the environmentally friendless, ease multiplexed analysis, and three tridimensional structures by folding and unfolding operations but has broken down barriers for delivering measurement without (i) addition of reagents, (ii) sample treatment for liquid, aerosol, and solid samples, and (iii) any additional pump for microfluidics*". In this plenary lecture, I will highlight how the intrinsic physicochemical properties, including porosity, foldability, and breathability can be harnessed to enable advanced printed electrochemical (bio)sensors for biomedical applications. Furthermore, I will point out that paper-based electrochemical (bio)sensors have also overcome several barriers in printed electrochemical devices, demonstrating the reliability of detecting target analytes in the aerosol phase, such as breath, for non-invasive diagnosis. Finally, in the roadmap of my group, I will report the results obtained within the Horizon Europe Pathfinder Project Phoenix-OoC, which I am coordinating, where we are now developing a paper-based (bio)sensing array to deliver a sensorized paper-based organ-on-a-chip for drug testing and precision medicine.

References

[1] F. Arduini, Anal. Chem., 2025, 19, 10126.

Acknowledgments: Horizon Europe Phoenix-OoC Project GA N. 101130395.

ABSTRACTS

KEYNOTE LECTURES

KN-01

Luminescence-based analytical approaches for pharmaceuticals: environmental and biological applications

Naoya Kishikawa

Nagasaki University, Graduate School of Biomedical Sciences, 852-8521 Nagasaki City, Nagasaki, Japan

Chemiluminescence (CL) is a phenomenon in which molecules, excited by the energy released from chemical reactions, emit light as they return to the ground state. CL-based analytical methods provide highly sensitive detection with simple instrumentation, making them a powerful platform for analytical applications in pharmaceutical, biological, and environmental samples. CL reagents such as luminol emit light upon oxidation by reactive oxygen species (ROS), and this ROS-triggered emission has been widely exploited for trace analysis. Our research focused on expanding the applicability of CL-based analytical approaches by integrating photochemical reactions that convert target analytes into ROS.

Ultraviolet (UV) irradiation of quinones induces photodecomposition to generate ROS capable of initiating luminol CL or peroxyoxalate chemiluminescence (PO-CL).[1] Utilizing this principle, we developed HPLC–PO-CL methods in which quinones are photoconverted into ROS and fluorescent photoproducts, enabling sensitive determinations after simple post-column mixing with diaryl oxalate. These methods were successfully applied to pharmaceutical analytes such as vitamin K and doxorubicin in biological matrices. Moreover, we found that quinone photoproducts significantly enhance luminol CL. The simultaneous photogeneration of ROS and this enhancer greatly increases luminol emission, enabling sensitive determination of polycyclic aromatic hydrocarbon quinones, including anthraquinone in airborne particulate samples.

We also established a novel CL derivatization strategy using anthraquinone-2-carbonyl chloride as a derivatizing agent for amines and exploiting the ability of the resulting quinone moiety to generate ROS under UV irradiation.[2] Additionally, although 2,4-dinitrophenylhydrazine (DNPH)-a widely used HPLC derivatization reagent-does not itself produce ROS, aldehydes derivatized with DNPH were found to generate ROS upon photoirradiation. Using this property, we developed an HPLC method for determining lipid peroxidation-derived aldehydes.[3]

More recently, we observed that simple UV irradiation of a mixed solution of luminol and tyrosine produces CL whose intensity increases proportionally with tyrosine concentration.[4,5] Notably, only tyrosine exhibits CL under these conditions, whereas structurally related analogues show little to no emission. We have utilized this selectivity to develop a rapid

and facile CL-based assay for enzymes involved in tyrosine conversion, such as alkaline phosphatase and tyrosinase.

Collectively, these studies expand the scope of CL-based detection by integrating photochemical ROS generation with luminescence-enhanced reactions, enabling versatile applications in pharmaceutical, biological, and environmental analysis.

References

1. N. Kishikawa and N. Kuroda, J. Pharm. Biomed. Anal. (2014), 87, 261-270.
2. N. Kishikawa et al., Molecules (2022), 28, 2146.
3. M. El-Maghrabey, N. Kishikawa et al., Talanta (2021), 233, 122522.
4. N. Kishikawa et al., Anal. Chem. (2022), 94, 33, 11529-11537.
5. A. Abdel-Hakim, N. Kishikawa et al., Talanta (2026), 297, 128560.

KN-02

Epitope mimetic peptide recognition technology promotes the in-depth analysis of antibody-drug conjugates

Z. Jiang, Q. Wang

Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, 510632 – Guangzhou

Antibody-drug conjugates (ADCs) are essential for targeted cancer therapy, yet their efficacy can be compromised by *in vivo* modifications such as deamidation, isomerization, and oxidation. Dynamic tracking of these biotransformations is crucial for understanding drug fate and ensuring therapeutic efficacy, but remains challenging due to low variant concentrations, complex biological matrices, and inherent limitations of conventional affinity methods that fail to capture variants modified within complementarity determining regions (CDRs). To address initial enrichment challenges, we developed a peptide-based affinity monolith using a one-step polymerization method with a methacrylate-modified tetrapeptide ligand (m-EDPW, K_d = 1.91 μM for trastuzumab), which exhibited excellent specificity and recovery (91.6% for trastuzumab, 98.37% for anti-HER2 ADC) from cell culture media and enabled preliminary monitoring of critical quality attributes. However, we identified that modifications occurring within CDRs weakened interactions with this single-ligand approach, leading to loss of key variants during analysis. Especially, the introduction of small molecule drugs can significantly enhance the steric hindrance between ADC and affinity ligands. To overcome this limitation, drawing inspiration from bispecific antibodies (BsAbs), we subsequently developed a multi-epitope affinity platform utilizing MOF@Au nanocomposites that simultaneously immobilize both CDR mimotope peptide and non-CDR mimotope aptamer. This strategy demonstrated significantly enhanced enrichment capabilities compared to mono-epitope methods and enabled detection of deamidation (LC-Asn-30) and isomerization (HC-Asn-55) ratios that single-ligand approaches failed to identify, with clinical application successfully monitoring trastuzumab modification variants in breast cancer patient sera and revealing faster modification trends in serum due to catalytic effects of plasma proteins. Building on this foundation, we further advanced the technology to achieve binding capacity ten to hundred times higher than traditional methods, enabling comprehensive monitoring of ADC biotransformations in serum including rapid drug-to-antibody ratio decline from 3.67 to 0.22, payload loss, and unexpected succinimide ring hydrolysis of the linker. Collectively, this integrated multi-epitope affinity strategy, progressing from initial monolith development to advanced nanocomposite platforms, provides a robust and versatile approach for in-depth biotransformation analysis, overcoming traditional recognition deficiencies and paving the way for improved ADC development, quality control, and personalized therapeutic monitoring.

References

1. C. Zhu, H. Han, Z Chen, Y Shen, Q Zhang, C Bao, J.-H. Qu, Q. Wang, Z. Jiang. Anal. Chim. Acta (2023), 1246, 340892.
2. C. Zuo, J. Zhou, S. Bian, Q. Zhang, Y. Lei, Y. Shen, Z. Chen, P. Ye, L. Shi, M. Mu, J.-H. Qu, Z. Jiang, Q. Wang. J. Pharm. Anal. (2024), 14, 101015.
3. Y. Lei, Y. Shen, F. Chen, R. He, Z. Zhang, Y. Zhou, J.-C. Yu, J. Crommen, Z. Jiang, Q. Wang. Acta Pharm. Sin. B (2024), 14, 4962-4976.

KN-03

Coral microbiome as a source for pharmaceuticals

Anelize Bauermeister[1]*, Ana Paula Schiavo[1], Mariana Rocha[1], Artur Oliveira Garcia Matos[1], Pedro Henrique Silva Candido[1], Laís Pina de Sousa1, Marcelo Kitahara[2].

[1] Institute of Chemistry, University of São Paulo; [2] Center for Marine Biology, University of São Paulo. * Corresponding author

Corals host highly diverse and complex microbial communities known as the microbiome, forming a holobiont that integrates the coral animal, symbiotic algae, and bacteria, fungi, and other microbes. This microbiome exhibits remarkable ecological complexity, with distinct microbial habitats in coral tissue, mucus, and skeleton influencing community structure and function. Coral-associated microbes produced several novel compounds in the last year, including terpenes, alkaloids, peptides, and polyketides with antitumoral, antibacterial, antifungal, anti-inflammatory, and antidiabetic activities. Metabolomics tools enable detailed profiling of small molecules in complex microbiome samples, revealing metabolic interactions and functions within microbial communities like coral holobionts, and contributing to the discovery of novel compounds with biological properties. In this context, metabolomics tools developed in the last decade have accelarated the analysis of these complex biological samples. Particularly, MicrobeMASST searches MS/MS spectra against a database of over 60,000 microbial monocultures to identify microbial producers without prior knowledge. Despite Brazil's extensive coastline and coral diversity, only a small fraction of native coral species has been chemically and biologically characterized, indicating a large untapped reservoir of metabolites. Future work is likely to integrate in situ-friendly sampling, advanced metabolomics and microbiome studies to discover new bioactive molecules from Brazilian corals while minimizing ecological impact and enabling sustainable drug-discovery pipelines. Herein, we have been investigation the metabolites diversity from corals from the Atlantic ocean from the Brazilian coast. Compounds such as Tubastrindole, Palythazine and, Dictazolin and possible novel derivatives have been annotated. Bacteria have been isolated from corals and have been cultured in order to find novel compounds with biological activity and to better understand their relationship with each other.

KN-04

Multifaceted LC-MS approach in drug discovery: from hit finding to binding and structural activity

Fernando G. Almeida[1,2], Srinath Krishnamurthy[1], Katharina L. Duerr [1,3] and Idlir Liko[1,2]

[1] OMass Therapeutics Ltd., Building 4000, Chancellor Court, John Smith Drive, Oxford Business Park, ARC, Oxford OX4 2GX, UK; [2] Current address: Ellison Institute of Technology, Schrödinger Building, Heatley Road, The Oxford Science Park, Oxford OX4 4GE, UK; [3] Current address: Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, Oxford, UK. Kavli Institute for Nanoscience Discovery, University of Oxford, Oxford OX1 3QU, UK

In early drug discovery, one of the most common strategies for identifying bioactive compounds for a given target is phenotypic assay based screening, in which large libraries of molecules are interrogated in cell-based assays to monitor changes in target relevant pathways. A key limitation of phenotypic assays is that their readouts often do not identify the molecular target directly. As a result, these screens frequently yield active hits with an unclear mechanism of action[1], and in some cases the observed activity arises from off-target effects or downstream signaling cascades, leading to false positives.

Within the context of target based screening, affinity selection–mass spectrometry (AS-MS) has emerged as an alternative approach for identifying potential ligands. AS-MS is an LC–MS–based high throughput screening method in which mixtures of small molecules are incubated with a purified target protein. Ligand-protein complexes are then separated from non binders, after which bound ligands are displaced, identified, and quantified by LC–MS[2].

Following hit identification, compounds typically undergo a validation workflow where many fail to demonstrate functional activity and are ultimately deprioritized. However, it is worth to mention that binders can affect the protein activity in underappreciated ways that are not covered by the traditional functional assays. For instance, binders can change the dynamic and structural features of proteins that can result in: a) novel interactions with other proteins acting as binding partners, b) changes in protein dynamics, stability and turnover rates[3].

Here, we present a case study demonstrating a multifaceted LC–MS centred workflow to interrogate protein–ligand interactions beyond binding alone. We combine AS-MS with complementary mass spectrometry approaches, including hydrogen–deuterium exchange mass spectrometry (HDX-MS) and native mass spectrometry, to obtain molecular level insight into ligand binding modes, conformational effects, and interaction determinants. This integrated strategy enables the identification of key ligand features and modifications that are important for a potential functional activity.

As a model system, we investigated the neuronal K^+/Cl^- cotransporter KCC2[4], which is essential for inhibitory neurotransmission, and whose dysfunction has been implicated in a range of neurological disorders. Enhancing KCC2 transport is a validated strategy for the treatment of various disease conditions but remains challenging due to limited understanding of druggable sites and regulatory mechanisms. Using AS-MS, HDX-MS, native-MS and cryo-electron microscopy, we identify and characterize small molecules that modulate KCC2 activity. We show that ATP is a positive allosteric regulator of KCC2 that couples regulatory segments, including key phosphosites, in the cytoplasmic domain (CTD) to the transmembrane ion channel. We uncover the molecular mechanism of action of how ATP-competitive and uncompetitive ligands inhibit ion transport.

References

1. J. A. Lee, E. L. Berg. SLAS Discovery. (2013), 18, 10, 1143-1155.
2. F. G. Almeida, Q. B. Cass. B.J. Anal. Chem. (2023), 10, 40, 13-16
3. S. L. Schreiber. I. J. Chemistry. (2018), 59, 1-2, 52-59
4. M. Agez, P. Schultz, I. Medina, D. J. Baker, Sci. Reports. (2017), 7, 16452

KN-05

Developing Analytical Procedures through Multivariate Risk Management Strategies: Tools and Advantages

Sandra Furlanetto[1], Serena Orlandini[1], Benedetta Pasquini[1], Lucrezia Floris[2], Roberto Gotti[2]

[1] Department of Chemistry "Ugo Schiff", University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino, Italy; [2] Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

The integration of risk management strategies and design of experiments, yields a risk-based scientific approach, aligned with the principles of Analytical Quality by Design (AQbD) [1] and compliant with major regulatory frameworks, including ICH Q8 (Pharmaceutical Development), ICH Q9 (Quality Risk Management), ICH Q14 (Analytical Method Development) [2], as well as guidelines from the European Medicines Agency [3] and the US Food and Drug Administration [4]. This approach is particularly relevant for biological drugs, where intrinsic variability and complex structures require rigorous analytical controls to ensure product safety and efficacy. From simple applications to the development of PAPs for the quality control of monoclonal antibodies, the presentation illustrates the evolution of analytical methodological development and the gradual affirmation of the concept of risk management, which has become essential to guarantee the quality of analytical data while providing the flexibility needed to face modern challenges.

Acknowledgements

This work was supported by Ministero dell'Università e della Ricerca Scientifica: PRIN project (QubyD4Prot) Prot. 2022AF8KZ3.

The support provided by the MUR - Dipartimenti di Eccellenza 2023–2027 (DICUS 2.0) to the Department of Chemistry "Ugo Schiff" of the University of Florence is also acknowledged.

References

[1] Ghizzani V., Orlandini S. et al. A Design of Experiments and Risk Management-Driven Analytical Platform for Charge Variant Analysis of Therapeutic Antibodies by Imaged Capillary Isoelectric Focusing, AAPS J 28, 27 (2026). https://doi.org/10.1208/s12248-025-01178-8

[2] https://www.ich.org/page/quality-guidelines

[3] https://www.ema.europa.eu/en/human-regulatory-overview/research-development/quality-design

[4] Pharmaceutical CGMPs for the 21st century — A risk-based approach Final report. Department of Health and Human Services, U.S Food and Drug Administration, Siler Spring, MD, 2004.

KN-06

GreenSOL: The first green solvent selection guide for analysis

Elia Psillakis, A. Mondello, E. Alladio, P. Oliveri, F. Pena-Pereira

Technical University of Crete, School of Chemical and Environmental Engineering, 73100 – Chania, Greece

Recent developments, guided by the principles of Green Analytical Chemistry and Green Sample Preparation, promote the adoption of environmentally friendly solvents as a key approach to reducing the environmental impact of analytical methods while also protecting laboratory personnel.[1–3] In this direction, efforts to identify "green" solvents in analytical chemistry have often relied on a limited set of environmental, health, and safety criteria, as well as on industrial solvent guides that are not specifically designed for analytical applications. To address this limitation, a new solvent guide called GreenSOL was developed, incorporating a comprehensive life-cycle perspective.[4] GreenSOL assesses 58 widely used, specialized, and deuterated solvents across three major stages: production, laboratory use, and waste management, using multiple environmental, health, and safety criteria. The presentation will highlight the trade-offs involved rather than suggesting the existence of a perfectly "green" solvent. It will also discuss practical approaches to solvent substitution, and key criteria for identifying suitable alternatives, including the potential of bio-based solvents.[5] While solvents and materials derived from renewable biomass are often viewed as inherently greener alternatives to petrochemicals, their bio-origin does not guarantee these attributes. Overall, this contribution emphasizes the necessity of verifying green claims through a life cycle approach, as solvents safe to use in a laboratory can still be energy-intensive to produce, toxic to the environment, or poorly degradable.

References

1. A. Gałuszka, Z. Migaszewski, J. Namieśnik, TrAC-Trends Anal. Chem. (2013) 50, 78.
2. Á.I. López-Lorente, F. Pena-Pereira, S. Pedersen-Bjergaard, V.G. Zuin, S.A. Ozkan, E. Psillakis, TrAC-Trends Anal. Chem. 148 (2022), 148, 116530.
3. E. Psillakis, F. Pena-Pereira, TrAC-Trends Anal. Chem. (2024), 175, 117686.
4. E. Stampolaki, A. Mondello, E. Alladio, P. Oliveri, A. Mazzoleni, F. Pena-Pereira, E. Psillakis, TrAC-Trends Anal. Chem. 194 (2026) 118531.
5. E. Psillakis, Anal. Bioanal. Chem. (2026) Under Review.

KN-07

Metabolomics Insights into Mindfulness: Multiblock Integration Reveals Stress-Associated Signatures in Healthcare Students

Serge Rudaz

University of Geneva, Switzerland

Healthcare students are exposed to sustained psychological stress that may impair empathy, prosocial behavior, and ultimately quality of care. Despite growing recognition of mental health challenges in medical education, biological correlates of stress reduction following Mindfulness-Based Interventions (MBIs) remain poorly characterized.

Within the Swiss National Science Foundation–funded *e-SMILE* project, we investigated the biological impact of Mindfulness-Based Cognitive Therapy for Life (MBCT-L) using a multi-omics strategy integrated with Perceived Stress Scale (PSS) scores.

Serum samples (n = 180) were analyzed by LC-HRMS metabolomics (460 Level 1 metabolites), lipidomics (1,167 lipids), extended steroid profiling (86 steroids converted into 148 pathway-based ratios), and electrochemiluminescent immunoassay quantification of 35 inflammatory biomarkers.

Single-block modeling using OPLS and non-linear approaches failed to generate predictive models for PSS scores. In contrast, a multiblock Consensus OPLS framework, combining multiple kernel learning with supervised latent variable modeling, successfully integrated complementary datasets and achieved strong predictive performance ($R^2(Y) > 0.8$).

The integrative model identified key metabolites, including serotonin, tyrosine, inosine, hypoxanthine, xanthine, and several acylcarnitines (palmitoyl-, tiglyl-, stearoyl-carnitine). Lipid enrichment analysis revealed overrepresentation of EtherPE, sphingomyelins, and LPC species, suggesting involvement of oxidative stress, membrane remodeling, and inflammatory signaling pathways. These findings were corroborated by inflammatory biomarkers. Steroid profiling demonstrated a strong biological sex effect, representing a major confounder in stress modeling. Application of iAMOPLS to pathway-based steroid ratios disentangled sex-driven variance and identified corticosteroids and corticosteroid-to-progestogen ratios as stress-associated markers.

This study demonstrates that multiblock integration substantially enhances detection of stress-related biological signatures and provides initial mechanistic insight into the metabolic impact of mindfulness-based interventions in healthcare students.

KN-08

Capillary electrophoresis for the analysis of the enantiomeric purity of drugs

Gerhard Scriba

Friedrich Schiller University, Department of Pharmaceutical/Medicinal Chemistry, 07743 Jena, Germany

The importance of the stereochemistry of pharmaceutical drugs is well recognized because stereoisomers often differ in their pharmacological, toxicological and/or pharmacokinetic profiles. As a consequence, the majority of small molecule drugs of the top 10 products according to their worldwide sales in 2024 are single enantiomer drugs.[1] For drug development and quality control, the determination of the stereoisomeric composition and purity of a compound requires sensitive and accurate analytical methods in order to determine the stereoisomer ratios in synthetic products, pharmaceutical formulations and biological samples. Besides high-performance liquid chromatography, capillary electrophoresis (CE) has become an attractive alternative for this purpose.

In CE, the chiral selector is added to the background electrolyte acting as a pseudostationary phase, which is also mobile in contrast to chromatographic methods. Consequently, two stereoselective principles contribute to stereoisomer separations, i.e. the formation of transient diastereomeric complexes between analyte enantiomers and the chiral selector (also referred to as the thermodynamic or chromatographic enantioselective mechanism) as well as the mobility of these complexes (electrophoretic enantioselective mechanism). Both principles can cooperate or counteract each other, which can be exploited for enantioseparations. Furthermore, selectors can be easily combined for optimization of the separation.

The presentation will discuss recent applications of chiral CE methods for the determination of the enantiomeric purity of pharmaceutical drugs and their application to the analysis of drug substances and pharmaceutical formulations using a quality by design (QbD) approach and design of experiment (DoE) methodologies as recommended by the ICH guidelines Q2(R2) and Q14.

References

1. N.A. McGrath, M. Brichacek, J.T. Njardarson J. Chem. Ed. 87 (2010) 1348-1349; https://sites.arizona.edu/njardarson-lab/top200-posters, Top 200 Small Molecule Drugs by Sales in 2024.

KN-09

A new era for microsampling in pharmaceutical and biomedical analysis

Laura Mercolini

Alma Mater Studiorum - University of Bologna, Department of Pharmacy and Biotechnology (FaBiT), Research Group of Pharmaco-Toxicological Analysis (PTA Lab), Via Belmeloro 6, 40126 Bologna, Italy

Pharmaceutical and biomedical analysis is undergoing a profound transformation, driven by the demand for greener, faster, patient-centric and decentralised workflows that must meet the analytical rigour required across medicinal chemistry, drug discovery and development, clinical pharmacology, pharmacokinetic and pharmacodynamic (PK/PD) studies, forensic applications, anti-doping and the emerging field of exposomics. Microsampling sits at the heart of this transition, but the field has been decisively moving beyond first-generation strategies such as classical dried blood spots (DBS) for some time [1], and nowadays even beyond second-generation, volumetrically reliable technologies. A new generation of fully customisable, self-3D-printable devices is starting to arrive on the market, promising unparalleled use flexibility and adaptability to clinical and analytical needs. When correctly designed, these innovations can be implemented in most established microsampling formats (absorptive, capillary-based, microfluidic) reshaping what microsampling can achieve, and where it can be deployed, and opening unprecedented opportunities for minimally invasive or non-invasive sampling, home- and community-based collection and longitudinal monitoring. Moreover, microsampling is inherently highly compliant with the principles of green sample preparation (GSP) and green analytical chemistry (GAC), thanks to the use of minimal amounts of sample and solvents. At the heart of this new era is the recognition that sampling, sample preparation and instrumental analysis must be jointly developed, optimised and validated as a single, fit-for-purpose workflow. Miniaturised sample preparation, based on micro-scale extraction strategies, seamlessly integrates low-volume specimens with high-performance (U)HPLC-MS/MS and HRMS analysis, delivering feasible, greener workflows and robust clean-up results. This co-design approach has been deployed across a broad range of pharmaceutical and biomedical scenarios, from precision medicine patient follow-up to forensic and anti-doping frameworks, *in vitro* and *in vivo* drug development studies, drug metabolism and biomarker discovery [2]. Across these scenarios, the jointly optimised workflows have consistently delivered reliable, reproducible and transferable methods, suitable for both research-grade and routine use. Looking ahead, the new era of microsampling is being further accelerated by automation, which reduces operator dependence and supports structured, scalable sample handling from collection to injection [3], now enhanced by the possibility of tailoring microsampling device design to the specific automation platform. Combined with intrinsic greenness and environmental friendliness [4], these developments outline a clear trajectory: microsampling is a

foundational enabler of next-generation pharmaceutical and biomedical analysis, greener, leaner, more reliable, genuinely subject- and patient-centric and nowadays customisable to the user's needs.

References
1. M. Protti, et al. Adv. Sample Prep. (2025), 13, 100161.
2. X. Shen, et al. Nat. Biomed. Eng. (2024), 8, 11-29.
3. M. Protti, et al. Anal. Chim. Acta (2025), 1359, 344018.
4. W. Wojnowski, et al. TrAC Trends Anal. Chem. (2022), 149, 116553.

KN-10

Redefining the analytical toolbox for biopharmaceuticals

Koen Sandra, Liesa Verscheure, Evert Van Hoestenberghe, Jelle De Vos, Jasmin Schairer, Gianni Vandenborre, Esther Devloo, Mabelle Meersseman, Pat Sandra

RIC group, President Kennedypark 6, 8500 Kortrijk, Belgium

Monoclonal antibodies (mAbs) leverage the immune system's natural precision to target disease pathways with high specificity and have become a major force in the pharmaceutical industry. Their success has spurred the development of various next-generation formats such as antibody-drug conjugates (ADCs), antibody-oligonucleotide conjugates (AOCs), bispecific antibodies (bsAbs), antibody fragments, Fc fusion proteins, etc. With vast therapeutic potential comes an immense structural and functional intricacy highly demanding towards analytics.

This contribution will reflect on recent advances in hyphenated and multidimensional analytical techniques to unravel the structural and functional intricacies of mAbs and related molecules. Topics covered will include high performance affinity chromatography-mass spectrometry (HPAC-MS), affinity resolved size exclusion chromatography with multi-angle light scattering detection (AR-SEC-MALS), imaged capillary isoelectric focusing-mass spectrometry (icIEF-MS), and the combination of ever more separation modes in multidimensional set-ups - optionally incorporating chemical or enzymatic reactors - to streamline characterization.

KN-11

Advancing LC-MS metabolomics and lipidomics for personalized health

Dajana Vuckovic

Concordia University, Canada

Liquid chromatography–mass spectrometry (LC–MS) has become the leading analytical platform for metabolomics and lipidomics, owing to the exceptional sensitivity of modern mass spectrometers. Current challenges in this field include: (i) maintaining sample integrity and ensuring that the measured lipidome reflects the true lipidome at the time of sampling, (ii) improving detection of low-abundance analytes and enhancing method coverage, (iii) strengthening quality control practices to improve data reliability. The objective of this talk is to discuss how my laboratory has addressed these challenges. First, I will discuss how lipid stability in biofluids can be improved with the addition of orlistat, and illustrate how we have applied this approach in a longitudinal study investigating early development of atherosclerosis in ApoE-/- mice fed high protein diet. Next, I will discuss the use of in vivo solid-phase microextraction for longitudinal profiling of tissues and biofluids, including challenging analytes such as oxylipins. Our results show that oxylipin stability on SPME devices exceeds what can be achieved by dried blood spots or volumetric absorptive microsampling devices. Finally, I will briefly share insights from a recent international harmonization effort led by Metabolomics Quality Assurance & Quality Control consortium (mQACC) to establish unified minimum and best practices for untargeted metabolomics.

KN-12

Dried Blood Microsampling in Therapeutic Drug Monitoring and Toxicology

Christophe Stove

Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Belgium; christophe.stove@ugent.be

Dried blood microsampling, originating in the newborn screening field, and also currently routinely applied in preclinical (animal) studies in the pharmaceutical industry, is increasingly receiving attention in the field of clinical chemistry. Many dried blood spot (DBS)-based methods have been developed for a variety of applications, e.g. phenotyping, therapeutic drug monitoring (TDM), doping analysis or toxicology. This presentation will cover dried blood microsampling in both TDM and toxicology, with selected applications highlighting the potential (but also limitations) of the technology.

In TDM, a major drawback for implementing DBS in routine analyses -despite the many advantages- is the presence of a hematocrit (hct) bias. In the past years several dried blood sampling techniques distinct from conventional DBS collection on filter paper cards have been developed that allow volumetric collection of blood, hence analysis of the complete dried blood microsample overcomes the impact of spreading on DBS-based quantitation. The last years, also non-fingerstick microsampling devices have been developed, based on (micro)needle technologies. Additionally, dried plasma devices have occurred on the market with the aim to cope with blood-plasma conversion issues. Apart from new devices, also approaches to predict the hct from non-volumetrically collected DBS have been developed, such as reflectance spectroscopy and near infrared spectroscopy. Despite all these advances, and despite a multitude of applications demonstrating the feasibility of dried blood microsampling for TDM purposes, widespread implementation of dried blood microsampling is still lagging behind. This presentation will provide an overview of the distinct requirements for (further & wider) routine implementation of dried blood microsampling. Several important steps towards this eventual goal have already been taken, and these will be outlined using concrete examples, covering all different aspects, from sampling, via analysis, up to interpretation of the result. Facilitators as well as hurdles will be covered, and -importantly- how to (potentially) tackle the latter in order to allow microsampling to become part of the available panel of patient-centric tools,.

Also in toxicology, dried blood microsampling offers opportunities, as will be exemplified by the Lab's implementation of a dried blood microsample workflow to analyze the direct alcohol biomarker phosphatidylethanol 16:0/18:1 (PEth). This marker allows to conclude compatibility with abstinence, 'social' drinking or chronic and excessive alcohol

consumption. Unlike traditional biomarkers such as carbohydrate-deficient transferrin (CDT) and gamma-glutamyl transferase (GGT), PEth is highly specific to alcohol intake and can be detected in blood for up to four weeks, allowing a more accurate assessment of alcohol consumption. The lab's procedure involves the application of volumetric absorptive microsampling (VAMS), allowing the volumetric collection of a drop of blood following a fingerprick. Incorporating VAMS for PEth analysis offers the benefit of a convenient sample collection and sample transport. For over 5 years now, the Ghent University Laboratory of Toxicology has been using an ISO 17025-accredited procedure to determine PEth in VAMS samples. This presentation will deal with the workflow, the experience gained while analyzing many thousands of samples, and how PEth analysis is being used in the context of driving license regranting. Overall, the integration of VAMS-based PEth analysis has proven to provide an incentive to the individuals to change their drinking behaviour, and provides the authorities with a more reliable and efficient evaluation of an individual's fitness to drive.

KN-13

Speed, Depth, and Quality: Building an Integrated LCMS Toolbox for ASO and siRNA Analytics in Early Drug Discovery

Tomas Leek[1], Manasses Jora[1], Christian Manz[1], Kathrin Stavenhagen[2] and Werngard Czechtizky[1]

[1] BioPharma Chemistry, Discovery Sciences, BioPharmaceuticals R&D AstraZeneca, Sweden
[2] Assay, Profiling & Cell Sciences, Discovery Sciences, BioPharmaceuticals R&D AstraZeneca, Sweden

Early oligonucleotide drug discovery programs operate on two intertwined requirements. From a project perspective, teams must triage hundreds to thousands of sequences quickly and consistently to keep campaigns moving, while selectively deploying deeper, tailored analytics to answer project-specific questions (e.g., chemistry improvements, duplex integrity, conjugate stability, impurity identity). From an analytical perspective, labs must deliver standardized, automation-ready, high-throughput release testing that is robust across diverse program chemistries, yet provide the flexibility to develop methods that address specific project questions. A chemistry-integrated LC–MS toolbox that balances standardized throughput with targeted depth is therefore essential to align project needs for both rapid screening and in-depth characterization.

We have therefore developed and implemented a modular LC–MS platform spanning discovery chemistries. For batch release, two semi-automated UV×MS methods with integrated data processing and reporting have been established: (i) a high-throughput workflow for rapid sequence verification and purity checks suitable for in vitro screening libraries; and (ii) an in-depth characterization workflow for single-sample oligonucleotides and their conjugates intended for in vivo studies. To broaden sequence and chemistry coverage and minimize sequence-specific development, we systematically evaluated separation modes compatible with UV and MS detection on large panels of modified and conjugated single- and double-stranded oligonucleotides.

The high-throughput workflow supports fit-for-purpose UV×MS analysis of hundreds of samples per day across diverse chemistries, leveraging ion-pair–free RP–LC and size-exclusion chromatography (SEC), to improve spectral quality and enable integration with automated data processing and reporting. For in-depth characterization, we developed a flexible methodology to cover the broad chemical space of final oligonucleotides (e.g spanning single strands, duplexes, and conjugates) with a high degree of automation in spectral deconvolution and impurity annotation. [1]
For tailored depth, hydrophilic interaction chromatography (HILIC) revealed that diastereomer resolution correlates with higher-order structural rigidity[2]. Adding MS-compatible salt to ion-pair reversed phase IPRP eluents preserved duplex formation and delivered tunable separation of duplexes from single strands across multiple chemistries

and conjugates[3]. Together, the toolbox reduces sequence-specific optimization, maintains duplex integrity when required, and enables impurity annotation for quality understanding.

This integrated LCMS toolbox aligns project and analytical needs by uniting standardized, rapid release for crude libraries with tailored, structure-preserving separations for detailed characterization, and by coupling molecular analytics to protein-level outcomes through streamlined proteomics. Integrated data pipelines and automated batch processing support reporting across both release and depth modes. The result is accelerated, high-confidence decision-making from synthesis to assay testing, with minimized sequence-specific tuning and broad compatibility spanning modified backbones, conjugates, and duplex states.

References

1 Stavenhagen et al.. J Chromatogr. A 2026 1765, 466517. DOI: 10.1016/j.chroma.2025.466517
2 Lardeux et al.. Anal Chem. 2024 96 (24), 9994-10002. DOI 10.1021/acs.analchem.4c01384
3 Enmark et al.. Anal Chem. 2024 96 (47), 18590-18595. DOI: 10.1021/acs.analchem.4c05248

ABSTRACTS

ORAL PRESENTATIONS

OP-01

Unraveling Aβ42 aggregation mechanisms under chemical exposome influence using Taylor Dispersion Analysis (TDA)

Marta Garrido Alvarez , Joseph Chamieh and Hervé Cottet

IBMM UMR 5247, Pôle Chimie Balard Recherche,1919 route de Mende,34293 Montpellier

Alzheimer's disease (AD) is a major cause of death. It exists in two forms: early-onset AD (EOAD), caused by rare mutations in APP and presenilin genes, and the more common late-onset form (LOAD), which results from a complex interplay of aging, genetic predisposition, and environmental exposures [3] . AD is characterized by neurofibrillary tangles (composed of hyperphosphorylated tau) and amyloid plaques (formed by Aβ42 aggregates), which led to two main hypotheses: the tau hypothesis, centred on cytoskeletal dysfunction, and the amyloid cascade hypothesis, which proposes Aβ aggregation as the primary cause. Our study adopts the latter to investigate how components of the chemical exposome influence Aβ42 aggregation.

Aβ42 fibrillation follows a three-phase sigmoidal kinetic trajectory: a nucleation lag phase, rapid elongation, and a saturation plateau [4]. Conventional ThT-based assays predominantly detect late-stage fibrils[5]. While early soluble oligomers which are diffusible, believed to be neurotoxic [6] , and capable of spreading across brain regions, remain poorly characterized. To address this gap, we use Taylor Dispersion Analysis (TDA) to monitor early aggregation events, focusing on oligomer formation.

We investigate how selected components of the chemical exposome influence Aβ42 aggregation dynamics over time and at varying concentrations. Aggregation kinetics were tracked at multiple time points using TDA, a technique that measures diffusion coefficients (D) and hydrodynamic radii (Rh) by analyzing solute dispersion under Poiseuille laminar flow. This approach enables parallel analysis of control and exposed samples under identical experimental conditions, allowing direct comparison of species evolution. TDA captures a broad range of molecular sizes, from small oligomers to large aggregates, providing insights into aggregation pathways [7,8].

Our results show that perfluorooctanoic acid (PFOA) accelerates Aβ42 aggregation, leading to rapid monomer depletion and promoting the early formation of large, insoluble aggregates, which are detected as non-diffusive spikes in the taylorgrams. Meanwhile, 2-phenylphenol appears to stabilize soluble oligomeric species, resulting in their prolonged presence and a noticeable delay in the appearance of mature fibrils. In contrast, EGCG, a polyphenol known for its anti-aggregation properties [9] , not only slows down the

aggregation kinetics but also significantly reduces the formation of large aggregates, suggesting a potential protective effect at the molecular level.

These findings show the value of TDA in resolving early Aβ42 aggregation and reveal how contaminants can reshape its pathways, offering insight into the molecular origins of Alzheimer's disease.

References

1.Liu, S. H.; Weber, E. S.; Manz, K. E.; McCarthy, K. J.; Chen, Y.; Schüffler, P. J.; Zhu, C. W.; Tracy, M. Assessing the Impact and Cost Effectiveness of Exposome Interventions on Alzheimer's Disease: A Review of Agent-Based Modeling and Other Data Science 2

2.Methods for Causal Inference. Genes 2024, 15 (11), 1457. https://doi.org/10.3390/genes15111457.

3.Monaco, M.; Torazza, C.; Fedele, E.; Grilli, M. The Impact of the Exposome on Alzheimer's Disease: The Influence of Nutrition. Int. J. Mol. Sci. 2025, 26 (7), 3015. https://doi.org/10.3390/ijms26073015.

4.Ferrone, F. Analysis of Protein Aggregation Kinetics. Methods Enzymol. 1999, 309, 256–274. https://doi.org/10.1016/s0076-6879(99)09019-9.

5.Meisl, G.; Kirkegaard, J. B.; Arosio, P.; Michaels, T. C. T.; Vendruscolo, M.; Dobson, C. M.; Linse, S.; Knowles, T. P. J. Molecular Mechanisms of Protein Aggregation from Global Fitting of Kinetic Models. Nat. Protoc. 2016, 11 (2), 252–272. https://doi.org/10.1038/nprot.2016.010.

6.Ahmed, M.; Davis, J.; Aucoin, D.; Sato, T.; Ahuja, S.; Aimoto, S.; Elliott, J. I.; Van Nostrand, W. E.; Smith, S. O. Structural Conversion of Neurotoxic Amyloid-β(1–42) Oligomers to Fibrils. Nat. Struct. Mol. Biol. 2010, 17 (5), 561–567. https://doi.org/10.1038/nsmb.1799.

7.Deleanu, M.; Deschaume, O.; Cipelletti, L.; Hernandez, J.-F.; Bartic, C.; Cottet, H.; Chamieh, J. Taylor Dispersion Analysis and Atomic Force Microscopy Provide a Quantitative Insight into the Aggregation Kinetics of Aβ (1-40)/Aβ (1-42) Amyloid Peptide Mixtures. ACS Chem. Neurosci. 2022, 13 (6), 786–795. https://doi.org/10.1021/acschemneuro.1c00784.

8.Deleanu, M.; Hernandez, J.; Cottet, H.; Chamieh, J. Taylor Dispersion Analysis Discloses the Impairment of Aβ Peptide Aggregation by the Presence of a Fluorescent Tag. ELECTROPHORESIS 2022, 44. https://doi.org/10.1002/elps.202200192.

9.Acharya, A.; Stockmann, J.; Beyer, L.; Rudack, T.; Nabers, A.; Gumbart, J. C.; Gerwert, K.; Batista, V. S. The Effect of (−)-Epigallocatechin-3-Gallate on the Amyloid-β Secondary Structure. Biophys. J. 2020, 119 (2), 349–359. https://doi.org/10.1016/j.bpj.2020.05.033.

OP-02

Optimization of an In-House Fluorometric Assay for Human Recombinant Cathepsin E as an Alternative to Commercial Kits

Rosaria Spagnuolo, Viviana Mitarotonda, Maria Laura Bolognesi, Elisa Uliassi, Manuela Bartolini

Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy

Lysosomal cathepsins play key roles in the regulation of neuroinflammatory responses associated with Alzheimer's disease. Cathepsin E, highly expressed in microglia, promotes neuroinflammation and enhances neuronal amyloid-β production via inflammatory signaling pathways, linking microglial activation to amyloid pathology.[1] Dysregulation of cathepsin-mediated lysosomal pathways contributes to microglial activation and the amplification of neuroinflammatory processes that drive neurodegeneration in Alzheimer's disease.[2]

Early drug discovery enzymatic screening, including that for cathepsin E inhibitors, often relies on the use of commercial assay kits, which can significantly increase experimental costs and limit assay scalability, particularly in academic laboratories with restricted budgets.[3] In this context, the development of reliable in-house assays, therefore, represents an important strategy to reduce costs and improve experimental flexibility in early-stage academic drug discovery. Furthermore, commercial kits also present methodological limitations because the substrates and the buffer composition are typically proprietary and undisclosed, preventing the evaluation of potential assay interference or solubility and stability issues, especially when testing chemically unstable compounds.

Given these premises, this work aimed to develop and validate a cost-effective in-house assay for cathepsin E for compound screening in early drug discovery campaigns. To this aim, a human recombinant cathepsin E enzyme and a fluorogenic MCA-based substrate were employed. The assay buffer composition and pH were selected based on literature data and subsequently optimized to maximize enzymatic activity. Particular attention was devoted to evaluating the influence of organic cosolvents (DMSO or methanol) and surfactants, both in terms of their effect on enzymatic activity and their ability to improve the solubility and stability of the compounds under investigation. The enzymatic response obtained with the optimized assay was then compared with that provided by a commercial kit. Although different fluorogenic substrates were used, resulting in expected differences in fluorescence intensity, a comparable enzymatic behavior was observed. The method was validated by determining the IC_{50} of pepstatin A, a well-known inhibitor of aspartic proteases, including cathepsins. Finally, a set of newly synthesized derivatives[4] was screened in parallel using both the commercial kit and the newly developed in-solution assay, yielding consistent inhibitory trends across the two platforms. Overall, the proposed

method represents a cost-effective and flexible alternative to commercial kits for screening new inhibitors of Cathepsin E.

Acknowledgments: This research was supported by EU funding within the NextGenerationEU-MIUR PNRR Extended Partnership 12 (Project no. PE00000006, "A multiscale integrated approach to the study of the nervous system in health and disease" MNESYS – SPOKE7.

References

1. Sun, Y., Chen, X., Liu, Y., & Zhang, J. Frontiers in Molecular Neuroscience, (2022), 15, 839682.
2. Nixon, R. A. Neurobiology of Disease, (2017), 105, 306–318.
3. Macarrón, R., Banks, M. N., Bojanic, D., Burns, D. J., Cirovic, D. A., Garyantes, T., Green, D. V. S., Hertzberg, R. P., Janzen, W. P., Paslay, J. W., Schopfer, U., and Sittampalam, G. S. (2011). Nature Reviews Drug Discovery, (2011), 10, 188–195
4.Constantin G, Terrabuio E, Pietronigro E, Mitarotonda V, Bartolini M, Uliassi E, Bolognesi ML. Cathepsin e-directed inhibitors and uses thereof. Patent n: 102025000023248

OP-03

A targeted mass spectrometry approach (MRM) to resolve protein analysis recovery challenges in solid dosage forms of therapeutic proteins

V. Vercruysse[1], M. Aerts[1], O. Coussens[1], H. Vissenaekens[1], A. Baan[1], M. De Beer[1], J. Voorspoels[1], J. Vercruysse[1]

[1] Eurofins CDMO, Drug Product Unit, 9052 – Zwijnaarde, Belgium

Solidification of therapeutic proteins into tablet dosage forms presents substantial analytical challenges. More specifically, accurate protein quantification can be challenging following standard assay/purity (based on RP-LC) sample preparation, especially when a substantial fraction of the protein is entrapped within insoluble excipient matrices through strong interactions. For an in-house case of a protein-containing tablet, RP-LC assay and dissolution consistently indicated an apparent loss of measured protein. This loss was not due to degradation during tableting, but due to incomplete extraction during analytical sample preparation caused by strong interactions with the drug product (DP) matrix, which resulted in a remaining insoluble pellet. Here, enzymatic digestion and Multiple Reaction Monitoring (MRM) mass spectrometry was used as a highly specific strategy to identify and quantify the protein directly in this insoluble pellet. This strategy allowed the recovery measurement of the apparent lost protein amount.

The MRM workflow started with comprehensive peptide selection of tryptic digests of the protein. A curated panel of seven specific peptides representing the protein was selected based on criteria such as ionization efficiency, chromatographic performance, susceptibility to modifications, and MS/MS fragmentation quality. For quantification, washed tablet pellets from 5 mg tablets were subjected to a robust tryptic digestion. Calibration curves were prepared by digesting protein reference solutions ranging from 0.1-18 µg protein, covering the expected protein content per pellet (~12 µg).

Method performance was evaluated in terms of linearity, specificity, repeatability, and miscleavage behavior. All peptides showed adequate linearity at both precursor and fragment level. Specificity was confirmed, as neither excipients nor pellet wash fractions produced interfering signals.

On the level of tablet pellet analysis, the more hydrophobic peptides showed lower recoveries compared to the more hydrophilic peptides. A protein-spiked excipient mixture exhibited a similar underestimation bias for these hydrophobic peptides, indicating the presence of unwanted effects such as remaining interaction with excipients or digestion ingredients. As a result, four of the seven peptides were reliable for MRM based quantification of entrapped protein.

In contrast, on the level of protein reference, digests demonstrated recoveries between 90–110% for all selected peptides.

MRM analysis of four independent tablet pellet aliquots clearly identified the protein and enabled accurate quantification. Measured amounts of protein in the tablet pellets corresponded well with the theoretical expected amounts.

Overall, the MRM-based approach successfully confirmed the presence and quantity of the protein entrapped in the tablet pellets. While hydrophobic peptides may require further optimization, MRM proved to be a powerful orthogonal tool for resolving assay discrepancies arising from protein–excipient interactions in solid protein drug products. This approach can establish a robust analytical platform for release testing of therapeutic proteins formulated in solid dosage forms.

OP-04

Integrating EAD and CID Fragmentation for Comprehensive Molecule Characterisation

Haichuan Liu, Jingwen Ding[1], Zoe Zhang[1] and Heather Chassaing[2]

1 Sciex , USA
2 Sciex, France

Comprehensive characterization of biotherapeutic product quality attributes (PQAs) requires high MS sensitivity for PQA detection and the ability of MS/MS to identify and localize labile post-translational modifications (PTMs), perform disulfide bond mapping and differentiate amino acid isomers.

An enhanced peptide mapping workflow is demonstrated using CID and electron-activated dissociation (EAD) for sensitive detection and confident characterization of low-abundant PQAs in biotherapeutics. The two methods work together to provide complementary information and extensive molecular characterisation with a LCMS configuration.

The significant MS sensitivity gain offered by the novel ZenoTOF 8600 system with enhanced hardware components, enables routine analysis of PQAs below the trace level (<0.1%). This analytical advancement expands the capabilities of EAD for routine to advanced PQA characterization, facilitating biotherapeutic development and important decision-making throughout the pipeline.

OP-05

Bioanalytical variability and identification criteria performance of low level microbial peptides in human plasma and saliva

P. Naumovski[1,2], A. De Spiegeleer[1,2], B. De Spiegeleer[1,2], E. Wynendaele[1,2]

1 Drug Quality and Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium.
2 Translational Research in Immunosenescence, Gerontology and Geriatrics (TRIGG) group, Ghent University Hospital, 9000 Ghent, Belgium.

Interest in peptides as diagnostic and therapeutic compounds is rapidly increasing across multiple biomedical disciplines. Studying the interindividual differences in peptide levels (biological variability) is essential to better understand their role in health and disease. To accelerate translational progress, early exploratory studies should efficiently generate reliable, matrix-dependent data on identification criteria performance and bioanalytical variability. However, key bioanalytical performance parameters in peptide research are frequently underreported which negatively impacts data transparency and quality of conclusions[1]. Here, the bioanalytical variability of 15 quorum sensing peptides (QSPs) at low-nanomolar concentrations in the plasma and saliva of 10 individuals over a two-month period was evaluated using a targeted multiple-reaction monitoring (MRM) UPLC-MS/MS method. Left-censored data emerged at concentrations near the limit of detection (LoD), and were addressed using an in-house validated maximum likelihood (ML) estimation method. The bioanalytical variability in peptide levels with no left-censored data across the study period was comparable to that predicted by the Horwitz equation. Deviations from the Horwitz relationship are more pronounced as the peptide LoD and proportion of left-censored data increase. Additionally, the variability of the product ion (PI) ratios, often applied as an additional identification criterion, was modelled across the concentration range, allowing construction of more accurate prediction intervals. Overall, this work provides a comprehensive framework for estimating bioanalytical variability and identification criteria performance in early-stage exploratory studies.

References

1. P. Naumovski, B. De Spiegeleer, A. Wakjira, et al., "Role of Peptides in Skeletal Muscle Wasting: A Scoping Review", *J Cachexia Sarcopenia Muscle* 16, no. 6 (2025): e70109.

OP-06

Method Development and Validation of Anthocyanin Quantification in Blueberry and Bilberry Powder: Analysis of the Market Integrity of Anthocyanin-Based Food Supplements

Lieselotte Jaspers, Kenn Foubert, Mart Theunis, Tania Naessens, Lissa Leus, Emmy Tuenter, Filip Kiekens and Nina Hermans

Natural Products & Food Research and Analysis – Pharmaceutical Technology (NatuRAPT), University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium

A shift towards health-conscious living has increased the popularity of plant-based food supplements, including those derived from blueberries (Vaccinium corymbosum) and bilberries (Vaccinium myrtillus). This is because blueberries and bilberries are often referred to as 'health-care fruits' due to their rich content of phytochemicals, of which anthocyanins are the most prominent. [1,2] As anthocyanins are known for their antioxidant properties and numerous health benefits, anthocyanin-containing food supplements are already established on the market and continue to rise in popularity. [3] However, the high cost of blueberries and bilberries as raw material and the lack of regulation regarding food supplements raises concerns about product quality and possible adulteration. [4]

In this context, a spectrophotometric assay was developed, optimised and validated to quantify anthocyanins in blueberry and bilberry powder, ensuring accuracy, precision and linearity. This method is based on ultrasonication-assisted-solvent-extraction and involves a hydro-ethanolic extraction solvent (classified as GRAS), aligning with the principle of green chemistry.

In parallel, a semi-quantitative HPLC-DAD method involving anthocyanin fingerprinting was employed to assess authenticity/adulteration of the investigated products. This approach is based on the principle that each berry type or red fruit species exhibits a characteristic anthocyanin profile, characterised by semi-quantitative differences in the presence, concentration and ratio of individual anthocyanins. These distinct profiles make anthocyanins suitable chemical markers for species identification and for detection of authenticity/adulteration. [5,6]

These two methods were then combined and applied to investigate thirteen anthocyanin-containing food supplements available on the Belgian market in terms of content- and authenticity conformity according to their label claims. Major discrepancies arose regarding both the anthocyanin content and authenticity of the supplements. Concerning anthocyanin content, deviations from the label claim ranged from approximately 5% to 300% and the daily dose of anthocyanins administered by each product varied significantly

(0.12 to 156 mg anthocyanins/day). When investigating authenticity, only 54% of the products could be verified as authentic. These findings raise serious concerns about the integrity of anthocyanin-based food supplements and underscore the need for stricter quality control and regulatory action. Without proper oversight, adulterated or mislabelled products mislead consumers and may pose a health risk.

References

1.Egan et al. Food and Function (2011)
2, 12, 747–752 2.Xue et al. Antioxidants, (2023) 12, 1
3.Mohammed & Khan et al. International journal of molecular sciences, (2022), 23, 2149
4.Lee et al. Food Science and Nutrition, (2016) 4(5), 742–752
5.Petreska Stanoeva et al. European Food Research and Technology, (2024), 250(3), 751–762
6.Primetta et al. Food Control, (2013), 30(2), 662–667

Funding

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them (Grant Agreement No. 101225248).

OP-07

Innovative SEC Affinity Method Using Natural Extracts with MSbased Metabolomics Approaches to Reveal PPAR-Bound Natural Metabolites

S. Salerno, [1] F. Rinaldi, [1] O. Kirchhoffer, [2,3] J.L. Wolfender, [2,3] E. Calleri. [1]

1 University of Pavia, Department of Drug Sciences, Via Taramelli 12, 27100, Pavia, Italy
2 Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU, 1211 Geneva, Switzerland.
3 School of Pharmaceutical Sciences, University of Geneva, CMU, 1211 Geneva, Switzerland.

In this work, we present a robust and high-content analytical strategy for the identification, characterization and validation of natural metabolites with affinity for biologically relevant protein targets. The approach was initially developed to investigate ligands of Peroxisome Proliferator-Activated Receptor γ (PPARγ) using complex plant extracts and was subsequently expanded to multiple botanical sources and protein targets, demonstrating excellent reproducibility and versatility.

The workflow combines size exclusion chromatography (SEC) with high-resolution mass spectrometry (HRMS) and the method relies on the systematic comparison of the target-bound fraction with the depleted extract, defined as the extract remaining after removal of metabolites exhibiting affinity for the target protein (unbound fraction). By assessing the relative abundance of the same metabolite in both fractions, the binding equilibrium can be investigated, clarifying whether it is mainly present in the bound or free form.

Plant extracts were incubated with purified target proteins under physiological conditions and injected into a SEC column, enabling the separation of protein–ligand complexes from non-binding metabolites. Bound fractions were subjected to protein and salt removal, while depleted fractions were desalted prior to HRMS analysis.

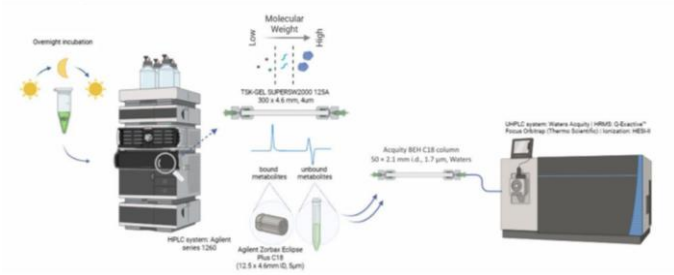

Figure 1. Analytical workflow of the instrumental setup used for the analysis

The strategy was first applied to Diospyros kaki leaf extract, where kaempferol, a known PPARγ ligand, exhibited a markedly higher signal in the bound fraction and a substantially lower signal in the unbound fraction, in line with a dynamic binding equilibrium and indicative of a strong affinity for PPARγ.

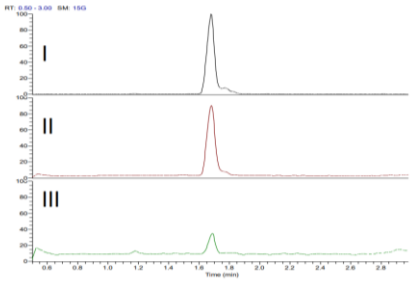

Figure 2. Comparison of the extracted ion chromatogram of kaempferol from Dyospiros Kaki extract in the crude extract (I), bound fraction (II) and unbound fraction (III).

HRMS data were processed using an integrated bioinformatic pipeline involving MZmine for feature detection and alignment, [1] GNPS for molecular networking, [2] SIRIUS for molecular formula annotation and structure prediction, [3] and Cytoscape for network visualization and comparative analysis. [4]

The method was then extended to a broader range of plant species, including Cananga latifolia, leading to the selection, isolation and purification of several bioactive compounds. Grating-coupled interferometry (GCI) experiments were performed to quantitatively characterize the binding affinity and determine the dissociation constants of the identified compounds toward PPARγ.

Furthermore, the applicability of the platform was demonstrated on a different and more challenging biological target, α-synuclein, a protein involved in neurodegenerative diseases due to its aggregation propensity. Despite experimental complexity related to protein aggregation, the approach successfully identified metabolites with anti-aggregating activity from Verbascum thapsus extract, including verbascoside isomers and saponins such as saponin class IV3 +2 hexoses +3 desoxyhexoses, saponin class IV3 +2 hexoses +1 desoxyhexose + 1 pentose and saponin class IV3 +2 hexoses +1 desoxyhexoses. [5]

Overall, this integrated SEC–HRMS and GCI-based strategy provides a reproducible, flexible, and information-rich platform for studying protein–metabolite interactions across diverse targets, significantly supporting bioactive compound discovery from complex natural matrices.

References
1.R. Schmid, S. Heuckeroth, A. Korf et al. Nat. Biotechnol. 2023, 41, 447–449.
2.M. Wang, J. Carver, V. Phelan et al. Nat. Biotechnol. 2016, 34, 828–837.
3.K. Dührkop, M. Fleischauer, M. Ludwig et al. Nat. Methods 2019, 16, 299–302.
4.P. Shannon, A. Markiel, O. Ozier et al. Genome Res. 2003, 13, 2498–2504.
5.A. Lambiase, G. Spandri, H. Moukham et al. Neurotherapeutics 2025, Dec 22, e00825.

OP-08

AS-MS-Based Approach for Tyrosinase Ligand Discovery

Quezia B. Cass[1], Thais Bertolino V. Dantas[2], Romário P. da Costa[2], Sandra Mota[3,4], Richele P. Severino[2], Marta Correia-da-Silva[5,6], Isabel Almeida[3,4] , Emília Sousa[5,6], Maria Elizabeth Tiritan[5,6]

1.Universidade Federal de São Carlos, Departamento de Química, 13565-905, São Carlos, SP, Brazil
2.Universidade Federal de Catalão, Instituto de Química, 75704-020, Catalão, GO, Brazil
3.UCIBIO—Unidade de Biociências Moleculares Aplicadas, Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade de Porto, 4050-313 Porto, Portugal
4.Laboratório Associado i4HB, Faculdade de Farmácia, Universidade do Porto, Portugal
5.CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, 4450-208, Matosinhos, Portugal
6.Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal

Tyrosinase, is an enzyme involved in several physiological processes in invertebrates, including immune responses, cuticle sclerotization, and wound healing. Tyrosinase is also an important target in cosmetology, particularly due to its role in melanocytes and melanoma cells[1]. To model an immobilization-based affinity selection–mass spectrometry (AS-MS) assay for tyrosinase, a synthetic library composed of two isobaric xanthones[2], butylresorcinol, four hydroxyphenyl esters (including two isomeric esters) was used. For the assay, tyrosinase was immobilized onto BcMag™ silica-based superparamagnetic particles coated with primary amine functional groups *via* a Schiff base reaction, using glutaraldehyde as a spacer. Control samples were prepared under identical conditions, with enzyme inactivation achieved by heating in water at 100 °C for 30 min. The desorbates were analyzed by LC-HRMS, and the affinity ratio (AR) was determined for each compound. The immobilization-based AS-MS assay for tyrosinase was also applied to the ethanolic extract of *Lippia origanoides* Kunth (Verbenaceae) and to ethanolic extracts from ripe fruits of *Byrsonima coccolobifolia* Kunth (Malpighiaceae), both from the Brazilian flora. The identified ligand metabolites from both extracts are currently being annotated. All these results will be presented and discussed in the oral presentation.

References.
1. D. Ram et.al. Mini-Reviews in Medicinal Chemistry, (2026) doi:10.2174/0113895575405163251027232908
2. J.R. Almeida et.al. Biomolecules (2020), 10, 1126; doi:10.3390/biom10081126

Acknowledgements
This research was funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., and by the European Commission's Recovery and Resilience Facility, within the scope of UID/04423/2025 (https://doi.org/10.54499/UID/04423/2025), UID/PRR/04423/2025 (https://doi.org/10.54499/UID/PRR/04423/2025), and LA/P/0101/2020

(https://doi.org/10.54499/LA/P/0101/2020) and by FCT/Mobility/1318494061/2024-25. The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Proc. Number 302464/2022-0 is also acknowledge.

OP-09

Immobilized Enzymes on Magnetic Beads: a Versatile Tool for Accelerated Drug Discovery in Alzheimer's Disease

Irene Castellino[1,2] , Rudolf Andrys[1] , Charline Monnier[1] , Manuela Bartolini[2] and Kamil Musilek[2]

1 Department of Chemistry, Faculty of Science, University of Hradec Kralove, 50011, Hradec Kralove, Czech Republic
2 Department of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, 40126, Bologna, Italy

The complex multifactorial nature of Alzheimer's Disease (AD) necessitates the development of novel treatments. Kinases, cholinesterases, and monoamine oxidases play key roles in the pathogenic hallmarks of AD, such as tau hyperphosphorylation, amyloid-beta aggregation, oxidative stress, and circadian clock dysregulation. While conventional in-solution biochemical assays are standard, they are frequently hindered by high enzyme consumption, lack of reusability, and their inherent instability over time. To address these challenges, this study investigates the immobilization of human recombinant enzymes utilizing functionalized magnetic beads (MBs). MBs allow rapid and easy separation using an external magnetic field, significantly streamlining the washing and recovery phases without the need for centrifugation or filtration.

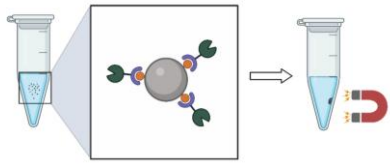

Figure 1. Representation of site-specific non-covalent enzyme immobilization on MBs.

In this study, six human recombinant enzymes, including two kinases (CK1δ, GSK3β), two cholinesterases (AChE, BChE), and two monoamine oxidases (MAO-A, MAO-B)[1] , were successfully immobilized on both porous and non-porous magnetic beads through site-specific non-covalent His-tag and GST-tag strategies. This approach ensures optimal orientation of the enzymes, preserving their structural and functional integrity. Comparative activity studies revealed that the catalytic activity of the immobilized enzymes remained consistent with their free counterparts. The immobilized enzymes retained good activity for over 20 days when stored at 4 °C and were successfully reused for 5 consecutive catalytic cycles. The reliability of the system was further validated through inhibition assays, where the IC50 values for standard inhibitors were in agreement

with those obtained for the corresponding free enzymes, confirming the robustness of the platform.

In conclusion, this versatile immobilization platform provides a powerful tool to overcome the limitations of conventional in-solution assays, offering a stable, reusable, and efficient method for studying multiple enzymes implicated in AD pathogenesis.

Acknowledgments: This project was supported by the European Union's programme under the Horizon Europe Marie Skłodowska-Curie Doctoral Network, grant agreement No 101072895 and the University of Hradec Králové (Specific research project PrF UHK 2026).

References

1. A. Andrys, Talanta. (2024), 276, 126263.

OP-10

Immobilized Animal Liver Microsomes: A Versatile Tool for Efficient Ester Hydrolysis in Chemo-Enzymatic Synthesis of Drug Candidate

Charline Monnier[1], Rudolf Andrys[1], Irene Castellino[1], Veronika Mickova[1], Annamaria Haleckova[1], Jan Loskot[1], Ondrej Benek[1], Lucie Zemanova[1]

1 University of Hradec Kralove, Department of chemistry, Hradecka 1227/4, 500 11 Hradec Kralove 3, Czech Republic

Enzymatic biocatalysis has become a cornerstone of modern pharmaceutical technology, valued for its high chemo-selectivity, specificity and alignment with green chemistry principles. However, enzymes limitations, like instability at high temperatures or in organic solvents and the logistical challenges of catalyst recovery, impede their wider applicability. Enzyme immobilization is a proven strategy to overcome these difficulties, transforming delicate biological catalysts into robust, reusable heterogeneous tools.[1] This study focuses on the innovative application of animal liver microsomes, a cost-effective complex source of membrane-bound hydrolases and cytochromes, for efficient ester hydrolysis in chemo-enzymatic synthesis within drug discovery.

In this work, microsomes derived from four animal species: house mouse (*Mus musculus*), wild boar (*Sus scrofa*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*) were covalently immobilized onto porous-magnetic microparticles.[2] This specific support was chosen to combine high surface area for enzyme loading and rapid magnetic separation. The resulting morphology was characterized using Scanning Electron Microscopy (SEM) to evaluate particle integrity and surface modification. To assess the functional benefits of immobilization, the catalytic activity of the microsomes for ester hydrolysis was monitored under various storage and working conditions via High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) and compared directly against free microsomes. Reusability of immobilized microsomes was also demonstrated.

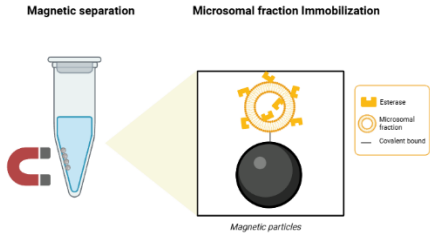

Figure 1. Non-oriented immobilization of microsomes on magnetic particles.

Our results demonstrate that immobilization significantly enhances the resilience of microsomal enzymes under diverse storage and operational conditions, including varied pH levels (5 – 9) and temperatures. Notably, immobilized fallow deer microsomes exhibited exceptional stability and maintained high catalytic efficiency across ten consecutive reaction cycles. This stability, coupled with a broad specificity towards xenobiotic substrates, allowed for the successful application of the biocatalyst in the chemo-enzymatic synthesis of a cyclophilin D inhibitor.[3] The process facilitated the preparation of this complex molecule with high purity (98%), validating the system's practical utility in drug discovery. By integrating magnetic separation to simplify downstream processing, this methodology provides a sustainable and economically viable alternative to traditional synthetic routes.

This research underscores the potential of immobilized animal liver microsomes as a sophisticated, reusable tool for efficient production of bioactive molecules.

This work was supported by the University of Hradec Kralove (SV UHK 2112/2025 and 21XX/2026), the Faculty of Pharmacy and the University of Hradec Kralove (BIPOLE project CZ.02.01.01/00/23_021/0008439), Horizon Europe Marie Skłodowska-Curie Doctoral Network grant agreement (No. 101072895) project.

References

1. P.Grunwald, ISBN: 978-981-4463-88-1 (2015).
2. Charline Monnier, Rudolf Andrys, Irene Castellino, Veronika Mickova, Annamaria Haleckova, Jan Loskot, Ondrej Benek, Lucie Zemanova, DOI: 10.1016/j.ymeth.2025.03.021 (2025)
3. Annamaria Haleckova, Katarina Jurkova, Jan Hurdalek, Katarina Chalupova, Veronika Syrova, Dominika Kozlov, Jana Svobodova, Rudolf Andrys, Oleksandr Kozlov, Charline Monnier, Rene Endlicher, Adam Skarka, Kamil Musilek, Lucie Zemanov, Ondrej Benek, DOI: 10.1016/j.bioorg.2025.108743 (2025)

OP-11

Prediction of Enantioseparations on Polysaccharide-Based Chiral Stationary Phases

D. Mangelings, P. De Gauquier, J. Peeters, F. Ameli, Y. Vander Heyden, K. Vanommeslaeghe

Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Laarbeeklaan 103, 1090 Brussels, Belgium

The separation of chiral drug molecules is an intensively studied domain in pharmaceutical research because enantiomers may have different pharmacological activities in the human body. While one enantiomer is therapeutically active, the other(s) can be ineffective, less active, show another activity or they even be toxic. For this reason, strict guidelines are imposed for the development of a chiral drug, strongly favoring enantiopure drugs. In all stages of drug development, analytical methods are needed that enable the separation of all enantiomers. Chiral separations are mostly performed using chromatography on chiral stationary phases, of which those with polysaccharide chiral selectors show the broadest enantioselectivity. However, of all selector types their enantiorecognition mechanisms are currently the least understood.

In this project, Quantitative Structure Enantioselective Retention Relationship (QSERR) models were created that allow distinguishing between the retentions of enantiomers and to gain insights in the recognition mechanisms on polysaccharide-based selectors in reversed-phase liquid chromatography.

Six polysaccharide-based chiral stationary phases and two mobile phases were used to analyze a test set of 48 structurally diverse chiral molecules. Retention times and elution sequences of the individual enantiomers were determined as responses. The retention or separation was modelled as a function of achiral or chiral descriptors by means of stepwise multiple linear regression (sMLR). Initially, the global retention of the molecules was modelled using only achiral descriptors. Next, chiral models were constructed using in-house defined conformation-dependent chiral descriptors that were calculated from molecular dynamics simulations. Chiral models were built to predict the enantioseparation and elution sequence. Finally, the results from achiral and chiral models were combined to predict the retention times of individual enantiomers.

The achiral models allowed the prediction of the global retention, while the chiral models showed a good predictive ability for the enantioselectivity, the separation and the elution sequence. The selected descriptors in the chiral models provided information on the chiral recognition mechanisms. Relatively good predictions were obtained on most chromatographic systems when the results of achiral and chiral models were combined. In summary, acceptable linear models were obtained to predict the chiral chromatographic separations of a chemically diverse set of pharmaceuticals.

OP-12

Analysis of Residual Moisture Content in Tablets with NIR-SRS and 3D-MRT

B. Van Geyt[1], A. De Man[2], T.Vandeputte[2], J.S. Uytterstprot[2], F. Vandenbroucke[3], Z. Kelemen[3], T. De Beer[1]

[1]Laboratory of Pharmaceutical Process Analytical Technology, Faculty of Pharmaceutical Sciences, 9000 Ghent
[2]UCB, Pharmaceutical Development Sciences, Av. de l'Industrie 1420, 1420 Braine-l'Alleud
[3]Pharma Technology, Rue Graham Bell 8, 1402 Nivelles

Residual moisture in pharmaceutical tablets can affect product stability, dissolution behaviour and the overall quality of tablets.(1) Conventional techniques for moisture analysis, such as Loss on Drying (LoD) and Karl Fischer (KF) titration are destructive in nature. Therefore, this study evaluated the performance of Near-Infrared Spatially Resolved Spectroscopy (NIR-SRS) and 3D Microwave Resonance Technology (3D-MRT) (2), two non-destructive techniques suitable for quantifying residual moisture in tablets.
A standard tablet formulation platform (UCB, Belgium) containing 40%$_{w/w}$ Active Pharmaceutical Ingredient (API) was used in this study. The base formulation, initially containing 2.5%$_{w/w}$ moisture, was supplemented with additional water (ranging from +1%$_{w/w}$ to +4%$_{w/w}$) using a high-shear mixer. Tablet compaction was performed using a compaction simulator. The resulting tablets were analysed with the CU-120 inspection unit (Pharma Technology, Belgium), a high-throughput instrument designed for 100% content uniformity and mass control. This inspection unit integrates a multipoint NIR-SRS probe for spatially resolved measurements and a 3D microwave resonator to determine the individual mass of tablets. Reference moisture content data was obtained using KF titration. A Partial Least Squares (PLS) regression model was developed from the NIR-SRS data, while multiple linear regression (MLR) was applied for the 3D-MRT data. For the latter, preprocessing of the microwave resonance data at three distinct frequencies enabled the estimation of moisture content.

NIR-SRS demonstrated sensitivity to moisture content, particularly in the spectral region around 1420 nm. A robust PLS model was developed with a Root Mean Squared Error of calibration (RMSEc) and prediction (RMSEp) of 0.06%$_{w/w}$ and 0.10%w/w, respectively. The 3D-MRT model performance appeared similar to its NIR-SRS variant (RMSEc = 0.10%$_{w/w}$, RMSEp = 0.13%$_{w/w}$).

Having demonstrated on average RMSEs around 0.1%$_{w/w}$, both NIR-SRS and 3D-MRT appeared suitable as PAT instruments for non-destructive and real-time analysis of tablet moisture content. Since both PAT techniques are integrated into a single inspection unit, the added value of data-fusion to model performance will be investigated in future research. The implementation of these techniques could eventually support quality control,

facilitate continuous manufacturing and inform formulation and packaging strategies for moisture-sensitive products.(1)

References

1. Lad S, Narkhede S, Luhar S, Prajapati A. EPRA International Journal of Research and Development (IJRD) REVIEW ON MOISTURE CONTENT: A STABILITY PROBLEM IN PHARMACEUTICALS. Available from: https://doi.org/10.36713/epra2016
2. Brouckaert D, Vandenbroucke F, Chauchard F, Dollinger M, Roggo Y, Pellegatti L, et al. Towards real-time release of pharmaceutical tablets: 100% in-line control via near-infrared spatially resolved spectroscopy and 3D microwave resonance technology. J Pharm Biomed Anal. 2022 Feb 5;209.

OP-13

Quantification and characterisation of monoclonal antibodies by the coupling of imaged capillary isoelectric focusing and chemometrics.

V. Sorret[1], A. Rayyad[1], S. Tfaili[2], D. Jouan-Rimbaud Bouveresse[3], L. Talbot[4], Y. Surrocca[4], M. Taverna[1] and C.Smadja[1].

1. Institut Galien Paris Saclay, Université Paris-Saclay, CNRS UMR 8612, Microscale Analytical Science for Therapy and Diagnostics, 17 Avenue des Sciences, 91300 Orsay, France
2. Université Paris-Saclay, Stratégies d'investigations analytiques: Médicaments, bioMatériaux, tissus et Matrices biologiques, 91400, Orsay, France.
3. AgroParisTech - INRAE - Campus Agro Paris-Saclay, 22 place de l'Agronomie, 91120, Palaiseau, France
4. Bio-Techne France, 19 Rue Louis Delourmel, 35230 Noyal-Châtillon-sur-Seiche, France

Monoclonal antibodies (mAbs) are large macromolecules used in the pharmaceutical industry as a therapeutic response against cancer, autoimmune or infectious diseases. They are widely employed for their ability to select a biological target. mAbs are highly complex macromolecules and represent a high immunological risk due to degradation and aggregation issues that may occur during the compounding process and manipulations at the hospital. Compounded mAbs at hospitals are typically submitted to visual controls only [1]. However, a thorough characterization process assessing their identity, dose and structural integrity before their administration to patients is mandatory to prevent mAbs adverse effect or administration mistake. Therefore, our goal is to propose a fast and straightforward method to comprehensively control compounded mAbs. For this purpose, we have developed an approach based on imaged capillary isoelectric focusing (icIEF) combined with chemometrics analysis. These methods have already been successfully employed to discriminate mAbs [2]. First, a generic method based on icIEF has been developed to analyze native mAbs, followed by a specific preprocessing method, then a Partial Least-Squares (PLS) [3] regression model has been created to correlate the concentration of the analysed mAb to its electropherograms. This strategy has been applied for three mAbs. Infliximab, Atezolizumab and Adalimumab at different concentrations and calibration models have been developed for these three mAbs, after signal pre-processing by an adapted Matlab® code. Characteristic peaks have been extracted from the electropherograms (mAbs icIEF profiles), and a model that predicts the concentration of a randomly chosen mAb sample with high accuracy has been created. UV-exposed mAbs have also been submitted to this approach, and the results obtained showed that we are able to identify mAbs and determine their concentration with high accuracy while discriminating between a native and a degraded mAb from a single analysis.

This new approach provides a promising strategy to control the therapeutic mAbs routinely used in hospitals.

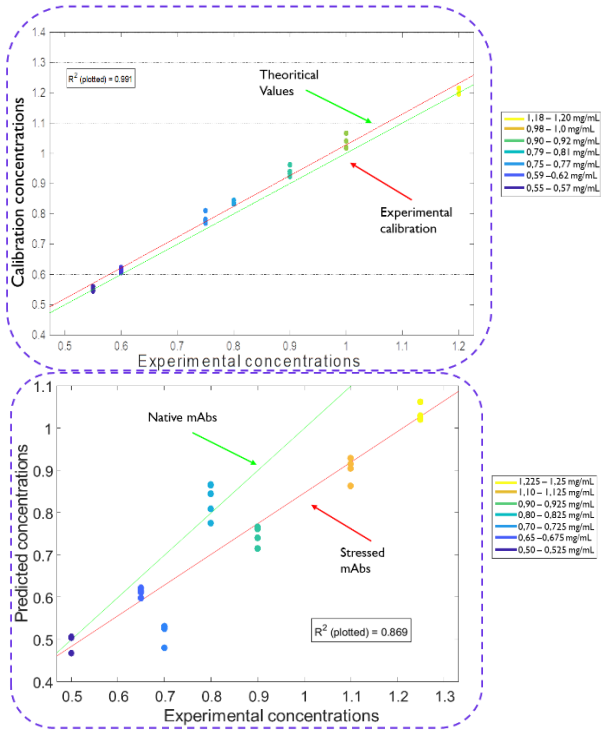

Figure 1a and 1b : Regression models of native (1a) and stressed (1b) samples.

References

1. Rana Fayek, BSc, Mostafa Soleyman, PharmD, Wim Jiskoot, PharmD, PhD, Mirjam Crul, PharmD, PhD, "Evaluation of post-production handling practices of monoclonal antibodies throughout the world", European Journal of ONCOLOGY PHARMACY, 2021

2. Cécile Tardif, Emmanuel Jaccoulet, Jean-François Bellec, Yannick Surroca, Laurence Talbot, Myriam Taverna, Claire Smadja, " Imaged capillary isoelectric focusing associated with multivariate analysis: a powerful tool for quality control of therapeutic monoclonal antibodies", Talanta, 2023

3. P. Geladi, B.R. Kowalski, Partial least squares regression: a tutorial, Analytica Chimica Acta, 185 (1986), 1-17

OP-14

In Situ Analysis of Drug–Target Interactions

Xiaofei Chen

Naval Medical University, Department of Pharmacy, 2002433, Shanghai, China

Characterization and analysis of drug–target interactions are crucial for innovative drug development, pharmacodynamic evaluation, and the elucidation of pharmacological mechanisms. However, because of the complexity of the physiological microenvironment and the conformational diversity of target molecules, drug–target interactions observed in vitro differ significantly from their actual interaction states in vivo. Therefore, how to achieve in situ and dynamic analysis of drug–target interactions under native or real conditions has become a key issue that urgently needs to be addressed in this research field. We integrate and advancing methodologies from multiple disciplines, including biochromatography, synthetic biology, biomimetic materials, molecular probes, and super-resolution imaging to solve these problems. At both the molecular target level and the subcellular level, the authentic states of drug–target interactions were restored in situ. On this basis, a new technology for precise targeted drug screening, namely receptor in situ synthesized biochromatography[1-3], was established, and a new in situ strategy for drug target identification, namely subcellular dominant target-region localization[4], was developed. These approaches were applied in research practice at two levels, namely target-based drug discovery and drug-based target identification, through which multiple active lead compounds and potential new targets were screened and identified[5]. This work has significantly improved the efficiency of new drug development and provides a reproducible research paradigm for the precise screening of targeted drugs and the efficient identification of drug targets.

References

1. Huifen Wu, Yanqiu Gu, Yanting Li, Shuya Jiang, Wei Dai, Haoran Wang, Kunmiao Feng, Dandan Guo, Dan Jia, Fei Cai, Yue Liu*, Keyun Zeng, Weilan Lu, Yuanyuan Dai, Wenyu Liu, Qixin Chen, Ting Han*, Xiaofei Chen*, *Adv. Funct. Mater.* 2026, e30995 .

2. Y. Li, Y. Gu, W. Zhang, T. Li, C. Chen, C. Wang, Y. Chai, X. Ma, Xiaofei Chen*, *J. Pharm. Anal.* 2026, 16(2): 101259

3. Wang C, Gu Y, Chen C, Li Y, Li L, Chai Y, Jiang Z*, Xiaofei Chen*, Yuan Y*. *Anal. Chem.* 2024, 96(28):11479-11487.

4. Fang G, Liu D, Bai Q, Shao X, Liu Z, Yao Q, Yuan Y, Zeng K, Chen X, Xiaofei Chen*, Q. Chen*. *Adv. Mater.* 2025, 37 (29): e2503220.

5. Yanqiu Gu; Fang Fan; Yue Liu; Yifeng Chai; Yongfang Yuan*; Xiaofei Chen*; *Trends Analyt. Chem.*, 2023, 161, 117015

OP-15

Extending Stability Testing of Drug Substances to Nitrosamine Formation in Online Design.

M.K. Parr[1], F. Bredendiek[1,2] and S. Schmidtsdorff[3]

1 Freie Universität Berlin, Institute of Pharmacy, 14195 Berlin, Germany
2 Freie Universität Berlin, Core Facility BioSupraMol, 14195 Berlin, Germany
3 German Federal Institute for Risk Assessment, Department Risk Communication, GLP Federal Bureau, Berlin, Germany

Following the discovery of nitrosamines as impurities in drug substances (APIs) and drug products, companies are requested by regulatory authorities to perform risk assessments regarding the formation of nitrosamines.[1] This includes small nitrosamines such as those monitored by procedures of the pharmacopoeias, but also the formation of nitrosamine drug substance-related impurities (NDSRI). For the latter, we propose to perform a nitrosation assay[2] as part of the stability testing procedures in addition to the classically required tests.[2] A fast and easy procedure is suggested that can be performed at the micro scale as an online procedure in the auto sampler of a HPLC system. This approach also enables fast and easy evaluation of the formation kinetics. Coupling to a QTOF mass spectrometer even allowed for recording of product ion spectra. The application of this online assay was successfully demonstrated using various drug substances. This assay may easily be transferred to other APIs as well as their related impurities, thus, allowing for an integration in the risk assessment process. Furthermore, the procedure may be used in an upscaled design after initial observations of the stability. This will also allow for collection of the relevant reference compounds for later use in quantitation of the NDSRI in case of observed probability of formation. Such data are essential for proper evaluation of potential risk mitigation strategies. Additionally, the generated NDSRIs may be used for further structure confirmation (e.g. using NMR) as well as for toxicity testing.

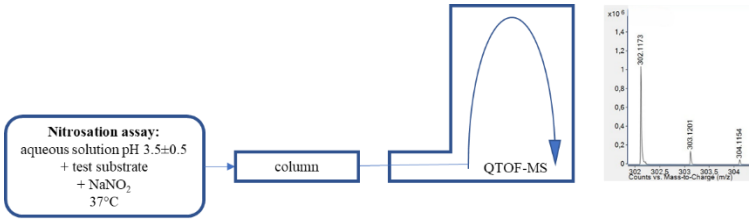

Figure 1. Design of the online nitrosation assay.

References
1. European Medicines Agency EMA/425645/2020, https://www.ema.europa.eu/en/documents/referral/europeanmedicines-regulatory-network-approach-implementation-chmpopinion-pursuant-article-53/2004-nitrosamine-impurities-humanmedicines_en.pdf. (2021), accessed 2026/02/09.
2. S. Schmidtsdorff, J. Neumann, A.H. Schmidt, M.K. Parr Arch. Pharm. (2022), 355, e2100435.
3. ICH Q1A (2003) and Q3A (2006) guidelines, Geneva, Switzerland, https://www.ich.org/page/quality-guidelines. acessed 2026/02/09.

OP-16

Practical, Low Cost Capillary Electrophoresis Solutions for Resource Limited Laboratories

H. Gebretsadik Kidanemariam[1,2], E. Adams[1] , A. Van Schepdael[1]

1 KU Leuven - University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, Herestraat 49, O&N2, PB 923, 3000 Leuven, Belgium
2 Mekelle University, College of Health Sciences, School of Pharmacy, Department of Pharmaceutical Analysis and Quality Assurance, P.O. Box: 1871, Mekelle, Ethiopia
Email address of presenting author: hailekiros.kidanemariam@kuleuven.be

Ensuring the quality of essential medicines remains a major challenge in many low- and middle-income countries. According to a WHO report, approximately one in ten medical products in these settings is substandard or falsified. [1] This is attributed to the limited capacity of regulatory agencies, resulting from shortages of trained personnel, inadequate resources, and the lack of affordable analytical methods. Capillary electrophoresis (CE), with its low reagent consumption, minimal instrumentation requirements, and compatibility with green analytical chemistry principles, offers a promising alternative to conventional chromatographic methods. In this work, affordable CE methods are presented as practical tools for assessing the quality of commonly used medicines in resource-limited settings.

The first study established a robust capillary zone electrophoresis method for insulin analysis. A multilayer capillary coated with polybrene and poly(sodium 4-styrenesulfonate) was used to enhance analytical repeatability by minimizing insulin adsorption to the capillary wall. The method was optimized by systematically evaluating running buffer composition, pH, ionic strength, and voltage, achieving optimal separation with a 60 mM phosphate buffer at pH 8.0. Validation of the method demonstrated excellent precision, with migration time and intra-day peak area RSDs below 2% and inter-day RSDs below 3%. Analysis of commercial insulin products confirmed compliance with pharmacopoeial specifications, indicating that this method provides a reliable and accessible tool for insulin quality control.

The second study focused on developing an affordable micellar electrokinetic chromatography (MEKC) method for analyzing six antiepileptic drugs: ethosuximide, phenobarbital, lamotrigine, phenytoin, carbamazepine, and diazepam. Optimization of surfactant concentration, buffer composition, organic modifier, and pH resulted in a running buffer consisting of 80 mM sodium dodecyl sulfate, 5% methanol, 2.5 mM phosphate, and 10 mM borate at pH 8.7. Under these conditions, all analytes showed well-resolved and symmetrical peaks. Robustness testing showed that minor variations in electrophoretic conditions did not significantly affect the resolution of the lamotrigine–phenytoin and carbamazepine–diazepam pairs, which consistently remained above 1.5. The

method was successfully applied to analyse five antiepileptic tablet formulations obtained from the Ethiopian market, demonstrating its suitability as a rapid and cost-effective screening tool.

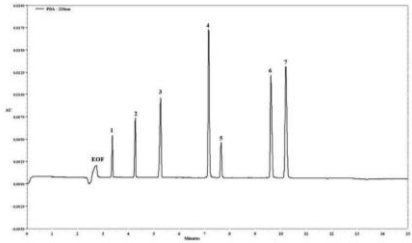

Figure 1. Representative electropherograms under the optimized conditions. Peak identification: (1) ethosuximide, (2) phenobarbital, (3) salicylic acid (internal standard), (4) lamotrigine, (5) phenytoin, (6) carbamazepine, (7) diazepam.

The third study explored an MEKC-UV method for rapid screening of five antimalarial drugs: chloroquine, quinine, mefloquine, primaquine, and doxycycline. Initial SDS-MEKC experiments resulted in long migration times and broad peaks due to strong ionic interactions between the basic analytes and SDS micelles. Adjustments to pH, SDS concentration, and organic modifiers provided limited improvement. The inclusion of the non-ionic surfactants Brij 35 resulted in faster and better-resolved separations. The optimized dual SDS-Brij-35 surfactant system with phosphate buffer achieved rapid separation of all five drugs. Given the widespread circulation of counterfeit antimalarials in low- and middle-income countries, this method provides a valuable analytical tool for reliable quality screening.

In conclusion, these studies demonstrate that CE-based methods can provide practical, affordable, and reliable analytical solutions for pharmaceutical quality control in resource-limited environments.

References

1. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/substandardand-falsified-medical-products (Accessed on Dec 2, 2025).

OP-17

Pharmacometabolomics reveals notable differences between real-world and expected drug metabolism patterns in humans

F. Klont[1,2]

1 Unit of PharmacoTherapy, Epidemiology and Economics, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands
2 Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands

Drug potency depends not only on the intrinsic activity of the administered substance but also on the body's ability to transform it into (in)active metabolites and to efficiently excrete the drug and its metabolites. Knowledge of such metabolism and excretion patterns is typically derived from early-phase clinical trials conducted in young, healthy males receiving single doses. These findings likely have limited generalizability to the intended drug users, who represent a considerably more heterogeneous group. This suggests a need for larger-scale drug metabolism and excretion studies in real-world patient populations, where we aim to confirm expected metabolites and (potentially) identify previously unreported ones.

To address this need, we established an untargeted pharmacometabolomics workflow, combining reversed-phase liquid chromatography and 'SWATH' data-independent acquisition mass spectrometry. Our method was applied to 24-hour urine samples of 1,421 kidney transplant recipients and 283 (potential) living kidney donors from the TransplantLines studies (NCT03272841, NCT02811835).[1,2] Data analysis was subsequently performed to assess the alignment of observed metabolite profiles with established consensus metabolic patterns of various immunosuppressants (*e.g.,* azathioprine, mycophenolate mofetil)[1,2,3], cardiovascular drugs (*e.g.,* clopidogrel, enalapril, furosemide, hydrochlorothiazide, metoprolol, perindopril)[4,5,6], and antibiotics (*e.g.,* sulfamethoxazole, trimethoprim)[7].

The real-world metabolic patterns obtained frequently diverged from consensus expectations, generally with more metabolites identified than expected.[1-7] For example, for metoprolol, 9 metabolites were anticipated, 7 were confirmed, and 10 previously unreported metabolites were discovered.[4] We occasionally failed to confirm expected metabolites, mostly intermediate metabolites for which downstream variants were detected. Interestingly, several studied drugs are prodrugs requiring activation by carboxylesterases, which are assumed to enable rapid and full conversions. Our findings, however, contradict this assumption, as the prodrugs themselves and their glucuronide counterparts were detected in most users, suggesting impaired prodrug activation. To

illustrate, we recently identified substantial and highly variable urinary presences of the prodrug mycophenolate mofetil and its previously unreported glucuronide.[3] Pharmacoepidemiologic analyses furthermore revealed a positive association with increasing drug dose and kidney function, as well as with the pharmaceutical excipient Kolliphor® EL, which is, for example, present in cyclosporine A capsules.[8]

In conclusion, our work offers new insights into the metabolic fate of various drugs in kidney transplant recipients and (potential) living kidney donors. Thereby, it confirms the limited generalizability of clinical trial-derived metabolic patterns. Moreover, it underscores the need for real-world drug metabolism and excretion studies that could contribute to improved drug therapies, for which untargeted pharmacometabolomics holds significant potential. Lastly, these findings are also relevant for environmental research, notably because incomplete knowledge of human drug metabolism and excretion may lead to underestimation of drug residues entering aquatic environments, as assessed through conventional (targeted) biomonitoring efforts.

References

1. F. Klont, S. Stepanović, D. Kremer, R. Bonner, D.J. Touw, E. Hak, S.J.L. Bakker, G. Hopfgartner. Food Chem Toxicol (2022), 165, 113188.
2. F.B. Nijdam, M.A.J. Hof, H. Blokzijl, S.J.L. Bakker, E. Hak, G. Hopfgartner, F. Klont. Metabolites (2025), 15, 39.
3. F. Klont, P. Sosnowski, D. Kremer, T.J. Knobbe, R. Bonner, H. Blokzijl, R.K. Weersma, S.J.L. Bakker, E. Hak, D.J. Touw, G. Hopfgartner. Metabolites (2022), 12, 942.
4. W.A. Heddema, M.A.J. Hof, P. Sosnowski, S.J.L. Bakker, G. Hopfgartner, F. Klont. Clin Pharmacokinet (2025), 64, 779-789.
5. C. Piccolotto, S.J. L. Bakker, V.E. de Meijer, G. Hopfgartner, P. Fodran, F. Klont. *Submitted*.
6. S. Han, M.A.J. Hof , S.J. L. Bakker, G. Hopfgartner, E. Hak, F. Klont. *Submitted*.
7. M.A.J. Hof & H. de Haan & S. Stepanović, S.J.L. Bakker, E. Hak, G. Hopfgartner, F. Klont. Metabolites (2025), 15, 473.
8. F.B. Nijdam, M.A.J. Hof, D. Kremer, T.J. Knobbe, G. Hopfgartner, S.J.L. Bakker, E. Hak, F. Klont. Eur J Drug Metab Pharmacokinet (2025), 50, 341-352.

OP-18

Alternative Surrogate Calibrants for Extended Internal Calibration in LC-MS/MS Quantitative Analysis

Oriane Strassel[1,2], Julien Boccard[1,2], Serge Rudaz[1,2]

1 School of Pharmaceutical Sciences, University of Geneva, CMU – Rue Michel-Servet 1, Geneva, Switzerland
2 Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU – Rue Michel-Servet 1, Geneva, Switzerland

Absolute quantification is essential in biomarker assessment for characterizing metabolite concentrations as indicators of physiological states or diseases, with applications in clinical research and diagnostics. Multi-targeted internal calibration[1] (IC) using stable isotope-labelled standards (SILs) as calibrants has recently emerged as an attractive approach for endogenous analyte quantification, avoiding the need for blank matrix assessment. However, commercial SIL availability is limited, costs are high, and purity can be restricted. Heterologous internal calibration (^{H}IC), employing alternative SILs as surrogate calibrants for multiple analytes, addresses these limitations through response factor determination between target metabolites and selected SILs.

A first study applied ^{H}IC to plasma samples from a chronic kidney disease clinical study, quantifying 18 endogenous metabolites by LC-MS/MS, primarily from the tryptophan pathway. Response factors between non-matching SIL-analyte pairs were established using standard addition approach in a pool of the sample to analyze. A comprehensive evaluation of various SIL-analyte combinations demonstrated that a reduced panel of five heterologous SILs could effectively quantify all 18 analytes with trueness and precision comparable to matched homologous SILs. Validation results showed trueness generally within 70–130% and precision below 10% for most analytes. Application to another small independent cohort confirmed the practical utility of this approach.

This methodology was further improved in the present work to enable the quantification of an extended steroid panel. Here, we have incorporated a post-column infusion correction for sample-specific matrix effects[2]. This technique involved continuous post-column infusion of a solution containing standards for real-time signal normalization. A key advantage of this approach is that response factors are determined in clean solvent, while matrix effects are corrected individually for each sample. The correction is applied point-by-point across the chromatogram: each data point of the analyte peak is normalized by the corresponding signal of the continuously infused standard at that retention time. The corrected data points are then used to reconstruct the chromatographic peak, and the resulting corrected peak area is used for quantification.

In the context of steroid analysis, the use of exogenous internal calibrants, structurally similar non-endogenous compounds, as surrogate standards were investigated. This strategy showed potential to address limited SIL availability while maintaining quantitative performance, with post-column correction appearing valuable for compounds exhibiting matrix effects.

References

1. Visconti, G. *et al.* Multitargeted Internal Calibration for the Quantification of Chronic Kidney Disease-Related Endogenous Metabolites Using Liquid Chromatography–Mass Spectrometry. *Anal. Chem.* **95**, 13546–13554 (2023).
2. Zhu, M. *et al.* Absolute quantification of polar metabolites using matrix-free calibration curves and post-column infusion of standards in HILIC-MS based metabolomics. *J. Chromatogr. A* 466507 (2025) doi:10.1016/j.chroma.2025.466507.

OP-19

Isomer Selective Metabolomics and Lipidomics

M. Lämmerhofer, A. Brun, M. Su and P. Seyfried

University of Tübingen, Institute of Pharmaceutical Sciences, 72076 – Tübingen, Germany

Lipidomics and metabolomics are analytical approaches widely adopted in biomedical research with the aim to obtain mechanistic insights in biochemical processes, to find diagnostic and prognostic biomarkers, and generate new hypothesis about biological processes or validate concepts from other experimentation. Although common untargeted methodologies allow simultaneous analysis of hundreds of metabolites, they often fall short in full coverage of pathways in which isomeric species play a major role, e.g. in oxylipidomics, the phosphoinositide network, glycolysis and pentosephosphate pathways. Nowadays, multiple tools have become available which can support the confident structural annotations of isomeric metabolites such as data-independent acquisition mass spectrometry, ion-mobility mass spectrometry, multimodal fragmentation with electron-activated dissociation complementing collisional induced dissociation, and double-bond derivatization. Additionally, chiral chromatography may be beneficial for constitutional isomers and is required for enantiomer separations. This presentation will focus on challenging lipid and metabolite classes in targeted and untargeted lipidomics and metabolomics, respectively, for which biological interpretations rely on a separation of their structural isomers.[1,2]

In the first part, the structural complexity of the oxylipidome with its numerous isomers will be elucidated. For untargeted analysis the structural annotation of the oxidized lipids requires a powerful library with isomer-characteristic fragments. Standards are only available for a few selected oxidized lipids. Hence, spectra must be generated in silico. This presentation will show the power of data-independent SWATH acquisition in combination with in silico libraries for the structural annotation of the oxylipidome in untargeted analysis of clinical samples such as from platelets of coronary artery disease patients where oxidative stress is observed.

In the second part, the focus will be on the phosphoinositide pathway. Phosphoinositides are regulating key metabolic processes including signal transduction. Arising from dynamic phosphorylation/dephosphorylation on the inositol ring of phosphatidylinositols (PI), phosphoinositides (PIPx) comprise seven classes according to phosphate position and number. Combined with various fatty acid side chains high structural complexity arises. For a comprehensive understanding of the phosphoinositide signaling, analytical coverage of the entire metabolic network is necessary. Here, we describe an integrated workflow for

these metabolites using a single sample aliquot. The application of PIPs profiling to clinical platelet samples will be shown.

In the third part, the challenges of an integrated workflow covering the entire glycolysis and pentosephosphate pathways with their numerous phosphorylated carbohydrate structural isomers will be discussed. In spite of new HILIC columns with deactivated hardware surface, HILIC separations of the entire sugar phosphate isomer sets remained troublesome. All metabolites of the glycolysis and pentose phosphate pathway are covered with good peak shapes and isomer selectivity and a simple solution to the reported repeatability problem of HILIC will be presented

References

1. P. Li, M. Lämmerhofer Anal. Chem. (221), 93, 9583.
2. K. Serafimov, M. Lämmerhofer J. Appl. Crystallogr. (2024), 96, 17271.

OP-20

Cell surface proteomics of 3D cell culture models: optimization, comparison, and critical evaluation of different enrichment strategies

A. Laurent[1] , V. Nerinckx[2], and M. Fillet [1]

1 Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, 4000 Liège, Belgium
2 GIGA Neurosciences, Department of Biomedical and Preclinic Sciences, GIGA, University of Liege, 400, Liège, Belgium

Cell surface proteins (CSPs) represent invaluable resources in biomedical research. Located at the interface between the cell and its environment, they orchestrate numerous physio-pathological mechanisms and constitute prime candidates for therapeutic targets and diagnostic development. Yet, obtaining a comprehensive and unbiased characterization of the surfacome remains challenging. Recent analytical innovations have substantially improved our capacity to explore the surfacome using targeted strategies. Nevertheless, some of the most common strategies nowadays for untargeted CSP enrichment and high-resolution mass spectrometry (HR-MS) analysis are biotinylation-based affinity purification and plasma membrane fractionation [1]. However, the growing diversity of chemistries and workflows has also highlighted a major finding: different extraction and labelling strategies yield different protein identification profiles, and no strategy is able to give a comprehensive one-shot picture of the surfacome. Selecting the most suitable approach is therefore critical to develop a time- and cost-effective highthroughput workflow able to be scaled-up to larger sample numbers and sources and offer the most comprehensive surfacome characterization possible.

This study is part of a broader project aiming to decipher the surfacome of glioblastoma (GBM), the most widespread brain tumor in adults, with an annual incidence of over 3 per 100,000 individuals and a median age at diagnosis of 65 years. GBM's median survival is only a few months, and its resistance to current state-of-the-art care treatments underscores the urgent need for new therapeutic strategies and therapeutic targets identification [2].

In this context, the best strategy for the extraction of CSP from GBM stem-like cell (GSC) threedimensional culture models was evaluated by comparing different biotinylation-based enrichment strategies targeting glycans on glycoproteins, primary amine groups on lysins, and N-terminal moieties, or carboxyl groups on aspartic acid, glutamic acid, and C-terminal moieties. Following biotin-based enrichment, the protein profiles obtained from these strategies were compared with those derived from membrane precipitation workflows and whole-cell lysates, allowing us to evaluate differences in proteome

coverage in terms of nature and number of identifications. This cross-comparison revealed differences in the extracted protein repertoires, highlighting the method-dependence of CSP identification and the risk of drawing incomplete conclusions when relying on a single enrichment approach.

Overall, this study allowed us to select the most effective strategy for CSP extraction from GSCs and demonstrates the importance of tailoring analytical workflows to specific clinical contexts and research objectives. Beyond its immediate application to GBM study, this work reinforces the critical role of advanced analytical techniques in refining our understanding of the surfacome and accelerating the discovery of clinically relevant biomarkers and therapeutic targets.

References:
1. A. Laurent, A. Allard, M.Fillet. TrAC. 184 (March 2025), 118143.
2. C. McKinnon, M. Nandhabalan, S.A. Murray, P. Plaha. BMJ 2021, n1560.

OP-21

Characterization of HPV virus-like particles using SEC and AF4 coupled with MALS

A. Boclinville[1] , N. Thelen[2] , M. Thiry[2] , N. Jacobs[3] , M. Fillet[1] and A.-C. Servais[1]

1 Laboratory for the Analysis of Medicines, Center of Interdisciplinary Research on Medicines, University of Liège, 4000 – Liège, Belgium
2 Cellular and Tissular Biology, GIGA-Neurosciences, University of Liège, 4000 – Liège, Belgium
3 Cellular and Molecular Immunology, GIGA-Research, University of Liège, 4000 – Liège, Belgium

Cervical cancer, the fourth most common malignancy in women, is caused by human papillomavirus (HPV) infection. [1,2] HPV is a small, non-enveloped, double-stranded DNA virus that infects stratified squamous epithelial tissues. Its capsid consists of 72 pentamers of L1 and of up to 72 L2 proteins. [3] Because HPV in vitro production is technically difficult and leads to low virus titers, HPV studies have used virus-like particles (VLPs) as model. [4,5] HPV-VLPs result from the self-assembly of L1 protein or L1 and L2 proteins and are morphologically and immunologically similar to native virions.[6]HPV-VLPs are of great interest as model in HPV research studies but also for prophylaxis. Indeed, three HPV-VLP L1 based vaccines, Cervarix®, Gardasil® and Gardasil®9, have been approved by the FDA.

Quality control presents a critical challenge in VLP-based vaccine production. Following recombinant expression and purification, VLPs must undergo rigorous characterization to ensure vaccine efficacy, stability, and batch consistency. [7] Among critical quality attributes, size distribution plays a key role. The classical technique for VLP size characterization, i.e. transmission electron microscopy, requires drying for sample preparation, limiting its efficiency in high-throughput vaccine production environments. [8] We present an integrated analytical platform combining size-exclusion chromatography (SEC) and asymmetric flow field-flow fractionation (AF4) with multi-angle light scattering (MALS) detection for comprehensive HPV-VLP characterization. The mobile phase composition was found to be a key parameter for the SEC method optimization, while AF4 conditions were refined through cross-flow optimization to enhance separation performance. This platform is complemented by nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) to provide orthogonal measurements of hydrodynamic radius, particle size distribution, and structural integrity.

Consistent particle size values were obtained, demonstrating strong agreement between the different techniques. In addition, stress condition studies enabled the detection and characterization of VLP aggregation, confirming the applicability of the applied techniques for monitoring size variants. By enhancing the efficiency and accuracy of HPV-VLP quality assessment, this approach supports vaccine process development, manufacturing, and regulatory compliance, ultimately facilitating the advancement of next-generation VLP-based vaccines.

References
1. F. X. Bosch, S. de Sanjose, Disease markers (2007), 23, 213-227.
2. L. Denny, Annals of surgical oncology (2015), 22, 728-733.
3. C. B. Buck et al, Journal of virology (2008), 82, 5190-5197.
4. S. C. Fausch et al, Cancer research (2003), 63, 3478-3482.
5. V. M. Renoux et al, Eur. J. Immunol. (2011), 41, 3240-3252.
6. P. Le Cann et al, FEMS microbiology letters (1994), 117, 269-274.
7. C.L. Effio et al, Biotechnology Journal (2015), 10, 715-727.
8. A. Rathore et al, LCGC International (2024), 1, 14-18.

OP-22

Affinity Capillary Electrophoresis to Probe Drug Interactions with Membrane Proteins in Their Native Lipid Environment

C. Davoine[1] , L. Pochet[2] , and M. Fillet[1]

1 Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, Avenue Hippocrate 15, B36 Tour 4 +3, 4000 Liège, Belgium.
2 Namur Medicine & Drug Innovation Center (NAMEDIC – NARILIS), University of Namur, Rue de Bruxelles 61, 5000, Namur, Belgium

Membrane proteins are major pharmacological targets. Our previous work demonstrated that affinity capillary electrophoresis (ACE) allows robust hit validation and accurate quantification of dissociation constants when applied to purified soluble proteins.1–5 Extending this methodology to membrane proteins, however, introduces a distinct set of challenges. Their intrinsic instability in solution and reliance on native lipid environments continue to limit affinity measurements. Here, we present the first application of ACE to probe molecular interactions directly at the surface of native Golgi membrane fractions isolated from human cancer cells (Figure 1). Using this approach, we quantified the binding affinities of two inhibitors of UDP-glucose ceramide glucosyltransferase (UGCG), an intracellular membrane enzyme implicated in cancer progression and chemoresistance. ACE enables direct measurement of target engagement for low-abundance membrane proteins while preserving their native lipid context, providing a robust analytical platform suitable for complex biological samples. This methodology broadens current strategies for membrane-protein drug discovery and addresses a key gap in evaluating pharmacological interactions under pathologically relevant conditions.

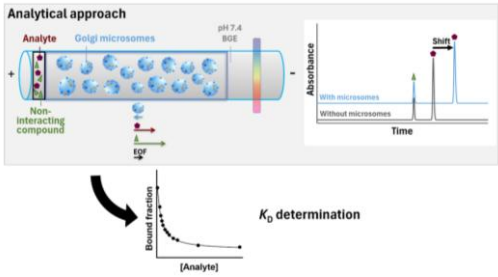

Figure 1. Overview of the Affinity Capillary Electrophoresis approach using Golgi membrane fractions isolated from cancer cells.

References
1 E. Farcaş, C. Bouckaert, A.C. Servais, J. Hanson, L. Pochet, M. Fillet, Anal. Chim. Acta 984 (2017) 211–222.
2 E. Farcaş, J. Hanson, L. Pochet, M. Fillet, Anal. Chim. Acta 1034 (2018) 214–222.
3 C. Davoine, M. Fillet, L. Pochet, Talanta 226 (2021) 122163.
4 C. Davoine, A. Pardo, L. Pochet, M. Fillet, Anal. Chem. 93 (2021) 14802–14809.
5 C. Davoine, M. Fillet, Anal. Chem. 97 (2025) 3988–3995.

OP-23

Generic Capillary Electrophoresis Methodology for Quality Control of Histidine Epimerization in Therapeutic Peptides

Q. Lorenzi[1] , C. Enjalbal[1] , C. Perrin[1]

1 IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

Peptides exhibit various biological activities, such as hormones, growth factors, neurotransmitters, ion channel ligands and antibacterial agents. Since the landmark synthesis of insulin in 1921, the importance of this molecular class has steadily increased, with peptides representing nearly 8% of all FDA-approved drugs between 2016 and 2024[1]. As natural ligands to receptors found in living organisms, peptides offer several advantages over small organic molecules, including high selectivity and specificity and low toxicity.

As with any therapeutic compound, controlling peptide chirality is essential. A particular case is the change in the absolute configuration of a single residue in the structure, known as epimerization. Epimerization can occur during synthesis, formulation, or storage, leading to subtle yet critical alterations in secondary and tertiary structures. Even minor conformational changes can significantly impact biological activity, safety profiles, and physicochemical properties such as solubility and bioavailability. Thus, the detection and quantification of unwanted epimers in peptide-based therapeutics pose a major analytical challenge for quality control (QC) laboratories.

To address this issue, we developed a generic capillary electrophoresis (CE) methodology to monitor and control epimerization of therapeutic peptides. CE was selected by a comprehensive literature review that emphasized significant changes in the charge and structure of peptide epimers, two key factors in electrophoretic separations. Our study focused on peptides containing histidine, one of the most epimerization-prone amino acids, due to its imidazole side chain's susceptibility to enolate formation through an intramolecular mechanism. For this study, 11 model tri- and hepta-peptides containing either L- or D-His and residues with a wide range of physicochemical properties (e.g., logP, pI) were synthesized according to conventional Fmoc-based solid phase peptide synthesis (SPPS), and purified prior to analyses.

The electrophoretic mobility of each epimer was determined using phosphate buffers across a pH range encompassing the peptides' estimated isoelectric points. Mobility-pH curves were constructed for each epimer, enabling the identification of optimal pH conditions where mobility differences were maximized for direct, efficient, and robust separation.

Significant differences in mobility were observed for all tri-peptides. Additionally, our data supported the hypothesis of negligible three-dimensional conformation effects, as mobility variations correlated strongly with changes in charge as a function of pH. In

contrast, the hepta-peptides exhibited slightly more complex behavior. Lower mobility differences were observed between epimers, and the correlation with the expected charge variations was occasionally weaker. These discrepanciessuggests that histidine epimerization induces conformational changes in longer peptides.

These insights from this study enabled the definition of optimal separation conditions for all epimer pairs tested. This study proposes a generic CE approach for finely tailoring optimal separation conditions to control traces of histidine epimerization in therapeutic peptides, support process optimization, formulation, stability assessment, and regulatory compliance in a QC context.

References

1.de la Torre, B.G.; Albericio, F. The Pharmaceutical Industry in 2024: An Analysis of the FDA Drug Approvals from the Perspective of Molecules. Molecules 2025, 30, 482. • DOI: 10.3390/molecules30030482

OP-24

Electropolymerized pABA/Aniline Molecularly Imprinted Sensor for Indirect Electrochemical Detection of Enalapril Maleate

S. N. Samanci[1,2,3], S. I. Kaya[4*] M. A. Unal[5], S. A. Ozkan[1*]

1 Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye
2 Ankara University, Graduate School of Health Sciences, Ankara, Türkiye
3 Afyonkarahisar Health Sciences University, Faculty of Pharmacy, Department of Analytical Chemistry, Afyon, Türkiye
4 University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye
5 Stem Cell Institute, Ankara University, Balgat, Ankara, Türkiye

Enalapril maleate is widely prescribed as an angiotensin-converting enzyme (ACE) inhibitor for the treatment of hypertension and heart failure; therefore, its accurate and sensitive determination in pharmaceutical formulations and biological samples is essential for therapeutic drug monitoring and quality control[1,2,3].

In this study, a molecularly imprinted polymer (MIP)-based electrochemical sensor was developed for the selective determination of enalapril maleate via electropolymerization in phosphate-buffered saline (PBS, pH 7.4). p-Aminobenzoic acid (pABA) and aniline were employed as functional monomers, while enalapril served as the template molecule. Systematic optimization studies were conducted to enhance sensor performance, including the optimization of template-to-monomer ratios (pABA/template and aniline/template), removal solution composition, removal time, rebinding time, electropolymerization cycle number, and scan rate.

The optimized conditions were determined as follows: 0.001 M template, 0.001 M pABA, and 0.020 M aniline prepared in PBS (pH 7.4). Electropolymerization was carried out by cyclic voltammetry (CV) in the potential range of −0.2 to +0.9 V for 4 cycles at a scan rate of 0.05 V s^{-1}. Template removal was achieved using a 9:1 (v/v) methanol:acetic acid solution for 5 min. Rebinding was performed for 5 min in a thermo-shaker. Indirect electrochemical measurements were conducted using differential pulse voltammetry (DPV), CV, and electrochemical impedance spectroscopy (EIS) in a ferri/ferrocyanide redox probe system. Surface morphology was characterized by scanning electron microscopy (SEM).

Calibration studies were performed in standard solutions and human serum samples. The developed sensor exhibited high sensitivity, reaching picomolar (pM) levels for the limit of detection (LOD) and limit of quantification (LOQ). Recovery studies were carried out in serum and pharmaceutical tablet formulations containing enalapril (Enapril®, Eneas®,

Zanipress®, and Konveril Plus®), demonstrating satisfactory analytical performance. Selectivity studies were performed using structurally similar drug molecules (e.g. Lercanidipine and Nitrendipine), and interference studies were conducted with commonly encountered interfering species. The results indicate that the proposed MIP-based electrochemical sensor provides a rapid, sensitive, and highly selective platform for the determination of enalapril maleate in complex matrices.

References

1. M. Piponski, T. Balkanov, L. Logoyda, Pharmacia (2021), 68, 69–77.
2. M.A.-A. Mohammad, M.A. Mahrouse, E.A.H. Amer, N.S. Elharati, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. (2021), 244, 118894.
3. C.F. Valezi, E.H. Duarte, G.R. Mansano, L.H. Dall'Antonia, C.R.T. Tarley, E.R. Sartori, Sens. Actuators B Chem. (2014), 205, 234–243.

OP-25

Synergistic Enhancement of an Electrochemical Sensor via NIR-Responsive Polydopamine Nanostructures for Pharmaceutical Analysis

S. Irem KAYA[1], Ozge Nur SARIKAYA[1], Hakan ERDOGAN[1]

1 University of Health Sciences, Gulhane Faculty of Pharmacy, Department of Analytical Chemistry, 06010 – Ankara

Electrochemical sensors are widely used tools in analytical applications thanks to their advantageous properties, such as low cost, high sensitivity, portability, and user-friendliness. However, the studies are still focusing on performance enhancement, specifically, rapid electron-transfer kinetics and higher sensitivity. Therefore, this study reports a photothermally enhanced electrochemical sensing platform based on polydopamine (PDA) nanostructures-modified screen-printed gold electrodes (SPAuEs). PDA nanostructures are advantageous materials for surface modification due to their optoelectronic features, biocompatibility, and adhesiveness. Utilizing these properties to induce laser-mediated interfacial restructuring presents a novel approach to improving charge-transfer kinetics and electrochemical signal intensity [1,2]. PDA nanostructures were deposited onto SPAuEs via drop-casting, and their morphology and chemical composition were confirmed by SEM, DLS, and FTIR analyses. The sensing interface was dynamically activated via near-infrared (NIR) laser irradiation to exploit PDA's photothermal conversion capability. Critical parameters, including PDA volume for drop-casting, NIR laser power, and pH of the working media, were optimized. Scan rate studies were conducted to investigate electron-transfer mechanisms. Electrochemical performance was evaluated using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). Paracetamol and caffeine were selected as model pharmaceutical analytes to demonstrate the sensor's improved performance. PDA modification increased the oxidation current response by 2.5-fold compared to bare SPAuEs. Under NIR photothermal stimulation, the current increased by approximately 4-fold, attributed to accelerated mass transport and enhanced charge-transfer kinetics at the interface. The proposed electrochemical sensor demonstrated low limits of detection (LOD) and quantification (LOQ), a wide linear range, and good recovery values in pharmaceutical formulations. These findings provide a proof-of-concept for NIR-stimulated photothermal activation as an effective strategy for improving electrochemical sensor performance, offering a rapid, sensitive, and versatile approach for pharmaceutical and bioanalytical applications.

References

1. H. Erdogan et al. J. Photochem. Photobiol. B (2024), 253, 112889.
2. S. Gao et al. Coord. Chem. Rev. (2024), 501, 215564.

OP-26

Highly Selective Electrochemical Sensing of Naringenin in Plant-Derived Samples via a Nanocomposite-Modified Molecularly Imprinted Polymer

Seyda Yayla[1], Marija Milićević[2], Ahmed Isa[3,4], Danica Bajuk-Bogdanović[5], Damjan Vengust[6], Matjaž Spreitzer[6], Ahmet Cetinkaya[7], M. Mesud Hurkul[1], Sonja Jovanović[2], Sibel A. Ozkan[3]

(1) Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Türkiye
(2) Laboratory of Physics, Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia
(3) Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye
(4) Ankara University, Graduate School of Health Sciences, Ankara, Türkiye
(5) Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia
(6) Advanced Materials Department, Jožef Stefan Institute, Ljubljana, Slovenia
(7) University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye

Naringenin (NAR) is a prominent flavanone found in citrus fruits, tomatoes, and other edible plants. Its chemical name is 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one ($C_{15}H_{12}O_5$). Its significant hepatoprotective, anti-atherogenic, anticancer, and antimicrobial activities have been demonstrated by extensive pharmacological studies [1]. This study reports the development of a novel molecularly imprinted polymer (MIP)-based electrochemical sensor for the selective determination of naringenin (NAR) in diverse real samples, including plant extracts, wine, and herbal supplements. To enhance the effective surface area and porosity of the glassy carbon electrode (GCE), the sensor design was engineered by incorporating a 2D/0D nanocomposite consisting of graphene oxide (GO) and cobalt ferrite (CFO) nanoparticles (CFO_GO). 4-aminobenzoic acid (4-ABA) was selected as the functional monomer for the fabrication of MIP. The polymerisation process was carried out using ethylene glycol dimethacrylate (EGDMA) as the crosslinker, 2-hydroxyethyl methacrylate (HEMA) as the basic monomer, and 2-methylpropiophenone as the photoinitiator. The resulting NAR/CFO_GO/4-ABA@MIP-GCE sensor is designed to detect NAR electrochemically in real samples, including *Solanum lycopersicum* L. (tomato) fruit, *Citrus × limon* (L.) Osbeck (lemon), oak (Quercus) bark, red wine, and commercial herbal supplements. Morphological and electrochemical characterisations of the fabricated sensor were performed using scanning electron microscopy (SEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). An indirect detection approach was used, utilising a 5.0 mM redox probe of $[Fe(CN)_6]^{3-/4-}$. The sensor exhibited a linear response range of 1.0×10^{-13} to 1.0×10^{-12} M, with a calculated limit of detection (LOD) of 2.84×10^{-14} M and a limit of quantification (LOQ) of 9.47×10^{-14} M. The fabricated MIP-based electrochemical sensor showed excellent specificity, selectivity, and sensitivity for detecting NAR. Studies conducted through recovery experiments demonstrated the sensor's accuracy in analyzing

real samples. Furthermore, these findings demonstrate the sensor's practical applicability for reliably determining NAR in complex matrices, including food and herbal products.

References

[1] Ke JY, Banh T, Hsiao YH, et al (2017) Citrus flavonoid naringenin reduces mammary tumor cell viability, adipose mass, and adipose inflammation in obese ovariectomized mice. Mol Nutr Food Res 61:. https://doi.org/10.1002/mnfr.201600934.

OP-27

Ion chromatography for the analysis of pharmaceutical formulations

E. Adams, K. Wolfs, A. Van Schepdael, Z. Wen

KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, Herestraat 49, O&N2, PB 923, 3000 Leuven, Belgium

A range of pharmaceutical formulations prepared in hospital pharmacies contain electrolytes to support vital physiological functions in vulnerable patients. To minimize risks associated with compounding errors, rigorous quality control is essential. However, commonly used techniques such as potentiometry and photometry/spectrometry often suffer from poor reproducibility and are labour intensive. Among various analytical techniques for the determination of ions, ion chromatography (IC) is generally preferred over atomic absorption spectroscopy, capillary electrophoresis, and inductively coupled plasma mass spectrometry. Despite its advantages, IC is still not widely adopted in routine pharmaceutical analysis.

This study focuses on several pharmaceutical formulations, including solutions for parenteral nutrition, cardioplegia, and nephroplegia. These solutions contain not only electrolytes such as sodium, potassium, magnesium, calcium, and chloride, but also additional components like glucose, mannose, and amino acids, that may interfere with the determination of the ions. Although IC is the method of choice for quantifying inorganic ions, its selectivity is sometimes insufficient to separate analytes from matrix components, necessitating additional sample pretreatment. For example, a calcination step was introduced prior to the analysis of electrolytes in the parenteral nutrition solution. This procedure was optimized with respect to calcination conditions, crucible type, and pickup solvent.

Another analytical challenge is the wide variation in analyte concentrations. Fortunately, IC coupled with conductivity detection offers a broad dynamic range. Sensitivity can be enhanced by using a suppressor. A comparison between suppressed and non-suppressed systems is included. An additional benefit of IC is its compatibility with multiple detection modes, for example conductivity detection for ions and pulsed electrochemical detection for carbohydrates.

All methods were successfully validated with respect to selectivity, range/linearity, sensitivity, precision, accuracy, and robustness. The latter was evaluated using a design-of-experiments approach. Finally, the methods were applied to real pharmaceutical samples.

OP-28

An Integrated Analytical Workflow for the Isolation and Structural Characterization of Bioactive Saturated Hyaluronic Acid Oligomers

S. Manzi[1], G. Mosconi[1], S. Messinese[1], G. Fumagalli [1], A. Bassi[1], L. Michelotti[1], M. Tucci[1], R. Taheribaviliolyaei[1], R. Ferrisi[1], C. Mocchetti[1], S. Vaccaro,[2] R. Inturri,[2] M. Soato,[3] A. Mario,[3] M. Filippetto,[3] C. Dallanoce[1], A. Altomare[1], G. Aldini[1], G. Baron[1]

1 Department of Pharmaceutical Sciences (DISFARM), Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milan, Italy
2 Fidia Farmaceutici S.p.A., Contrada Pizzuta, 96017 Noto, Italy
3 Fidia Farmaceutici S.p.A., Via Ponte della Fabbrica 3/A, 35031 Abano Terme, Italyy

Hyaluronic acid (HA) oligomers are increasingly recognized as biologically active molecules whose properties critically depend on chain length, end-group chemistry, and ionization state [1]. A key but often overlooked distinction exists between unsaturated and saturated HA fragments. Unsaturated oligomers, generated by enzymatic cleavage, contain a terminal Δ4,5-unsaturated uronic acid residue and have been extensively investigated [2]. In contrast, saturated HA oligomers are primarily formed through non-enzymatic oxidative depolymerization occurring in vivo under conditions of oxidative stress and inflammation. These species retain fully saturated sugar residues and intact reducing ends, resulting in distinct chemical reactivity and analytical behaviour. Despite evidence of their physiological relevance, saturated HA oligomers—particularly at low degrees of polymerization—remain poorly characterized, largely due to difficulties in their controlled generation, purification, and unambiguous structural validation [3].

Here, we report an integrated and reproducible analytical workflow for the production, isolation, and comprehensive structural characterization of a saturated HA disaccharide (2-mer), conceived as a minimal and chemically defined model for studying HA fragmentation under oxidative conditions. The disaccharide was generated by optimized acidic hydrolysis of high-molecular-weight HA, with reaction parameters carefully tuned to maximize disaccharide yield while minimizing deacetylation, monosaccharide formation, and thermally induced rearrangement products [4].

The resulting complex mixture was first fractionated by size-exclusion FPLC, allowing efficient removal of residual polymeric HA and inorganic salts generated during neutralization. Low-molecular-weight fractions were subsequently purified by preparative hydrophilic interaction liquid chromatography (HILIC). The orthogonality between size-exclusion and HILIC proved essential for resolving the target disaccharide from closely related oligomers and highly polar UV-absorbing by-products, highlighting the importance of multi-dimensional chromatographic strategies in carbohydrate drug analysis.

Structural identity and purity were confirmed using a complementary analytical platform combining high-resolution LC-ESI-Q-TOF mass spectrometry, FT-IR spectroscopy, and ^{1}H-NMR. HRMS unequivocally confirmed molecular composition and high chemical purity, while chromatographic and spectroscopic analyses revealed the presence of distinct anomeric forms in equilibrium. ^{1}H-NMR data confirmed the glycosidic linkage, stereochemistry, and preservation of the N-acetyl group, excluding artifacts associated with deacetylation or degradation [5].

Overall, this work establishes a robust analytical and preparative framework for isolating structurally validated saturated HA disaccharides and provides well-defined molecular tools for investigating the chemical and biological consequences of oxidative HA degradation.

Such chemically defined saturated HA oligomers may represent valuable starting points for the rational design of novel biologically active molecules and HA-derived therapeutic candidates.

References

1. J. M. Cyphert, C. S. Trempus, S. Garantziotis Int. J. Cell Biol. (2015), 2015, 563818.
2. S. Matou-Nasri, J. Gaffney, S. Kumar, M. Slevin Int. J. Oncol. (2009), 35, 761–773.
3. R. J. Dodd, C. D. Blundell, B. M. Sattelle, J. J. Enghild, C. M. Milner, A. J. Day J. Biol. Chem. (2024), 300, 107668.
4. N. Volpi, F. Maccari, R. J. Linhardt Electrophoresis (2008), 29, 3095–3106.
5. J. R. Fraser, T. C. Laurent, U. B. Laurent J. Intern. Med. (1997), 242, 27–33.

OP-29

A polyamide-based 3D-printed sorbent in a validated sample pretreatment for the analysis of amino acids in mice plasma

P. Verding[1,2] , D. Kroll[3] , M. Belka[3] , T. Bączek[3] , Y. Vander Heyden[1] , A. Van Eeckhaut[2] , D. Mangelings[1]

1 Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling (FABI), Laarbeeklaan 103, 1090 Brussels, Belgium
2 Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Research group of Experimental Pharmacology (EFAR), Center for Neurosciences (C4N), Laarbeeklaan 103, 1090 Brussels, Belgium
3 Medical University of Gdańsk, Department of Pharmaceutical Chemistry, J. Hallera 107, 80-416 Gdańsk, Poland

The quantification of endogenous compounds, such as amino acids, in biological samples can be further challenged by their small volumes (≤ 100 µL), obtained during preclinical studies. This research focuses on improving and optimizing sample preparation techniques for amino acids in volume-restricted mice plasma samples. Quantification of the amino acids is performed using liquid chromatography with fluorescence detection after an automated on-line pre-column derivatization with o-phtalaldehyde and 2-mercapto ethanol. In this study, a reference protein precipitation method was optimized and validated. Next, this sample preparation method was compared to a novel one based on solid-phase microextraction with a 3D-printed sorbent. 3D-printed devices have the advantage of being highly customizable and inexpensive. In the present method, a 3D-printed cone-shaped microextraction device was fabricated to fit tightly in a 0.5 mL Eppendorf® tube. A polyamide-based composite was chosen as best match with the polarity of the targeted amino acids. The adsorption and desorption times of the novel method were optimized with a central composite design. Rather short sample preparation times were obtained, with 35 min for adsorption and only 15 min for desorption. The complete method, including sample preparation and analysis, was validated for linearity, precision and accuracy. The obtained repeatability and intermediate precision were below 20% for the majority of the amino acids investigated.

When comparing the results on the novel 3D-sorbents to those of protein precipitation, the precision and ease of use of the reference method were slightly better. However, the total sample preparation time was shorter for the 3D-printed sorbents, as those samplessolvents needed considerably less time to evaporate prior to reconstitution. One hour could be saved because there is no aqueous fraction left in the sample after using the 3D-printed sorbent. Furthermore, the customizability of the 3D-printed sorbent creates possibilities for further miniaturization or to increase the sample throughput.

OP-30

Toward a high-quality human albumin formulation: analytical assessment of commercial albumin damage and structural recovery by glutathione treatment

M. Naldi[1], M. Baldassarre[2], Enrico Pompili[2,3], Giacomo Zaccherini[2,3], Daniele Tedesco[4], Marta Nugnes[1], Edoardo Fabini[1], Katharina Pock[5], Oliver Hegener[5], Paolo Caraceni[2,3], Manuela Bartolini[1]

1 Department of Pharmacy and Biotechnology, University of Bologna, 40126, Bologna, Italy
2 Unit of Semeiotics, Liver and Alcohol-related Diseases, 40138, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
3 Department of Medical and Surgical Sciences, University of Bologna, 40138, Bologna, Italy
4 Institute for Organic Synthesis and Photoreactivity, National Research Council, 40129, Bologna, Italy
5 Octapharma Pharmazeutika Produktions GesmbH, >A-1100, Vienna, Austria

Human albumin administration represents a cornerstone in the treatment of decompensated cirrhosis, and accumulating clinical evidence has highlighted its importance in patient management. [1] However, commercial albumin formulations contain structurally altered molecular forms that may limit recovery of the full functional properties of the protein. [2] This study aimed to determine when these alterations arise in pharmaceutical albumin and to develop a practical strategy to obtain a high-quality formulation enriched in native albumin.

Albumin molecular integrity was mainly investigated by high-performance liquid chromatography-electrospray ionization mass spectrometry [3] during both the manufacturing process and shelf-life. The effects of cold storage and supplementation with pharmaceutical-grade glutathione (GSH), alone or in combination, were also evaluated.

Manufacturing had a limited impact on albumin integrity, whereas storage at room temperature promoted progressive oxidation and truncation, leading to a marked reduction of the native form over time. Cold storage significantly limited structural deterioration, while GSH supplementation rapidly restored the reduced Cys34 form. The combination of storage at 2-8°C and GSH supplementation resulted in the highest recovery of native albumin and the best overall preservation of molecular integrity. Complementary analyses by circular dichroism, surface plasmon resonance, and untargeted LC-MS showed that albumin conformation and its binding properties were preserved upon GSH supplementation, and no unexpected impurities were detected. Safety was further assessed in preclinical models. Overall, this study identifies shelf-life as the main source of albumin damage and provides an analytically supported, clinically feasible strategy to improve the quality of commercial albumin formulations. Storage at 2-8°C combined with pre-infusion GSH supplementation represents a simple approach to obtain a high-quality human albumin solution from standard commercial vials.

References
1. P. Caraceni et al.The Lancet. (2018), 391, 10138, 2417.
2. J.L. Plantier et al. Biologicals (2016), 44, 226.
3. M. Baldassarre et al. Hepatology (2021), 74, 4, 2058.

OP-31

Forced degradation studies of small oligonucleotide therapeutics: IPRP-HRAMS as a stability-indicating analytical methods to decipher high complexity samples

Silvia Millán-Martín[1], Felipe Guapo[1], Sara Carillo[1], Ulrik H. Mistarz[2], Ken Cook[3], and Jonathan Bones[1,4]

1 National Institute for Bioprocessing Research and Training, Dublin, A94 X099, Ireland
2 Thermo Fisher Scientific, Allerød, 3450, Copenhagen NV, Denmark
3 Thermo Fisher Scientific, Morpeth, NE61, United Kingdom
4 School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, D04 V1W8, Ireland

Oligonucleotide based therapeutics are an emerging class of new therapeutic modalities which offer great potential for previously untreatable diseases with the ability for tailored treatments, rapid development potential and broad applicability [1-4]. During their synthesis multiple modifications are introduced at different structural components to enhance their stability, increase protein production, extend circulation time and improve target specificity [5]. Chemical stability and degradation studies are regulatory requirements to provide insights into decomposition pathways and the identification of potential degradation byproducts. The complexity of the oligonucleotides and their degradation products demand more specific and sensitive analytical platforms, where IP-RP-MS stands out as the gold standard for oligonucleotide analysis.

This work shows a forced degradation study of several small oligonucleotide therapeutics with the aim to further explore the stability indicating potential of a high specific and sensitive ion-pairing reversed-phase (IP-RP) liquid chromatography coupled with high resolution accurate mass spectrometry (HRAMS) method [6] using a moderate ion pairing reagent (pentylamine) together with HFIP, and a mild ESI ionization source which allowed for reduced adduct formation and elimination of in-source generated impurities. Selected molecules included small DNA-based ASOs of different lengths and with extensive chemical modifications at the backbone, sugar and nucleobase level.

Following regulatory requirements and ICH guidelines [7], forced degradation studies focused on the evaluation of acidic (10 mM HCl), thermal (80 °C) and oxidative (0.05% H_2O_2) stress over 24 hours, on individual samples at 0.5 µg/µL. Control samples and forced degraded samples after 1, 4 and 24 hours were subsequently analysed on the developed analytical platform using an Orbitrap™ mass analyser. Degraded samples resulted in high complex chromatographic profiles with multiple peaks eluting across a 27-minute chromatographic gradient. Although full chromatographic resolution couldn't be achieved for all detected species, HRAMS allowed for accurate identification of multiple degradants and/or impurities at the intact level down to 0.1 % of relative abundance. Forced degradation revealed several critical chemical modifications and break down products with different fractional abundances depending on the type of molecule, including N-shortmers

from the 3' and 5' termini, loss of purine bases, conversion of phosphorothioate (PS) linkages to phosphodiester (PO) linkages, or multiple break down products. A special focus was paid on how the different chemical modifications of the studied molecules would impact degradation rates during these studies. Under harsher conditions, acid stress induced the break down, at different levels, from total to partial depending on the molecule, while oxidative stress created multiple PO impurities for PS-modified molecules. Heat stress resulted in chain shortening with species detected up to N-14 from both termini. For confident sequence assignment of all detected impurities and degradant species, MS/MS analysis were performed using Higher-energy collisional dissociation (HCD) in an Orbitrap™ Exploris™ mass analyser and a stepped normalized collisional energy (NCE) of 17–19–21. Acquired spectra were analysed using BioPharma Finder™ software version 5.4, using 5 ppm mass accuracy and Chromeleon CDS software version 7.4 for compliant and automated data processing including reporting options.

References

1. M.D. de Smet, C.J. Meenken, G.J. van den Horn. Ocul Immunol Inflamm 7(3-4) (1999) 189-98.
2. R. McCartan, O. Khorkova, C.H. Volmar, C. Wahlestedt. Front Genet 14 (2023) 1250276.
3. G. Kolovou, V. Kolovou, N. Katsiki. J Clin Med 11(4) (2022).
4. H. Xiong, R.N. Veedu, S.D. Diermeier. Int J Mol Sci 22(7) (2021).
5. J. Gao, B. Nutan, D. Gargouri, N.D. Pisal, V. Do, M. Zubair, H. Alanzi. Adv. Therap. (2400231) (2024) 1-29.
6. S.Millan-Martín, F. Guapo, S. Carillo, U. H. Mistarz, K. Cook, J. Bones. J. Pharm. Biomed. Anal. 268 (2026) 117097.
7. EMA/CHMP/CVMP/QWP/262313/202424. Guideline on the development and manufacture of oligonucleotides. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-development-manufacture-oligonucleotides_en.pdf (2024).

OP-32

Mass Spectrometry–Based Characterization of Structural Heterogeneity in Recombinant and Plasma-Derived Ceruloplasmin

Aurora Tini [1], Ruolan Cheng[2], Igor Kaltashov[2], Ilaria Nardini[3], Andrea Denardo[3], Andrea Caricasole[3] and Caterina Temporini[1]

1 University of Pavia, Department of Drug Science, Via Taramelli 12, 27100 – Pavia, Italy
2 University of Massachusetts-Amherst, Department of Chemistry, 240 Thatcher Way, 01003 – Amherst, MA, USA
3 Researchand Innovation, Kedrion S.p.A., Via di Fondovalle, Loc. Bolognana, 55027 – Gallicano (LU), Italy.

Ceruloplasmin (CP) is a ~130 kDa protein that exhibits ferroxidase activity and serves as the primary copper-binding protein required for its enzymatic function. There are two main forms of CP: holo-CP, which is copper-loaded and catalytically active, and apo-CP, which is copper-depleted and inactive. The administration of CP is currently being investigated as a replacement therapy for aceruloplasminemia, a disorder characterised by CP misfolding that leads to pathological iron accumulation.[1,2] Plasmaderived replacement therapies restore missing proteins but exhibit molecular heterogeneity, whereas the evolution of Haemophilia treatments exemplifies how recombinant technologies enable more standardized, tightly controlled, and potentially functionally optimized protein replacement products. [3] Clearly, molecular profiles of the same protein purified from plasma or produced through recombinant technology in a human cell line can be different, and this may have functionally relevant impacts. Recently, a recombinant CP prototype was reported with functional properties comparable to CP purified from plasma [4] . In the present study, a preliminary comparative analysis of the molecular profiles of plasma-derived CP (pCP) and HEK293 cells-derived recombinant CP (rCP) prototypes is reported. Structural characterization was conducted at intact, de-glycosylated and peptide level using Native MS and LC-MS techniques.

Several challenges had to be address for intact analysis due to the nature of the compound, since CP is a multi-copper oxidase with extensive glycosylation. First, to achieve a high-resolution characterization, Native Mass Spectrometry using Nano ESI-QTOF was used. [5] The application of non-conventional MS technique was then necessary to reduce the broadening signals obtained. Limited Charge Reduction (LCR)[6] technique was selected to reduce spectral crowding and peak overlapping in rCP significantly improving the resolution of the mass spectra and enabling a more precise comparison of the glycan and proteoforms profiles between the samples. MS data revealed the rCP as the most heterogeneous, possibly related to the production host, as HEK cell expression is known to result in extensive and variable glycosylation. [7]

To remove the extreme heterogeneity due to glycan variability, the two samples have been analyzed in their deglycosylated forms better focussing on the proteoform distributions and highlighting possible differences in the backbone structure. Native MS revealed similar proteoform distributions for both products, indicating that the observed differences in intact products might be primarily driven by glycosylation. To confirm this observation, Peptide Mapping was performed. Optimization of denaturing and digestion conditions were necessary due to the presence of copper atoms stabilizing the folding. Analyses were carried out on deglycosylated and glycosylated sample to compare all posttranslational modifications including glycosylation sites. [8,9]
MS data have been also analysed to derive information on protein folding, which is a critical attribute of CP. Indeed, heterogeneity in charge state may also arise from incomplete protein folding due to the partial incorporation of copper ions, resulting in inactive protein (apoCP). [4]

Collectively these data represent an example of a detailed comparative molecular characterization of a candidate therapeutic protein purified from two commonly used starting points for protein replacement therapies, namely human plasma and a recombinant source.

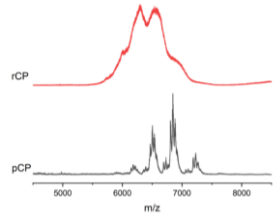

Figure 1. Nano ESI QTOF profile in Native conditions of the Plasma derived product (black) compared with the recombinant Ceruloplasmin product (red).

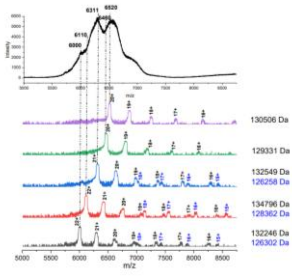

Figure 2. Limited Charge reduction technique applied to Recombinant Ceruloplasmin product.

References

1. Miyajima, H. Aceruloplasminemia, an iron metabolic disorder. in Neuropathology vol. 23 345– 350 (2003).

2. Zanardi, A. et al. New orphan disease therapies from the proteome of industrial plasma processing waste- a treatment for aceruloplasminemia. Commun. Biol. 7, 1–17 (2024).

3. Ozelo, M. C. & Yamaguti-Hayakawa, G. G. Impact of novel hemophilia therapies around the world. Res. Pract. Thromb. Haemost. 6, (2022).

4. Ziliotto, N. et al. Functional characterisation of missense ceruloplasmin variants and real-world prevalence assessment of Aceruloplasminemia using population data. EBioMedicine 113, 105625 (2025).

5. Cramer, D. A. T., Franc, V., Caval, T. & Heck, A. J. R. Charting the Proteoform Landscape of Serum Proteins in Individual Donors by High-Resolution Native Mass Spectrometry. Anal. Chem. 94, 12732–12741 (2022).

6. Yang, W., Ivanov, D. G. & Kaltashov, I. A. Extending the capabilities of intact-mass analyses to monoclonal immunoglobulins of the E-isotype (IgE). MAbs 14, (2022).

7. Böhm, E. et al. Differences in N-glycosylation of recombinant human coagulation factor VII derived from BHK, CHO, and HEK293 cells. BMC Biotechnol. 15, (2015).

8. DeWilde, A., Sadilkova, K., Sadilek, M., Vasta, V. & Si, H. H. Tryptic peptide analysis of ceruloplasmin in dried blood spots using liquid chromatography-tandem mass spectrometry: Application to newborn screening. Clin. Chem. 54, 1961–1968 (2008).

9. Harazono, A. et al. Site-specific N-glycosylation analysis of human plasma ceruloplasmin using liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Biochem. 348, 259–268 (2006).

OP-33

Mycotoxins or doping? The intertwined story of Zearalenone and Zeranol

Lia Visintin[1,2], Monica Mazzarino[2], Marthe De Boevre[3], Evelien Wynendaele[1], Peter Van Eenoo[2]

1 Drug Quality and Registration, Department of Pharmaceutical Analysis, Ghent University, 9000 Ghent, Belgium
2 Doping Control Laboratory, Department of Diagnostic Sciences, Ghent University, 9000 Ghent, Belgium
3 Centre of Excellence in Mycotoxicology and Public Health, Department of Bioanalysis, Ghent University, 9000 Ghent, Belgium

Zeranol, also known as α-zearalanol (α-ZAL), is classified by the World Anti-Doping Agency (WADA) as a prohibited anabolic agent at all times [1] . However, α-ZAL can occur endogenously in human urine as a metabolite of zearalenone (ZEN), a Fusarium-derived mycotoxin commonly and naturally found in food commodities such as wheat, maize, rice, fruits, vegetables, and animal-derived products [2,3] . WADA recommends that, when zeranol is detected in urine, analytical confirmation should include testing for ZEN and its metabolites, and evaluation of specific metabolite ratios to distinguish between food-derived exposure and intentional administration. However, the absence of scientifically validated cut-off values for these ratios leads to uncertainty in reporting Adverse Analytical Findings (AAFs) for zeranol. Discriminating between ZEN intake and zeranol administration is exacerbated by the lack of human toxicokinetic data for both compounds. To establish scientifically robust and legally defensible cut-offs, human intervention trials combined with toxicokinetic (TK) modeling are essential. The present work aimed to elucidate and compare the human toxicokinetics and metabolism of α-ZAL and ZEN through two independent controlled human intervention trials (n = 10 per group)[4] . Following a three-day wash-out period under a controlled diet, volunteers received an aqueous bolus of either ZEN or α-ZAL at the tolerable daily intake (TDI) level of 250 ng/kg body weight of ZEN equivalents. Post-exposure, individual full-void urine and capillary blood samples will be collected for 72 hours using volumetric absorptive microsampling (VAMS®, Mitra). Analytes including ZEN, ZAN, α/β-ZEL, and α/β-ZAL (free and conjugated forms) were quantified via validated ultra-trace liquid chromatography–high resolution mass spectrometry (UHPLC-Orbitrap MS/MS). The experimental data will be used to fit a hierarchical Bayesian population TK model [5,6] to characterize inter-individual variability and to support the derivation of evidence-based concentration ratios and cut-off values for distinguishing exogenous zeranol administration from natural exposure. The model will be further used for reverse dosimetry estimation of ingested doses from urinary or blood biomarker data, providing a physiologically grounded framework for doping case evaluation and risk assessment of ZEN.

References
1 WADA. TL23 (2021)
2 EFSA Panel. EFSA Journal 9, (2011)
3 EFSA Panel. EFSA Journal 14, (2016)
4 Visintin, L. et al. Sci. Rep. 15, 39096 (2025)
5 Visintin, L. et al. Toxins 17, (2025)
6 Visintin, L. et al. J. Expo. Sci. Environ. Epidemiol. (2025)

OP-34

Quantification of ADHD Medication in Plasma and Breast Milk: An LC-MS/MS Analysis

L. De Hondt, J. Muyldermans, E. Tommelein and A. Van Eeckhaut

Experimental Pharmacology Research Group, Department of Pharmaceutical and Pharmacological Sciences, Center for Neurosciences, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Jette, Belgium

Attention-deficit/hyperactivity disorder (ADHD) has long been considered as only a paediatric concern. However, in about 50% of children with ADHD, symptoms persist into adulthood and may require medication. A subgroup within the adult ADHD population are breastfeeding women, of which the treatment has become more prevalent during the last years. Methylphenidate and (lis)dexamphetamine are the first-line treatment, followed by atomoxetine. Guanfacine and bupropion are also used. Information about ADHD medication milk levels during breastfeeding is however very limited. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify these active substances simultaneously in plasma and breast milk has not yet been described. In addition, no information exists on the correlation between maternal plasma and breast milk ADHD medication concentrations. The aim of this study was to develop and validate a sensitive LC-MS/MS method able to quantify the different ADHD medications simultaneously in plasma and breast milk to be applied in clinical lactation studies.

Blank plasma and breast milk samples have been collected and spiked with ADHD medication, as well as deuterated internal standards. After protein precipitation, an LC-MS/MS analysis was carried out on an Acquity UPLC® system, coupled with a Xevo TQ MS triple quadrupole system, equipped with a Zspray™ electrospray ionization source (all from Waters). We used an Acquity UPLC® BEH C18 column (2.1 x 100 mm, 1.7 µm) at 60°C, gradient elution with ultrapure water/acetonitrile with 0.1% formic acid (FA) as mobile phase A/B at a flow rate of 0.4 mL/min, and an injection volume of 5 µL.

We have developed a method for the simultaneous quantification of methylphenidate, dexamphetamine, atomoxetine, guanfacine, and (hydroxy)bupropion. First, the compound-specific MS parameters were optimized, i.e. ionization mode, selection of a precursor and two product ions (quantifier and qualifier), collision energy and cone voltage. Second, the LC parameters were optimized. Several gradients were tested, resulting in a run time of 15 min and elution of all compounds within 7.5 min. Last, the sample preparation method was optimized for both breast milk and plasma by precipitating 100 µL sample with 400 µL acetonitrile containing 0.1% FA, drying the supernatant, and reconstituting in 50 µL ultrapure water + 0.1% FA before injection. The method has been validated for both matrices by testing the limit of quantification, linearity (5-500 ng/mL), precision, accuracy, matrix effects, recovery, dilution integrity, and stability.

An LC-MS/MS method for simultaneous quantification of ADHD medication was developed and validated for a 5-500 ng/mL range in plasma and breast milk. Currently, this method is applied in clinical lactation studies in order to correlate maternal plasma and breast milk concentrations.

OP-35

A Sensitive and Accurate 1536-based Screening Platform Coupled with Cosine Similarity-driven Algorithm for High-throughput Screening of Bioactive Compounds from Complex Matrices

Jincai Wang, Hanlin Zeng, Zhengjin Jiang

Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, 511436, Guangzhou, China

A miniaturized and automated screening platform can effectively speed up the discovery of bioactive compounds from natural products[1]. In this work, a novel 1536 well plate-based screening platform was developed by integrating HPLC-HRMS, bioassay and homemade fractionation collector. The collector with a minimal tip can rapidly and accurately transfer micro-volume LC eluate into wells without any well-to-well cross-contamination. Coupled with minimal bioassay, it can generate a high-resolution bioactivity profile for sensitively recognizing bioactive compounds. In addition, an in-house written algorithm was designed to automatically identify the bioactive compounds based on MS and bioassay data. It employs a two steps identification strategy: the bioactive peaks were first recognized according to their gaussian distribution characteristics, and then cosine similarity was developed to calculate the shape similarity between the bioactivity peaks and corresponding extracted-ion chromatogram (XICs), which could accurately identify the real bioactive compounds, and effectively avoid the interference from co-elute compounds. As for neuraminidase inhibitors (NAIs) screening, the bioactivity peak height of oseltamivir, hyperoside in this platform was measured to be 2-4.25 times higher than that using conventional 384 well-plate system[2]. And a total of 10 NAIs were successfully identified from *Selaginella tamariscina* (P. Beauv.) Spring. (Only 3 NAIs were identified in 384 well-plate based platform), which fully verified the higher sensitivity and accuracy of the proposed platform.

References

1. Wölcke J. Drug discovery today. (2001), 6, 12.
2. Z.X. Chen[1], J.C. Wang[1], J.M. Yuan1, Z. Wang, Z.C. Tu, J. Crommen, W.H. Luo, J.L. Guo*, T.T. Zhang, Z.J Jiang*. J. Chromatogr. A. (2023), 1687, 463693.

OP-36

Eco-Friendly MXene/Chitosan-Supported Molecularly Imprinted Platform for Selective Detection of Abemaciclib

A. Cetinkaya[1], Z. Dogan[2,3], E. Piskin[2,3], A. Türkoglu[4], E. Zor[5], S. Akbayrak[6], E. Bellur-Atici, and S. A. Ozkan[2]

1 University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye
2 Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkiye
3 Ankara University, Graduate School of Health Sciences, Ankara, Turkiye
4 University of Health Sciences, Gülhane Institute of Health Sciences, Ankara, Türkiye
5 Necmettin Erbakan University, A. K. Education Faculty, Department of Science Education, Konya, Turkiye
6 Necmettin Erbakan University, Faculty of Engineering, Department of Basic Sciences, 42140, Konya, Türkiye
7 DEVA Holding A.S., R&D Center, Tekirdağ, Türkiye
8 Gebze Technical University, Department of Chemistry, Kocaeli, Türkiye

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults and is characterized by rapid proliferation, diffuse infiltration, and strong resistance to conventional therapies. Dysregulation of cell-cycle control, particularly abnormal activation of cyclin-dependent kinases (CDKs), plays a critical role in GBM progression, making CDK4/6 inhibitors promising therapeutic agents. Among them, abemaciclib (ABE) has attracted attention due to its high central nervous system penetration, sustained target inhibition, and ability to induce programmed cell death pathways, including apoptosis and pyroptosis. However, sensitive and reliable analytical methods are still required to quantify ABE at trace levels in complex biological matrices. In the present study, a green and molecularly imprinted polymer (MIP)-based electrochemical sensing platform for the determination of ABE was developed on a glassy carbon electrode (GCE). The MIP layer was fabricated using ABE as the template molecule and 1H-indazole-5-boronic acid (1H-I-5BA) as the functional monomer. To enhance the physicochemical characteristics of the sensing interface, a Cht@Mel–$Ti_3C_2T_x$ composite was prepared, in which chitosan (Cht) was combined with the melamine–MXene (Mel–$Ti_3C_2T_x$) component at a 1:1 ratio prior to use. This Cht@Mel–$Ti_3C_2T_x$ composite enabled electropolymerization (EP) on the GCE surface, generating a uniformly distributed, highly conductive, and mechanically stable imprinting matrix, while simultaneously reducing solvent consumption in accordance with the principles of green analytical chemistry. Following template removal, the analytical performance of the sensor was evaluated using differential pulse voltammetry (DPV) in the presence of the redox probe $[Fe(CN)_6]^{3-/4-}$. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were employed to monitor the stepwise formation of the polymeric film and to confirm target recognition events. In addition, the morphological and elemental characteristics of the fabricated sensing

interface were investigated using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). Calibration studies were performed using standard solutions and subsequently validated in commercial serum samples. The fabricated ABE-1H-I-5BA/Cht@Mel–$Ti_3C_2T_x$/MIP-GCE sensor exhibited a linear analytical response within the concentration range of 1.00–10.00 pM, with a limit of detection (LOD) of 0.24 pM and a limit of quantification (LOQ) of 0.80 pM, demonstrating good sensitivity. These results highlight the sensor's potential as a reliable and eco-friendly tool for the selective and ultrasensitive detection of abemaciclib in complex biological samples, supporting its future application in clinical and pharmaceutical analysis.

References

1. Alkaç, B. Ç., Soyöz, M., Ayna, T. K., Pehlivan, M., & Pirim, İ. *Gene Reports*, (2026), 42, 102413

OP-37

Beyond Dissolution Testing: Optical Coherence Tomography for Non-Destructive Evaluation and Regulatory Advancement of Enteric-Coated Dosage Forms

Jesús Alberto Afonso Urich [1,2], Matthias Wolfgang [1], Raymar Andreina Lara Garcia [1], and Johannes Khinast [1,2]

1 Research Center Pharmaceutical Engineering GmbH, Inffeldgasse 13, 8010 Graz, Austria.
2 Institute of Process and Particle Engineering, Graz University of Technology, Inffeldgasse 13, 8010 Graz, Austria.

Enteric coatings are widely used to protect acid-labile active pharmaceutical ingredients and ensure targeted intestinal release. Their performance depends on coating thickness, integrity, and long-term stability, which are traditionally evaluated using destructive and resource-intensive acid-stage dissolution testing. Optical Coherence Tomography (OCT) offers a non-destructive, high-resolution alternative capable of directly assessing coating characteristics, yet its regulatory acceptance remains limited [1-3].

This work evaluates OCT as a quantitative analytical tool for enteric-coated solid oral dosage forms, integrating experimental validation, stability assessment, and regulatory considerations. Three commercially relevant enteric polymers (Acryl-Eze®, Aquarius™ Control ENA, and Nutrateric®) were investigated across multiple manufacturing batches to assess operator and batch variability. OCT was used to measure coating thickness and the results were correlated with pharmacopeial acid-stage dissolution testing [1].

Critical coating thickness thresholds between 65 and 69 µm were established for the three polymers, ensuring compliance with USP <711> acid resistance criteria. OCT-based measurements showed strong agreement with dissolution performance, supporting the use of thickness specifications as an alternative or complementary control strategy for product release. These findings demonstrate that OCT can provide rapid, non-destructive, and clinically relevant information on enteric coating performance [1].

The applicability of OCT during stability testing was further evaluated under ICH Q1A (R2) accelerated and long-term conditions. Coating thickness remained generally consistent over time and continued to correlate with acid-stage dissolution results. While all formulations met pharmacopeial acceptance criteria, increased variability was observed for Aquarius™ Control ENA and Nutrateric®, highlighting the need for risk-based interpretation and appropriate regulatory justification in stability submissions [2].

In parallel, this work discusses the current status of OCT within pharmaceutical quality frameworks, including Quality by Design (QbD), Process Analytical Technology (PAT), and Good Manufacturing Practices (GMP). Although the lack of pharmacopeial monographs has

limited widespread adoption, increasing engagement from regulatory agencies and industry is driving progress toward method standardization and acceptance [3].

Overall, the results support OCT as a robust, non-destructive tool for evaluating enteric coating performance throughout the product lifecycle. By demonstrating equivalence with pharmacopeial dissolution testing and alignment with modern regulatory expectations, OCT has the potential to enhance quality assurance, reduce laboratory burden, and contribute to more efficient pharmaceutical development and manufacturing [1-2].

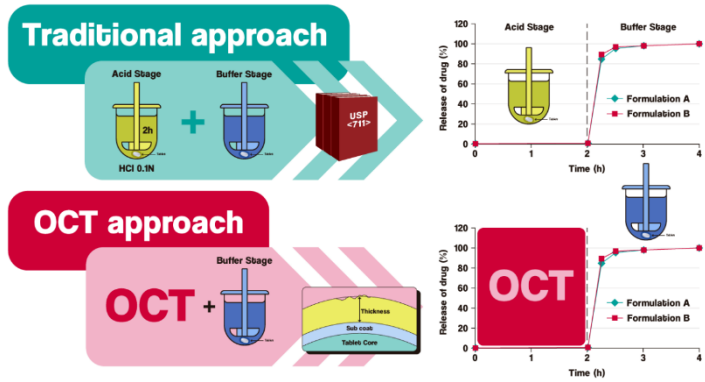

Figure 1. Graphical Abstract.

References

1. Afonso Urich, J. A., Wolfgang, M., Lara Garcia, R. A., West, H., & Khinast, J. (2025). Technical and regulatory perspective on acid stage dissolution assessed via optical coherence tomography (Part 1: Release Scenario). Int. J. Pharm. (Vol. 683, Article 126044). https://doi.org/10.1016/j.ijpharm.2025.126044
2. Afonso Urich, J. A., Lara Garcia, R. A., Wolfgang, M., & Khinast, J. (2025). Technical and regulatory perspective on acid stage dissolution assessed via optical coherence tomography: (Part 2: Stability scenario). Int. J. Pharm. (Vol. 686, Article 126352). https://doi.org/10.1016/j.ijpharm.2025.126352
3. Afonso Urich, J. A., Lara Garcia, R. A, Wolfgang, M. & Khinast, J. (2026) Regulatory implementation of Optical Coherence Tomography as an analytical technique in pharmaceutical fillings. Front. Med. (12:1693159). https://doi.org/10.3389/fmed.2025.1693159

OP-38

Development of a surface-enhanced Raman chemical imaging method for pharmaceutical active ingredient tracking in the context of in-vitro skin equivalent-model permeation studies

Kemik K.[1] , De Bleye C.[1] , Sacré P.-Y.[2] , Hubert Ph. [1] , and Ziemons E.[1]

1 University of Liège (ULiège), CIRM, ViBra-Santé HUB, Laboratory of Pharmaceutical Analytical Chemistry, Department of Pharmacy, Avenue Hippocrate 15, 4000 Liège – Belgium
2 University of Liège (ULiège), CIRM, Research Support Unit in Chemometrics, Department of Pharmacy, Avenue Hippocrate, 15, 4000 Liège - Belgium

In-vitro permeation testing is one of the mandatory steps in the development of a topical drug formulation. In this context, regulatory guidelines from the European Medicine Agency (EMA) and the Organism for Economic Cooperation and Development (OECD) currently recommend the use of a diffusion platform such as a Franz diffusion cell, coupled with a separative technique to quantify the total permeated drug amount in the receptor fluid of the cell. However, that methodology, despite widespread adoption, suffers from major limitations: destructive sampling, time-consuming analysis and inability to provide spatial distribution data of the active pharmaceutical ingredients (API) within the skin layers of the used model [1-3] .

Surface-enhanced Raman Chemical Imaging (SER-CI) offers new insights in the context of in-vitro permeation studies. Combining molecular specificity through the deposition of metallic nanostructures with spatial resolution, it constitutes a non-destructive alternative analytical technique, bringing complementary data to the current gold standard protocol [4]. However, two main challenges need to be addressed before implementing SER-CI: (i) the non-uniform distribution of nanoparticles during deposition yielding high variability in intensities, independent from the local API concentration; (ii) the absence of standardized, reproducible calibration matrices with homogeneous distribution of an API for method development, since biological tissues exhibit uncontrolled inter-sample variability [5] .

This project addresses thus this gap by proposing a rigorous method development by SER-CI with inhouse developed biomimetic matrices. Following ICH Q14 guidelines [6], a dried gelatin-agarose hydrogel substrate was designed to meet predefined performance criteria: spatially uniform analyte distribution, reproducible physicochemical properties, and spectral compatibility with Raman and SERS imaging. Drying kinetics were characterized through mixed-effects modelling and a bimodal Weibull fitting, revealing a two-phase dehydration process [7]. API distribution was assessed by Raman imaging and the use of the Distributional Homogeneity Index (DHI) metric, confirming uniform distribution across the samples [8] .

Using this validated matrix as an analytical platform, systematic optimization of automated spraycoating deposition technique is undergoing to achieve uniform SERS substrate deposition. Following a Quality-by-Design (QbD) framework, critical parameters were investigated, such as nozzle-to-sample distance, nanoparticle flowrate, deposition type and nozzle geometry. SERS acquisition parameters will subsequently be optimized, transitioning from conventional point-mapping to global imaging modes to balance spatial resolution with acquisition throughput. This methodology will establish quantitative performance metrics for spatial reproducibility, and analytical sensitivity, positioning SERS-CI as a complementary imaging technique to conventional methodologies for skin permeation studies in topical product development.

References

1.C. Huo et al., Talanta, vol. 234, (2021), pp. 122678
2.S. Bielfeldt et al., TrAC Trends Anal. Chem., vol. 156, (2022), pp. 116709
3.OECD, Guidance Document for the Conduct of Skin Absorption Studies. (2004). Accessed: Feb. 3rd, 2026. [Online]
4.Zsikó et al., Sci. Pharm., vol. 87, no. 3, (2016) pp. 19
5.S. Lohumi et al., TrAC Trends Anal. Chem., vol. 93, (2017), pp. 183–198
6."ICH Q14 Analytical Procedure Development - Scientific Guideline | European Medicines Agency (EMA)." (2022). https://www.ema.europa.eu/en/ich-q14-analytical-proceduredevelopment-scientific-guideline, last consulted on February 10th 2026
7.B. Janković et al., Chem. Eng. Res.Dev., vol 89, no. 4, (2011), pp. 373-383 8.P.-Y. Sacré et al., Anal. Chim. Acta, vol. 818, (2014), pp 7-14

OP-39

In vitro inhibition assay for human sulfotransferases expressed in Schizosaccharomyces pombe

Annika Lisa Jendretzki[1] , Prof. Dr. Matthias Bureik[2] , Prof. Dr. Maria Kristina Parr[1]

1 Freie Universität Berlin, Institute of Pharmacy, Königin-Luise-Str. 2+4, 14195 Berlin, Germany
2 Tianjin University, School of Pharmaceutical Science and Technology, Faculty of Medicine, 92 Weijin Road, Nankai District, Tianjin 300072, China

Sulfotransferases (SULTs) are enzymes that take part in phase-II metabolism reactions, leading to enhanced water solubility of endogenous compounds or drugs. Other compounds may alter the activity of SULTs or compete for sulfoconjugation which can result in an increased effect and/or a higher risk of adverse drug reactions of therapeutically used drugs metabolized by SULTs. To investigate the inhibition of SULTs, an in vitro assay based on genetically modified strains of the fission yeast Schizosaccharomyces pombe was developed. The yeast cells are modified to contain genetic information coding for the expression of human SULTs and are capable to functionally express the enzymes [1], with each constructed strain expressing one specific human SULT.

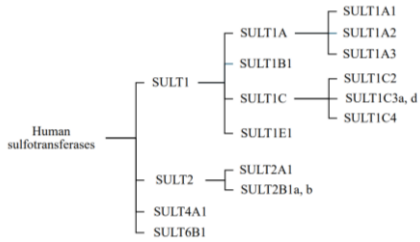

Figure 1. Human sulfotransferase subfamilies available in Schizosaccharomyces pombe

To allow for an efficient screening of inhibitors, it was desirable to find a compound that is accepted as substrate by a majority of human SULTs. As p-nitrophenol and a-naphthol have been reported in literature to be substrates for a wide range of SULTs [1-5], the activity of all human SULTs towards these two substrates was tested. A biotransformation technique using permeabilized cells referred to as “enzyme bags” was employed for the activity assay. This method facilitates the transport of small molecules (such as SULT substrates and inhibitors) through the cell wall and cell membrane while keeping large molecules (such as enzymes) inside [6, 7]. It also allows for production of the cofactor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) in situ by adding ammonium sulfate,

adenosine-5'- triphosphate and magnesium chloride to the reaction buffer, thus minimizing experimental costs [8]. Analysis with high resolution LC-MS demonstrated, that p-nitrophenol is substrate for ten of the fourteen human SULTs while eleven SULTS have been shown to act on a-naphthol. Fortunately, all of the SULTs that fail to metabolize one of the two probe substrates do show activity towards the other. Therefore, all 14 human SULTs are covered. Based on the activity assay, an inhibition assay was performed with p-nitrophenol and a-naphthol. It has been reported that the plant flavonol quercetin has an inhibitory effect on some sulfotransferases such as SULT1A1, 1A3, 1E1 and 2A1[9-11]. Therefore, quercetin was used as a positive control for the inhibition assay. Another compound that inhibits SULT1E1 and was hypothesized to inhibit other SULTs as well is the cyclooxygenase-2 (COX-2) inhibitor etoricoxib [12, 13], which was to be tested in this assay. Quercetin significantly inhibited all fourteen human SULTs to a varying extent, whereas etoricoxib not only showed a significant inhibition of p-nitrophenol sulfation by SULT1E1 (as expected) but also significantly inhibited sulfoconjugation of a-naphthol by either SULT1C3d or SULT4A1.

Table 1. Mean extent of inhibition of sulfoconjugation of p-nitrophenol (p-NP) or a-naphthol (a-N) relative to control (0% inhibition) ± SD

SULT	Substrate	% inhibition by etoricoxib	% inhibition by quercetin
SULT1A1	p-NP	- 8.42 ± 1.62 **	98.39 ± 0.22 ***
SULT1A2	p-NP	- 8.15 ± 3.20 **	95.76 ± 0.75 ***
SULT1A3	p-NP	1.09 ± 2.73 NS.	96.12 ± 0.66 ***
SULT1B1	p-NP	- 3.61 ± 2.84 *	83.77 ± 2.40 ***
SULT1C2	p-NP	- 13.78 ± 9.72 *	69.84 ± 11.03 ***
SULT1C3a	α-N	9.58 ± 21.46 NS.	91.23 ± 6.18 ***
SULT1C3d	α-N	38.06 ± 16.07 ***	96.91 ± 1.88 ***
SULT1C4	p-NP	- 1.06 ± 5.21 NS.	84.83 ± 8.20 ***
SULT1E1	p-NP	32.07 ± 4.95 ***	97.81 ± 0.77 ***
SULT2A1	p-NP	- 27.36 ± 7.17 ***	65.59 ± 5.67 ***
SULT2B1a	p-NP	- 12.77 ± 19.87 NS.	94.06 ± 2.39 **
SULT2B1b	p-NP	- 20.30 ± 8.54 ***	94.13 ± 3.39 ***
SULT4A1	α-N	54.77 ± 10.93 ***	90.41 ± 7.98 ***
SULT6B1	α-N	3.21 ± 4.40 NS.	99.12 ± 1.46 **

NS. – not significant $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, $n = 6$

To the best of our knowledge, the capability of quercetin to inhibit all human SULTs, as well as the inhibitory effect of etoricoxib on SULT1C3d and SULT4A1 are hereby reported for the first time, showing potential of the assay for future discovery of SULT inhibitors.

References

[1] Sun Y, Machalz D, Wolber G, Parr MK, Bureik M. Functional Expression of All Human Sulfotransferases in Fission Yeast, Assay Development, and Structural Models for Isoforms SULT4A1 and SULT6B1. Biomolecules 10 (2020) 1517

[2] Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, Mcmanus ME. Human sulfotransferases and their role in chemical metabolism. Toxicol Sci 90 (2006) 5-22

[3] Brix LA, Barnett AC, Duggleby RG, Leggett B, Mcmanus ME. Analysis of the Substrate Specificity of Human Sulfotransferases SULT1A1 and SULT1A3: Site-Directed Mutagenesis and Kinetic Studies. Biochemistry 38 (1999) 10474-10479

[4] Cook I, Wang T, Leyh TS. Sulfotransferase 1A1 Substrate Selectivity: A Molecular Clamp Mechanism. Biochemistry 54 (2015) 6114-6122
[5] Teubner W. Charakterisierung von Sulfotransferasen im Gastrointestinaltrakt von Mensch und Ratte und Aktivierung von Promutagenen in V79- Zellen, die eine intestinale Form (1B1) des Menschen und der Ratte exprimieren. in, Universität Potsdam, (2001)
[6] Yang F, Machalz D, Wang S, Li Z, Wolber G, Bureik M. A common polymorphic variant of UGT1A5 displays increased activity due to optimized cofactor binding. FEBS Letters 592 (2018) 1837-1846
[7] Weyler C, Bureik M, Heinzle E. Selective oxidation of UDP-glucose to UDP-glucuronic acid using permeabilized Schizosaccharomyces pombe expressing human UDP-glucose 6-dehydrogenase. Biotechnol Lett 38 (2016) 477-481
[8] Sun Y, Harps LC, Bureik M, Parr MK. Human Sulfotransferase Assays With PAPS Production in situ. Frontiers in Molecular Biosciences 9 (2022)
[9] Eaton EA, Walle UK, Lewis AJ, Hudson T, Wilson AA, Walle T. Flavonoids, potent inhibitors of the human P-form phenolsulfotransferase. Potential role in drug metabolism and chemoprevention. Drug Metab Dispos 24 (1996) 232- 237
[10] James MO, Ambadapadi S. Interactions of cytosolic sulfotransferases with xenobiotics. Drug Metabolism Reviews 45 (2013) 401-414
[11] Nishimuta H, Ohtani H, Tsujimoto M, Ogura K, Hiratsuka A, Sawada Y. Inhibitory effects of various beverages on human recombinant sulfotransferase isoforms SULT1A1 and SULT1A3. Biopharmaceutics & Drug Disposition 28 (2007) 491-500
[12] Schwartz J, Hunt T, Smith WB, Wong P, Larson P, Crumley T, Mehta A, Gottesdiener K, Agrawal N. The Effect of Etoricoxib on the Pharmacokinetics of Oral Contraceptives in Healthy Participants. The Journal of Clinical Pharmacology 49 (2009) 807-815
[13] Moore HP, Chen K-F, Heikkinen AT, Neuhoff S, Ezuruike U, Dinh J, Gardner I, Jones HM, Stader F. Investigating Sulfotransferase Mediated Drug Interactions of Ethinylestradiol using a Physiologically Based Pharmacokinetic Model. The AAPS Journal 28 (2025) 13

ABSTRACTS

POSTER PRESENTATIONS

PP-01

Studying LDHB Oligomerization Dynamics by size exclusion chromatography

Océane Bauwens[1] , Caroline Mathieu[2] , Clara Davoine[1] , Johan Wouters[3] , Quentin Spiller[2] , Lionel Pochet[4] , Raphaël Frédérick[2] , Marianne Fillet[1]

1 Laboratory for the Analysis of Medicines (LAM), University of Liege, Belgium
2 Medicinal Chemistry Research Lab, Louvain Drug Research Institute (LDRI), University of Louvain, Belgium
3 Laboratory of Structural Chemical Biology (NARILIS), University of Namur, Belgium
4 Namur Medicine & Drug Innovation Center (NAMEDIC - NARILIS), University of Namur, Belgium

Drug discovery has traditionally focused on active-site inhibition; however, this approach sometimes proves ineffective, particularly for multimeric targets. As a result, alternative strategies are gaining increasing attention, exploiting the close relationship between protein structure and function. Targeting protein–protein interactions (PPIs) has emerged as a promising approach to modulate enzymatic activity by disrupting functional assemblies. While most efforts have focused on heteromeric interactions, homomeric interfaces are now recognized as valuable and largely untapped therapeutic targets.

Lactate dehydrogenase B (LDH-B), a key enzyme in cellular metabolism and cancer progression, exemplifies such a challenging target1,2. Its active site is considered poorly druggable, limiting the success of conventional inhibitors. LDH-B functions as a tetramer, and its enzymatic activity is tightly linked to this quaternary structure. Consequently, interfering with its oligomerization represents an attractive alternative strategy for inhibition. However, accurately characterizing LDH-B self-assembly remains difficult due to the dynamic and reversible nature of subunit interactions. These equilibria are highly sensitive to environmental conditions, including pH, ionic strength, and solvent composition, making experimental analysis particularly complex.

In this study, we developed a robust analytical method combining size-exclusion chromatography (SEC) with multi-angle light scattering (MALS) to monitor LDH-B oligomerization. SEC enables separation of oligomeric species based on hydrodynamic size, while MALS, coupled with UV and differential refractive index detection, provides reliable molar mass determination, allowing precise identification of oligomeric states. Using wild-type LDH-B and targeted single-point mutants, we systematically optimized experimental conditions to resolve distinct oligomeric species while preserving native structure. In parallel, we evaluated the influence of key physicochemical parameters on oligomerization behavior.

Overall, this work provides an analytical framework for studying protein assemblies by SEC and contributes to a better understanding of LDH-B oligomerization dynamics.

References

(1) Thabault, L.; Liberelle, M.; Koruza, K.; Yildiz, E.; Joudiou, N.; Messens, J.; Brisson, L.; Wouters, J.; Sonveaux, P.; Frédérick, R. Discovery of a Novel Lactate Dehydrogenase Tetramerization Domain Using Epitope Mapping and Peptides. J Biol Chem 2021, 296, 100422.

(2) Thabault, L.; Brisson, L.; Brustenga, C.; Martinez Gache, S. A.; Prévost, J. R. C.; Kozlova, A.; Spillier, Q.; Liberelle, M.; Benyahia, Z.; Messens, J.; Copetti, T.; Sonveaux, P.; Frédérick, R. Interrogating the Lactate Dehydrogenase Tetramerization Site Using (Stapled) Peptides. J Med Chem 2020, 63 (9), 4628–4643.

PP-02

Development of an untargeted profiling method for brain metabolomics using supercritical fluid chromatography-mass spectrometry

Sarah Bilal[1] , Ann Van Eeckhaut[2] , Yvan Vander Heyden[1] , Debby Mangelings[1]

1 Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Research group of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Laarbeeklaan 103, 1090 Jette, Belgium
2 Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Research group of Experimental Pharmacology (EFAR), Center for Neurosciences (C4N), Laarbeeklaan 103, 1090 Jette, Belgium

Untargeted metabolomics enables the characterization of comprehensive metabolic profiles without a priori hypothesis, enabling the comparison of healthy and diseased states. This approach may provide insight into underlying biological processes and support the identification of potential biomarkers. Liquid chromatography coupled to mass spectrometry (LC-MS) is widely used to generate comprehensive metabolic profiles. An alternative to LC is supercritical fluid chromatography (SFC), an efficient separation technique that uses supercritical fluids with low viscosity and high diffusivity as mobile phases, allowing rapid and high-resolution separations. To extend the range of analyzable compounds, a unified chromatography (UC)[1] approach, where a gradual transition from a supercritical to liquid mobile phase is made during the analysis, is sometimes applied. Hyphenating SFC with MS enhances detection capabilities and provides better sensitivity and selectivity. [2]

This work aims to establish a robust, untargeted SFC-MS method for the metabolic profiling of plasma samples to identify alterations in metabolites that are associated with neurological disorders.

To develop the method, a representative analyte mixture, comprising 52 neurologically relevant metabolites and covering a log P range of -5 to 10, was prepared. Seven dissimilar stationary phases[3] were systematically screened. Each stationary phase was evaluated using a 65 min linear gradient ranging from 2% till 100% co-solvent. The co-solvent consisted of methanol with 3% water and either ammonium formate, ammonium acetate, ammonium hydroxide, or a combination of ammonium fluoride/ammonium formate. Next, the mobile phase composition was further optimized using a facecentred central composite design with three factors: 1) the ammonium formate concentration, 2) the percentage water content, and 3) the concentration of ammonium fluoride in the co-solvent. Optimal conditions were selected primarily based on the number of separated peaks, but also peak shape, retention behaviour, repeatability and MS response were considered.

The best separation results were obtained on a diol stationary phase using a co-solvent with 20 mM ammonium formate, 0.5 mM ammonium fluoride and 3% water. Using these conditions, 45 compounds were fully or partially separated, eluting within 35 minutes.

Future work will evaluate the necessity of using UC conditions, besides selecting an optimal pretreatment strategy for plasma samples.

References

1. Taguchi K, Fukusaki E, Bamba T. J Chromatogr A. (2014), 1362, 270-277.
2. Desfontaine V, Losacco GL, Gagnebin Y, Pezzatti J, Farrell WP, González-Ruiz V, Rudaz S, Veuthey JL, Guillarme D. J Chromatogr A. (2018) 1562, 96-107.
3. Muscat Galea C, Slosse A, Mangelings D, Vander Heyden Y. J Chromatogr A. (2017), 1518, 78-88.

PP-03

To distinguish degradation products of eumelanin and pheomelanin: a chromatographic approach by HPLC- Vis.

M. Wells[1], P. R. Njanko Tchoujwen[1], C. S. Tchamakeu Yossa[1], B. Blankert[1]

[1] Laboratory of Pharmaceutical Analysis, Faculty of Medicine, Pharmacy and Biomedical Sciences, Research Institute for Health Sciences and Technology, University of Mons, Place du Parc 20, 7000 Mons, Belgium

Melanoma is an aggressive and treatment-resistant skin cancer that results from changes in melanocytes, which alter melanogenesis, particularly by modifying the proportion of eumelanin (EU) and pheomelanin (PM) in cells. This cancer is influenced by factors such as sun exposure and the activity of the MITF transcription factor. An accurate diagnosis of melanoma would be crucial for optimizing patient treatment. [1]

Melanogenesis produces a mixture of eumelanin and pheomelanin at different ratios. This composition is determined by tyrosinase activity. Related to black to brown pigments (mostly in dark skins) and insoluble, EU has antioxidant properties (neutralizes the oxygen reactive species (ROS), photoprotective and counteracts UV damages to the DNA of skin cells) by acting as a natural sunscreen. PM associated with the yellowish to reddish tones has pro-oxidant properties (especially under the effect of the sun), photosensitizing and is soluble in an alkaline medium. Unlike EU, PM is less effective at blocking UV rays, but it can, on the contrary, promote the production of ROS, which can increase the risk of skin damage and predisposition to melanoma

The amount and ratio of EU and PM are the main determinants of hair, skin, and eye color. A balance between these two types of melanin determines the skin's ability to protect itself against UV rays but can also represent a considerable risk factor in the development of melanoma.

The final aim of this study is to elaborate a HPLC-Vis method to distinguish the proportions of PM and EU in melanoma cells to assess their degree of malignancy.

PM and EU are biopolymers with noteworthy characteristics: low solubility and high stability of the bonds between its monomeric units. These properties prevent direct analysis by conventional analytical techniques. To circumvent these limitations, specific methods have been developed to chemically degrade the different forms of melanin, including eumelanin (EM) and pheomelanin (PM), into simpler and well-defined compounds. The analysis of melanin is therefore based on an indirect approach, which consists of identifying and quantifying these degradation products, considered as typical markers of each type of melanin.

In this context, we developed an HPLC-Vis method able to separate in a same run melanin degradation products, namely PTCA (pyrrole-2,3,5-tricarboxylic acid), PDCA (pyrrole-2,3-dicarboxylic acid), for EU; and TDCA (1,3-thiazole-4,5-dicarboxylic acid), TTCA (1,3-thiazole-2,4,5-tricarboxylic acid) for PM. Several relevant chromatographic parameters were studied to improve the experimental protocol. Typically, mobile phase composition, pH, elution gradient program, column length, oven temperature, and flow rate, were optimized to determine the optimal separation leading to a reliable quantification of melanin markers.

In fine, the described method could constitute a solid methodological basis for the development of a UPLC-Vis method dedicated to the analysis of the relative proportions of eumelanin and pheomelanin in complex biological matrices.

References

1. Soumoy L., Wells M., Najem A., Krayem M., Ghanem G., Hambye S., et al., Toad venom antiproliferative activities on metastatic melanoma: Bio-guided fractionation and screening of the compounds of two different venoms. Biology (Basel), 2020,Vol. 9(8), p. 1–16.

PP-04

Development of an ATR-FTIR spectroscopy PLSR model for the rapid screening of quality of enalapril tablets from selected African countries

Billy Chabalenge[1] , Tarsem Sahota, Irina Ermolina and Sangeeta Tanna

1 Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK

Introduction

Poor-quality medicines in Africa remains a major public health challenge, accounting for over 42% of global cases. [1] This is driven by increased disease burden, particularly non-communicable diseases such as hypertension, which have outpaced communicable disease in causing death on the continent. [2] The disease burden has increased the demand for medicines, leading to an influx of unregistered medicines due to poor regulatory oversight. For example, 16.3% of such unregistered medicines are antihypertensive. [3]

To protect public health, there is a need for rapid, simple analytical techniques for screening the quality of medicines in Africa. While conventional analytical methods used in Africa such as HPLC are accurate, they are costly, time consuming and unsuitable for rapid screening in many African settings. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) reduces sample preparation and analysis time. [4] This study aimed at developing a simple and rapid ATR-FTIR chemometric method, validated with UV–Vis spectroscopy, for quantitative analysis of single tablet enalapril maleate samples.

Materials and methods

- Materials: Enalapril reference standard, UK tablets, and African samples. Maize starch and lactose monohydrate were selected as common excipients from the literature.
- Sample Preparation: API-excipient calibration powders (1 – 30% enalapril) were mixed with a mortar and pestle for 90 seconds. Individual tablets were crushed uniformly and analysed directly.
- ATR-FTIR Analysis: Five spectra per calibration mixtures and tablet were recorded (4000 – 400 cm^{-1}, 2 cm^{-1} resolution, 16 scans) using the Bruker Alpha ATR-FTIR. Spectral reproducibility and key API fingerprint peaks were evaluated.
- Chemometric Modelling: Partial least square regression (PLSR) models were built in OPUS software using pre-processing methods; Straight Line Subtraction, Vector Normalization, Minmax Normalization, Multiplicative Scatter Correction (MSC), and First Derivative. Models included whole spectra, fingerprint region, and selected API peak without excipient interference. Performance was assessed by RMSECV, and R^2 values.

▪ Data analysis: Tablet spectra were qualitatively compared with the reference standard. Predicted concentrations were quantified in Excel and validated using a literature UV-vis method.[5]

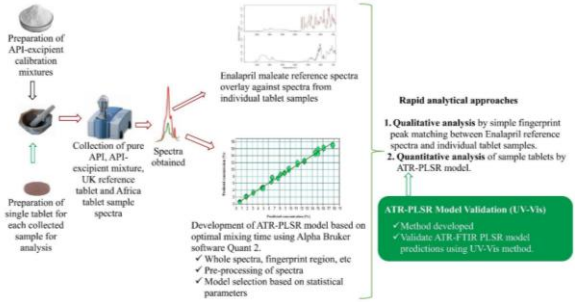

Figure 1. Methodology for preparation of calibration mixtures and analysis of enalapril samples

Results

Qualitative analysis

Not all characteristics enalapril peaks were observed in some African tablet samples, whereas all characteristic peaks were consistently detected in the three UK tablet brands.

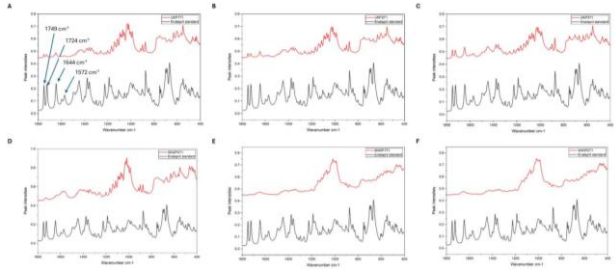

Figure 2. Representative overlay ATR-FTIR spectra in the fingerprint regions for enalapril reference (black) and selected crushed enalapril tablets in red (A) UKP1T1, (B) UKP2T1, (C) UKP3T1, (D) MOZP4T1, (E) MWIP1T1, and (F) MWIP2T1.

Quantitative analysis

Some African tablet samples had predicted enalapril content outside the expected range.

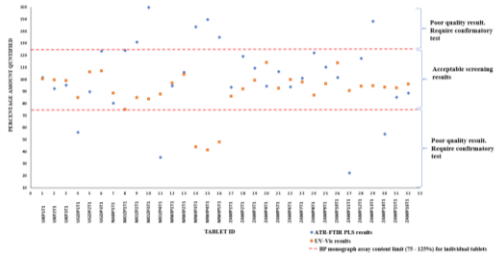

Figure 3. Comparison of percentage calculated amount for all enalapril tablet samples by both ATR-FTIR PLSR and UV-vis analysis methods

Discussion and conclusion

- A simple ATR-FTIR PLSR model was developed for rapid medicine quality screening.
- The optimal model used the whole spectra with first derivative and MSC pre-processing.
- Drug quality was classified based on the ratio of the measured to expected enalapril content: 75 – 125% as good quality and 125% as poor quality based on the content uniformity limit in the BP monograph.
- Out of 29 African samples, 10 (34.48%) were considered poor quality using the ATR-FTIR PLSR model and would require confirmatory testing.
- ATR-FTIR PLSR results above 125% my reflect formulation differences causing API-excipient interaction.
- The ATR-FTIR PLSR simplified sample preparation and reduced analysis time to less than 5 minutes per tablet, compared with over 30 minutes for UV-vis, making it suitable for rapid medicine screening of medicines.

References

1. World Health Organization. WHO Global Surveillance and Monitoring System for Substandard and Falsified Medical Products (2017), Geneva
2. J. Arnlov. The lancet. (2020), 396, 1204
3. B. Asrade Mekonnen, M. Getie Yizengaw, M. Chanie Worku J. Pharm. Policy Pract. (2024), 17, 2375267.
4. G. Lawson, J.Ogwu, S. Tanna. PLoS ONE (2018), 13, e0202059.
5. P.N.V.N Murthy, Y. Anand Kumar. World J. Pharm. Pharm. Sci (2016), 5, 1208 - 1218.

PP-05

Breaking the limits, An ultra-sensitive complementary fragmentation for the confident identification and characterization of drug metabolites

Rahul Baghla[1], Ebru Selen[1], Jason Causon[1], Eshani Galermo[1], Zoe Zhang[1] and Heather Chassaing[2]

1 Sciex , Redwood City,USA
2 Sciex, Luisant, France

Metabolite identification studies are essential to drug discovery and development, and they are often conducted using LC-MS due to their high sensitivity and characterization capabilities. Drug metabolite structures with labile functional groups can often lose information with collision-induced dissociation (CID). Thus, complementary fragmentation methods, such as electron activated dissociation have gained recognition as they preserve the labile functional groups and provide information rich spectra. EAD spectra consist of unique fragments for more reliable drug metabolite identification. Here, an enhanced sensitivity information rich fragmentation technique is demonstrated to achieve increased confidence in metabolite structure assignments.

PP-06

A Multi-Attribute Workflow for Antibody–Drug Conjugate Characterization Using High-Resolution Mass Spectrometry

Laura Corveleyn[1] and Geert Van Raemdonck[1]

1 AnaBioTec, 9940 – Evergem, Belgium

Antibody–drug conjugates (ADCs) are an emerging class of biotherapeutics that combine the specificity of monoclonal antibodies with the cytotoxic potency of small-molecule drugs. The antibodies are directed towards specific surface proteins on cancer cells, allowing precise delivery of the cytotoxic drug directly to tumor cells, thereby maximizing therapeutic efficacy while minimizing systemic toxicity to healthy tissues. A critical quality attribute (CQA) of ADCs is the drug-to-antibody ratio (DAR) distribution, which directly influences pharmacokinetics, stability and therapeutic efficacy. In cysteine-linked ADCs, conjugation occurs predominantly at inter-chain disulfide bonds, generating heterogeneous mixtures of antibody species bearing zero to four pairs of drug conjugates. Accurate characterization of this distribution is essential for process development, quality control, and understanding structure–function relationships.

Multiple Attribute Method (MAM) has emerged as a powerful LC–MS-based approach for the comprehensive characterization of monoclonal antibodies, enabling simultaneous monitoring of multiple CQAs at the peptide level such as post-translational modifications and glycosylations. However, for ADCs, this peptide-level approach could result in information loss such as the drug distribution profile. Therefore, a complementary approach based on intact mass analysis is necessary to provide both quantification of the different conjugated species and monitor the DAR.

In this study, we present a hybrid MAM LC–high-resolution mass spectrometry (LC–HRMS) workflow for the characterization of cysteine-linked ADCs, developed and optimized using a non-toxic antibody–drug conjugate mimic (“ADC-mimic”). In this ADC-mimic, the native cytotoxic payload is replaced with a dansyl-cadaverine–SMCC linker system, introducing a defined mass shift of 668 Da upon conjugation to cysteine residues. The ADC-mimic was measured using reversed-phase chromatography on a Vanquish Flex UHPLC system coupled to an Orbitrap Exploris 240 mass spectrometer operating in an untargeted acquisition mode. Data was analyzed using the Thermo Scientific BioPharma Finder software.

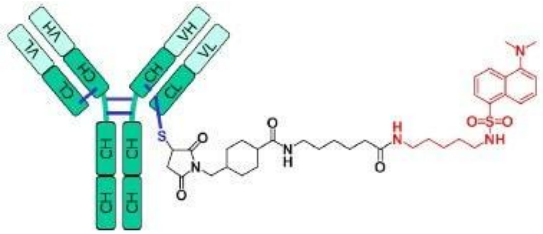

Figure 1. ADC-mimic

Before determination of the accurate masses of the ADC, the antibody was deglycosylated overnight using PNGase F treatment. Different conjugation states were defined after RP-C4 separation coupled to HRMS analysis. The DAR is calculated as the weighted sum of annotated peak intensities of the MS1 spectrum. For bottom-up analysis, the ADC mimic was reduced, alkylated, and digested with trypsin at 37 °C and peptides were analyzed using RP-C18 chromatography coupled to HRMS. After MS2 fragmentation, the precise amino acid containing the drug conjugate could be assigned. In addition, key PTMs relevant to ADC quality were monitored, including oxidation of methionine residues proximal to conjugation sites, deamidation of asparagine residues, aspartate isomerization, and N-terminal pyroglutamate formation. Relative abundances of glycoforms were also evaluated, allowing assessment of glycosylation heterogeneity.

This hybrid MAM approach provides a comprehensive characterization strategy, enabling simultaneous monitoring of site-specific modifications and accurate determination of DAR distribution.

PP-07

Analytical characterization of epigenetic regulation in Alzheimer-like astrocytes: LC-MS profiling of histone PTMs toward early diagnosis and targeted neuroprotection

A. De Simone,[1] E. Esposito,[2] L. Tapella,[3] A. Grolla,[3] M. Cavallo,[4] C. Casali,[4] D. Lim,[3] M. Naldi[5] and J. Fiori [2,6]

1 University of Turin, Department of Drug Science and Technology, 10125 – Turin
2 IRCCS Istituto delle Scienze Neurologiche, Bellaria Hospital, 40139 – Bologna
3 Università degli Studi del Piemonte Orientale, Department of Pharmaceutical Sciences, 28100 – Novara
4 University of Pavia, Department of Biology and Biotechnology "Lazzaro Spallanzani", 27100 – Pavia
5 University of Bologna, Department of Pharmacy and Biotechnology, 40126 – Bologna
6 University of Bologna, Department of Chemistry "G. Ciamician", 40126 – Bologna

Epigenetic mechanisms, including histone post-translational modifications (PTMs), play a central role in regulating gene expression and chromatin organization in the central nervous system. Increasing evidence indicates that dysregulation of histone modifications contributes to the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD), by altering transcriptional programs and cellular responses to stress.[1-3] In parallel, astrocytes have emerged as key regulators of brain function and disease, with their activity and reactivity tightly controlled by dynamic epigenetic programs.[4] However, the molecular characterization of histone PTMs in astrocytes remains limited, particularly at the proteoform level. In this study, we developed and applied integrated top-down and bottom-up LC-MS workflows to profile histone PTMs in immortalized astrocytes derived from a 3xTg-AD mouse model (3xTg-iAstro)[5] and wild-type controls (WT-iAstro). Histones were isolated from nuclear fractions and analyzed using high-resolution mass spectrometry, enabling both proteoform-level and site-specific characterization. Comparative analysis revealed a distinct phosphorylation profile in Alzheimer-like astrocytes, characterized by increased phosphorylation of linker histone H1 variants (H1.1 and H1.2) and reduced phosphorylation of H2A.X, a key component of the DNA damage response.[6] These alterations are consistent with a shift toward chromatin compaction and altered chromatin responsiveness. Overall, this study highlights the power of integrated MS-based approaches for the comprehensive characterization of histone PTMs, provide new insights into astrocyte epigenetic dysregulation in AD, and supports the identification of disease-associated PTM patterns as potential epigenetic biomarkers for early diagnosis and targeted intervention.

References

1. J. Park, K. Lee, K. Kim, S.J. Yi. Sig Transduct Target Ther. (2022), 7, 1.
2. M. Jakovcevski, S. Akbarian. Nat Med. (2012),18, 8.
3. A.J. Bannister, T. Kouzarides. Cell Res. (2011), 21, 3.
4. I.E. Morales Pantoja, C.D. Mintz. Front Mol Neurosci. (2026), 18, 1716805.

5. F. Rocchio, L. Tapella, M. Manfredi, M. Chisari, F. Ronco, F.A. Ruffinatti FA, et al. Cell Death Dis. (2019),10, 1.
6. K.S. Prabhu, S. Kuttikrishnan, N. Ahmad, U. Habeeba, Z. Mariyam, M. Suleman, et al. Biomedicine & Pharmacotherapy. (2024), 175, 116663.

PP-08

Characterization of IgG N-glycan patterns in COVID-19, sepsis and healthy subjects

Victoria Paredes-Orejudo[1,2$] , Yosra Helali[3$], Axelle Bourez[1,2], Alexandre Rousseau[4,5], Pierre Van Antwerpen[1,2] , Karim Zouaoui Boudjeltia[4] , Michael Piagnerelli[4,5], Arnaud Marchant[6] , Cédric Delporte[1,2]

1 RD3 Unit of Pharmacognosy, Bioanalysis and Drug Discovery, Faculty of Pharmacy, Université libre de Bruxelles, Campus Plaine, CP 205/09, 1050 Brussels, Belgium.
2 APFP Analytical platform of the Faculty of Pharmacy, Faculty of Pharmacy, Université libre de Bruxelles, Campus Plaine, CP 205/5, 1050 Brussels, Belgium.
3 CPBL, Crop Production and biostimulation Laboratory, Ecole de Bioingénierie de Bruxelles, Faculté des sciences, Université libre de Bruxelles, Campus Plaine, CP 245, 1050 Brussels, Belgium.
4 Laboratory of Experimental Medicine (ULB 222 Unit), CHU Charleroi-Chimay, Université libre de Bruxelles, Charleroi, Belgium.
5 Intensive Care, CHU-Charleroi Marie-Curie, Université libre de Bruxelles, Charleroi, Belgium.
6 Immunology laboratory, Faculty of medicine, Université libre de Bruxelles, Campus Erasme, CP580/45, 1070 Brussels, Belgium.
$ These authors equally contributed to the work.

Patients with severe infectious diseases may require admission to intensive care units (ICUs) when the infection progresses to severe forms, as observed in sepsis or, more recently, in COVID-19. Despite advances in critical care, the risk of progression toward severe or fatal outcomes remains difficult to predict, highlighting the need for reliable prognostic biomarkers.[1]

Immunoglobulin G (IgG) N-glycosylation is a key modulator of immune responses and may represent a valuable biomarker for assessing disease severity. Other studies demonstrate the N-glycosylation importance on other diseases such as meningococcal sepsis.[2] N-glycans are present on a highly conserved amino acid sequence found on IgG Fc fragment on IgG1, 2 and 4 subtype. On the other hand, IgG3 subtype show two N-glycosylation sequence on the Fc fragment. The different N-glycans each show specific FcγR binding properties. This receptor is especially important because of its proinflammatory properties upon activation by IgG. Different subsets can be found on different effector immune cell and are classified with numbers and letters. Among them, FcγRIIIb show a peculiar activity. N-glycans can also influence the IgG clearance and, by extension, its activity. Indeed, IgG lacking Nglycosylation show a lower affinity for the FcγR. Interaction between IgG Fc fragments and FcγR are important to enable immune responses such as phagocytosis, cytokine production, complementdependent cytotoxicity and antibody-dependant cellular toxicity. [3,4]

In this study, we investigate glycosylation profiles of IgG to evaluate their potential association with clinical outcomes in critically ill patients with sepsis or COVID-19. The study cohort included healthy controls, ICU patients with sepsis or severe COVID-19, the latter being further stratified according to survival outcome.[5,6]

A first approach was performed where serum IgG N-glycans were characterized after N-glycan release using online hydrophilic interaction liquid chromatography (HILIC) solid-phase extraction, followed by HILIC separation and fluorescence and mass spectrometry detection. [5]

Distinct IgG N-glycosylation patterns and relative abundances of specific N-glycan structures in serum were associated with disease etiology (sepsis versus COVID-19) as well as with patient outcomes for COVID-19 group.[7] These findings suggested that IgG glycosylation profiling may provide a promising approach for stratifying ICU patients according to their risk of developing severe or fatal diseases.

In a second approach, we optimized IgG glycopeptide analysis which enable the characterization of IgG subtype glyco-profiling. Analysing only released glycans yields less information especially concerning the glycan compositions and site-specific heterogenicity. By using tryptic digestion, we are able to differentiate the different IgG subtypes (IgG1, 2, 3 and 4). It was shown that those different subtypes might have different involvement in the inflammation pathways.[8] Using HILIC-MS/MS, we can obtain information about the glycan attachment site as well as N-glycan composition of the different IgG. This approach uses total IgG glycosylation in sepsis serum. These analyses will help to understand and refine the role of IgG glycosylation in sepsis.

References

1. Krištić J, Lauc G. Immunol Rev. (2024), 328, 143–70.
2. De Haan N, Boeddha NP, Ekinci E, et al. mBio. (2018), 9, e00546-18.
3. Wang H, Li H, Guo Z, et al. Front Immunol. (2025), 16, 1532145.
4. Vicente MM, Alves I, Gaifem J, et al. Eur J Immunol. (2022), 52, 946–57.
5. Helali Y, Bourez A, Marchant A, et al. Talanta. (2024), 270, 125541.
6. Helali Y, Sharma S, Vandeput M, et al. Methods Mol Biol. (2021), 2271, 57–71.
7. Petrović T, Alves I, Bugada D, et al. Glycobiology. (2020), cwaa102.
8. Zauner G, Selman MHJ, Bondt A, et al. Mol Cell Proteomics. (2013), 12, 856–65.

PP-09

LC-UV method development and validation for identity screening and quantification of selected antihypertensive medicines

H.K. Desta [1,2], A. Van Schepdael [1], G. Ketema [2], E. Adams [1]

[1] KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, 3000 Leuven, Belgium
[2] Wollo University, College of Medicine and Health Sciences, Department of Pharmacy, Dessie, Ethiopia

Antihypertensive therapy often requires lifelong adherence. So, good quality medicines are important for the prevention and management of cardiovascular diseases. However, substandard and falsified (SF) antihypertensive medicines pose a significant public health challenge in resource-limited countries, particularly in sub-Saharan Africa.[1] The use of SF cardiovascular medicines can cause morbidity, mortality, treatment failure, and loss of confidence in health systems, particularly in low income and middle-income countries.[2] The development of easily implementable analytical methods is therefore essential for the detection of SF medicines. High performance liquid chromatography (LC) is the most commonly used technique for quality control of pharmaceuticals.

In this study, a simple, affordable, and robust LC–UV method was developed and validated for the identity screening and assay of 14 antihypertensive compounds (methyldopa, atenolol, hydrochlorothiazide, lisinopril, captopril, metoprolol (tartrate), enalapril (maleate), furosemide, losartan, amlodipine (besilate), nifedipine, carvedilol, spironolactone and candesartan cilexetil) in tablet formulations. Separation was achieved using an XBridge C18 column (250 mm × 3 mm, 5 µm) with gradient elution. Mobile phases A and B contained 4 and 60% of acetonitrile, respectively, and 20 mM phosphate buffer (pH 3.0). The flow rate was 0.5 mL/min and UV detection was performed at 215 nm. The total analysis time was 42 min. The developed method was validated for specificity, sensitivity, range/linearity, accuracy, precision (repeatability and intermediate precision), and robustness. The results confirmed that the method was linear ($r^2 > 0.999$), precise (%RSD < 1 %), accurate (% recovery between 98 and 102%), sensitive, and specific. Robustness was verified using an experimental design. Next, it was successfully applied for the identification and assay of 19 commercial antihypertensive tablets collected from Ethiopia. Among the samples analysed, 16% of the products were SF. The developed and validated LC–UV method proved to be suitable for routine quality control of antihypertensive medicines in resource limited countries, avoiding to setup multiple methods for different compounds. The method can be applied for both identity screening (i.e., confirming the identity of analytes in a sample), and quantification of Active Pharmaceutical Ingredients in single-component or fixed-dose combination formulations. This approach supports efforts to ensure the quality and efficacy of cardiovascular medicines.

References
1. H.K. Hassen, Y.T. Mekasha, A.A. Tegegne, Y. Ozalp. Front. Med. (2024), 11, 1472495.
2. M. Antignac, I.B. Diop, D.M. de Terline, *et al.* J. Glob. Health. (2019), 9, 010308.

PP-10

A simple and affordable LC-UV method for routine quality control of antimicrobial medicines

H. K. Desta[1,2], A. Van Schepdael[1], G. Ketema[2], E. Adams[1]

1 KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, 3000 Leuven, Belgium
2 Wollo University, College of Medicine and Health Sciences, Department of Pharmacy, Dessie, Ethiopia

The circulation of substandard and falsified (SF) medicines remains a significant challenge in developing countries. Antimicrobial medicines, particularly antibiotics, are frequently found to be SF in low-income countries. [1,2] Quality control laboratories often fail to check the samples since no general screening method is available. Pharmacopoeial methods are different for each antibiotic and require different mobile phases, columns, and experimental conditions. Obviously, this is not very practical for the large-scale surveillance of the quality of essential medicines in the market. Therefore, there is a need to develop simple and affordable screening methods for the quality control of antimicrobial medicines.

In this study, a liquid chromatographic method with ultraviolet detection (LC-UV) was developed and validated for the identity screening and assay of 13 orally administered antimicrobial medicines (i.e. azithromycin, amoxicillin, amoxicillin/clavulanic acid, clarithromycin, co-trimoxazole, metronidazole, ciprofloxacin, cloxacillin, norfloxacin, cephalexin, cefixime, cefuroxime, and cefpodoxime proxetil) and 13 injectable antibiotics (i.e. cefepime, amoxicillin, cefazolin, ampicillin, chloramphenicol, ceftazidime, ceftriaxone, cefotaxime, vancomycin, flucloxacillin, cloxacillin, benzylpenicillin and meropenem) in pharmaceutical formulations. LC separation was carried out using gradient elution on a Kinetex C18 (150 mm × 4.6 mm, 2.6 µm) and an XBridge C18 (250 mm x 4.6 mm, 5 µm) column for oral antimicrobial medicines and injectable antibiotics, respectively. For orally administered antimicrobial medicines, the mobile phase consisted of mixtures of acetonitrile (ACN) - water - 10 mM phosphate buffer pH 3.5 at ratios of 3:92:5, v/v/v for mobile phase A and 50:45:5, v/v/v for mobile phase B. For injectable antibiotics, ACN and 20 mM phosphate buffer pH 8 with a ratio of 6:94 (v/v) for mobile phase A and 30:70 (v/v) for mobile phase B were used. The flow rate was maintained at 1.0 mL/min for injectable antibiotics and 0.5 mL/min for oral antimicrobial agents. In both cases, the analysis time was about half an hour. The screening approach was intended for confirmation of the identity of the actives and validated for specificity and robustness, whereas the quantification version was further validated in terms of linearity, accuracy, sensitivity and precision (repeatability and intermediate precision). For all compounds, the determination coefficients (r^2) were greater than 0.999. The relative standard deviations of the peak

areas for both repeatability and inter-day precision were less than 1%. The accuracy was expressed as percent recovery, with values between 98% and 102%. The developed methods were also found to be sensitive and robust. The latter was examined using an experimental design. Next, the methods were applied to analyse 12 samples of orally administered antimicrobial products and 17 injectable antibiotics collected from the Ethiopian market. One product was confirmed to be falsified.

References

1. T. Kelesidis, M.E. Falagas. Clin. Microbiol. Rev. (2015), 28, 443.
2. L. Höllein, E. Kaale, Y.H. Mwalwisi. et al. Anal. Chem. (2016), 76, 60.

PP-11

Electrochemistry–Mass Spectrometry Reveals Known and Unreported Oxidation Products of Olaparib and Inavolisib without Enzymatic Systems

F. Dontsi Tonleu, M. Wesner, N. Marczinczik and U. Karst

University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr.48,48149 Germany

Understanding the fate of drugs in the human body is a key step to ensure therapeutic efficacy, prevent adverse and toxic effects, and meet the regulatory requirements necessary for market authorization. In this study, a fully instrumental approach based on electrochemistry (EC) coupled online with electrospray ionization mass spectrometry (ESI-MS) was employed to mimic the oxidative metabolism of olaparib (Ola), a clinically relevant poly (ADP-ribose) polymerase-1 (PARP-1) inhibitor and Inavolisib (Ina), a phosphatidylinositol-3-kinase alpha (PI3Kα) used in breast cancers treatment. Using the online EC-MS approach, six electrochemical transformation products (ETPs) of Ola and five ETPs of Ina were rapidly generated and identified. Notably, one of the detected products of Ola was unambiguously identified as a known in vivo metabolite reported previously in the literature. In addition, five previously unreported oxidation products were detected for both Ola and Ina, two ETPs of Ola exhibit isomeric forms. To achieve enhanced separation and structural elucidation, liquid chromatography (LC), was integrated in an offline EC-LC-MS workflow and therefore highlights the ability of this approach to reveal metabolically relevant structural diversity. Structural elucidation was based on retention times, m/zratios in positive ion mode, and isotopic pattern analysis. Key metabolic reactions, including hydroxylation, dehydrogenation and C-N bond cleavage, were successfully reproduced. Overall, this study demonstrates that electrochemistry represents a powerful, enzyme-free and complementary tool for drug metabolism investigations, offering rapid access to both known and previously unidentified metabolites and supporting its potential application in early-stage drug development.

PP-12

Optimization of the sample preparation protocol for UHPLCMS/MS analysis of myelin basic protein size isoforms in cerebrospinal fluid

L. Donvil[1,2] , A. Van Eeckhaut[1] and M. Bjerke[2,3]

1 Vrije Universiteit Brussel, Research Group Experimental Pharmacology (EFAR), Center for Neurosciences (C4N), Jette, Belgium
2 Universitair Ziekenhuis Brussel, Department of Clinical Biology, Laboratory of Clinical Neurochemistry, Jette, Belgium
3 Neuroprotection and Neuromodulation (NEUR) Research Group, Center for Neurosciences (C4N), Vrije Universiteit Brussel (VUB), Brussels, Belgium

Myelin Basic Protein (MBP) has been identified as a marker that supports differential diagnosis of various neurodegenerative diseases[1],[2] . Human MBP exists in six different size isoforms, originating from alternative splicing. Of these six isoforms, four are found in central nervous system (CNS) myelin (isoforms 3,4,5 & 6). The aim of this study is to develop an ultra-high performance liquid chromatography - tandem mass spectrometry (UHPLC-MS/MS) method for the quantification of these different CNS MBP isoforms in cerebrospinal fluid (CSF).

All experiments were performed on an ACQUITY UPLC M-Class system with iKey separation device, coupled to a Xevo TQ-XS (all from Waters) run in multiple reaction monitoring (MRM) mode. In silico tryptic digestion of the MPB isoforms using Skyline yielded fivge isoform-specific MBP peptides, which were selected as characteristic markers. The UHPLC-MS/MS method was subsequently optimized using corresponding lyophilized peptide standards. The limits of detection (LOD) were in the 10 pM range for four of the peptides and in the 100 pM range for the fifth.

The sample preparation protocol for the CSF samples included trypsination followed by solid phase extraction for sample clean-up, vacuum centrifugation to dry the samples and reconstitution of the sample before injection. In this study, the first two steps of the protocol were optimized and evaluated by comparing spike recoveries of the peptides. First, different denaturing and reducing agents within the trypsination protocol were compared. The addition of denaturing agents such as guanidium chloride and urea improved the recovery of the targeted peptides, but the differences between the agents were minimal. Reducing the CSF samples with either dithiotreitol (DTT) or tris(2-carboxyethyl)phosphine gave similar results. Quenching the excess of iodoacetamide (which is used as an alkylating agent) with DTT prevented non-specific over-alkylation of amino acids and clearly improved the spike recovery. Second, the solid phase extraction protocol, performed using a Waters Oasis HLB µElution plate, was optimized by adapting the washing and elution solvents. The highest spike recovery was found using water as a washing solvent and methanol as a elution solvent. To determine the optimal sample

pretreatment conditions, additional experiments employing full-length recombinant MBP isoforms will be performed, followed by method validation and subsequent application.

References
1.Hjalmarsson, C., et al. J CNS disease (2014) 6, 51–58. 2.Bjerke, M., et al. JAD (2011) 27(3), 665–676.

PP-13

Mechanisms and Therapeutic Targeting of Aberrant CD81 Surface Expression in Malignancy

Mona Elhammoud[1], Cyril Couturier[1], and Meyling Cheok[1]

1 Univ. Lille, CHU Lille, IRCL, CNRS-UMR9020, Inserm-U1277, Oncolille Institute, Canther - Cancer Heterogeneity, Plasticity and Resistance to Therapies, Lille, France

Acute myeloid leukemia (AML) is a severe clonal malignant blood disorder in which synergistic genetic mutations drive excessive proliferation and survival of immature myeloid cells and suppress normal hematopoiesis[1]. Treatment typically involves intensive combination chemotherapy, usually consisting of cytarabine and an anthracycline, often followed by hematopoietic stem cell transplantation. Although several targeted therapies are now available for selected patients, AML mortality remains high, largely due to treatment resistance and disease relapse, which affect up to 40% of younger and 80% of older adults[2]. Recently, we newly identified the tetraspanin CD81 as an important cell surface determinant of AML progression and treatment resistance which represents a valid druggable target candidate[3]. In this context, our aim is to identify the exact molecular mechanisms of action by investigating two main axes. Firstly, we will screen the different domains and conformations of CD81 in the setting of aggressive cancer cells. In this regard, a set of specific mutants were designed to test the outcome on adhesion, migration and invasion of leukemia cells[4,5]. We expect to detect mutants which promote and others which hamper the level of leukemia aggressiveness. Secondly, we will identify partners of CD81 contributing to the malignant phenotype[6]. We will use the proximity labeling approach APEX3 (Engineered Ascorbate Peroxidase) to reveal proteins physically interacting with CD81 further refined by BRET technology (Bioluminescence Resonance Energy Transfer). With respect to therapeutic targeting, two available anti-CD81 antibodies (i.e., 5A6 and JS81) are able to suppress the metastatic behavior, a phenomenon of global interest in oncology. Therefore, we will also determine CD81 protein partners in the presence of 5A6 and JS81 antibodies separately. In combining the results of these two axes, we expect to reveal not only the mechanism of action on how CD81 induces an aggressive phenotype in leukemia cells but also how treatment with 5A6 and JS81 antibodies induce their anti-leukemic effect[7]. Moreover, we expect to characterize the molecular phenotype of anti-CD81 necessary for novel therapeutic approaches such as peptides or nanobodies based strategies[8]. Alternatively, a molecular model highlighting the aggressive phenotype, can easily be used to screen anti- metastatic small molecules. In summary, this project aims to gain molecular insights into CD81 oncogenic effects which are fundamental to propose biologically validated therapeutic strategies.

References

1. Wachter, F. & Pikman, Y. Pathophysiology of Acute Myeloid Leukemia. *Acta Haematol* **147**, 229–246 (2024).
2. Boyer, T. *et al.* Tetraspanin CD81 is an adverse prognostic marker in acute myeloid leukemia. *Oncotarget* **7**, 62377–62385 (2016).
3. Gonzales, F. *et al.* Tetraspanin CD81 promotes leukemia stem cell function and represents a new therapeutic vulnerability in acute myeloid leukemia. 2023.09.20.558656 Preprint at https://doi.org/10.1101/2023.09.20.558656 (2023).
4. Delandre, C., Penabaz, T. R., Passarelli, A. L., Chapes, S. K. & Clem, R. J. Mutation of juxtamembrane cysteines in the tetraspanin CD81 affects palmitoylation and alters interaction with other proteins at the cell surface. *Exp Cell Res* **315**, 1953–1963 (2009).
5. Palor, M., Stejskal, L., Mandal, P., Shepherd, A. J. & Grove, J. Conformational switching of CD81 controls its function as a receptor for hepatitis C virus. 542837 Preprint at https://doi.org/10.1101/542837 (2019).
6. Müller, S. *et al.* CD44 regulates epigenetic plasticity by mediating iron endocytosis. *Nat Chem* **12**, 929–938 (2020).
7. Abu-Saleh, N., Kuo, C.-C., Jiang, W., Levy, R. & Levy, S. The molecular mechanism of CD81 antibody inhibition of metastasis. *Proc Natl Acad Sci U S A* **120**, e2305042120 (2023).
8. Bailly, C. & Thuru, X. Targeting of Tetraspanin CD81 with Monoclonal Antibodies and Small Molecules to Combat Cancers and Viral Diseases. *Cancers (Basel)* **15**, 2186 (2023).

PP-14

DNA-labelled probe as a potential tool for in-vitro affinity measurements on membrane receptors

Szymon Faliński[1] , Jean-Luc Hayen[1] , Alain Brans[2] , Frédéric Kerff[2,3] , Jean-François Liégeois[1]

1 University of Liège, Laboratory of Medicinal Chemistry, CIRM, 4000 Liège
2 University of Liège, Centre for Protein Engineering, InBios, 4000 Liège
3 University of Liège, Laboratory of Crystallography, 4000 Liège

While approximately 20-30% of human proteome consists of membrane proteins[1] , due to their biological importance they constitute approximately 60% of all drug targets [2][3], despite their fragility and structural complexity[4][5] . There have been techniques developed to mitigate these challenges; however, the method of choice is highly dependent on the characteristics of the target protein, availability of specific equipment, financial constraints, and in-house expertise[6]. In our research targeting small-conductance calcium-activated potassium channels (SK), radiolabeled ligand binding assay is the performed method for drug affinity measurements. This approach is sensitive and possible to use on crude membrane extracts, but it requires a radioactive probe, which is created by modifying a known ligand with hazardous and expensive radioactive isotopes [7]. In the case of SK channels, the labelled ligand is apamin, a peptidic toxin and a potent SK channels blocker[8] . For binding studies, it is iodinated with 125I on the C-terminal His18 residue[9] . In our research, no other non-radioactive alternative has been sufficient to bring enough sensitivity to confidently determine binding values.

To address these limitations, we aim to develop a comparably sensitive probe. It will be based on apamin conjugated with a DNA chain instead of 125I. The DNA tag would serve as a template in a subsequent qPCR step following the binding reaction. Because the quantification cycle (Cq) value directly depends on the amount of DNA[10] , this approach should allow the determination of affinity values for tested drugs in dose-response assays. According to our preliminary qPCR results, DNA-labelled apamin can be detected at concentrations similar to, or even lower than, those typically used in radioligand binding assays, highlighting its potential as a safer and equally sensitive alternative for membrane receptor affinity measurements.

References

1. Almén M. S., Nordström K. J., Fredriksson R., Schiöth H. B., BMC Biol (2009), 7, 50.
2. Overington J. P., Al-Lazikani B., Hopkins A. L., Nat Rev Drug Discov (2006), 5, 993–996.
3. Sood V. D., Gross A. W., Advances in Membrane Proteins: Building, Signaling and Malfunction (2019), , 49–65.
4. Errey J. C., Fiez-Vandal C., Protein Expression and Purification (2020), 169, 105569.
5. Bajaj R., Journal of Structural Biology (2025), 217, 108251.

6. Jong L. A. A. de, Uges D. R. A., Franke J. P., Bischoff R., Journal of Chromatography B (2005), 829, 1–25.
7. Maguire J. J., Kuc R. E., Davenport A. P., Methods in Molecular Biology (2012), 897, 31–77.
8. Kuzmenkov A. I., Peigneur S., Nasburg J. A., Mineev K. S., Nikolaev M. V., Pinheiro-Junior E. L., Arseniev A. S., Wulff H., Tytgat J., Vassilevski A. A., Front Pharmacol (2022), 13, 977440.
9. Habermann E., Fischer K., Eur J Biochem (1979), 94, 355–364.
10. Ruiz-Villalba A., Ruijter J. M., Hoff M. J. B. van den, Life (2021), 11, 496.

PP-15

Paratope-Specific Recognition Biosensing Materials for Real-Time Monoclonal Antibody Detection and Personalized Therapy

Fang Fan, Yu Chen, Yuxin Zhang, Xiaofei Chen

Naval Medical University (Second Military Medical University), School of Pharmacy, 200433 – Shanghai

Monoclonal antibody (mAb) therapeutics are cornerstone treatments for cancers, autoimmune diseases, and infections, however, their efficacy is often compromised by anti-drug antibody (ADA) formation. Accurate and dynamic detection of mAbs and ADAs is essential for early immunogenicity evaluation and individualized dosing. Using the model adalimumab (ADL), we developed a dual biosensing strategy that enables the simultaneous quantification of mAb drugs and ADA. To overcome the lack of paratope-specific recognition elements in mAb assays, a bidirectional positive/negative phage display screening method based on $F(ab')_2$ fragments was established, removing interference from homologous IgG. Using this approach, a specific ADL-paratope recognition peptide, CP1 was successfully identified, demonstrating high affinity with K_D of 7.84 μM. For ADA recognition, an ADL-derived single-chain variable fragment (c-scFv) was used as an ADA paratope-targeting unit. CP1 and c-scFv were conjugated to AuNPs to construct biosensing materials: CP1.1@AuNPs and c-scFv@AuNPs for ADL and ADA detection, respectively, enabling rapid 2-min quantification that highly correlated with results of ELISA detection (R^2 = 0.99). These paratope-specific recognition element-functionalized biosensors were successfully applied for the real-time detection of ADL and ADA in patients with ankylosing spondylitis, supporting individualized therapeutic refinement and offering a practical approach toward the precision management of mAb therapies.

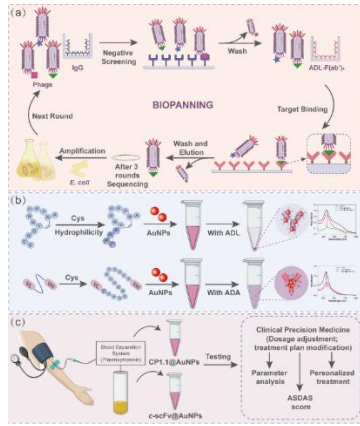

Figure 1. Overview of the screening process for ADL paratope-specific recognition peptides and their functionalization onto AuNPs to enable the precise detection of adalimumab (ADL) and anti-drug antibody (ADA).

PP-16

Extraction and Platform-Dependent Bias Reshapes the Observed Liver Glycolipidome: Implications for Lipidomics Harmonization. A Case Study.

Constanza Fernández-Hernández[1] , Helena Beatriz Ferreira[2,3], Elena Carceller-López[4,5], Patricia Rada[4,5], Francisco J Rupérez[1] , Ángela Valverde[4,5] , M. Rosário Domingues[2,3] and Antonia García[1]

1 Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28660, Boadilla del Monte, Spain
2 Department of Chemistry, Mass Spectrometry Center, LAQV-REQUIMTE, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
3 Department of Chemistry, CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal
4 Instituto de Investigaciones Biomédicas Sols-Morreale (IIBM), Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain
5 Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain

Glycolipidomics has emerged as a specialized field dedicated to the study of glycolipids, amphipathic molecules composed of a hydrophobic lipid moiety and a hydrophilic sugar moiety. Among them, glycosphingolipids (GSLs) represent the major subclass in animals. GSLs are primarily located in the outer leaflet of cellular membranes, driving key cellular signalling pathways. They have garnered great interest due to their associations with lysosomal storage disorders (e.g., Gaucher, Niemann-Pick), neurodegenerative conditions (e.g., Alzheimer's, Parkinson's), cardiovascular complications, and metabolic syndrome (obesity, diabetes, insulin resistance), as evidenced by recent glycolipidomic works linking GSL dysregulation to metainflammation, oxidative stress, and lipotoxicity. Recent studies have demonstrated that the transmembrane protein PKD2 has been implicated in the development of hepatic insulin resistance [1] . Therefore, this study aims to investigate the role of GSLs in liver samples from a PKD2-depleted mouse model compared with wild-type mice, both fed a high-fat diet.

Despite their biological importance, analytical methods used to study GSLs often suffers from structural information loss, due to their low abundance, poor ionization, reduced hydrophobicity and volatility, and high structural diversity. Moreover, no single extraction method currently enables comprehensive recovery of all glycosphingolipid classes[2]. In this context, liquid chromatography coupled with highresolution mass spectrometry (LC-HRMS) is considered as the most powerful approach to address some of these analytical limitations. Here, we report a comparative LC-HRMS workflow to measure GSLs in mouse liver samples, achieving annotation of 128 GSLs species at the MS1 level. These confirmed species are usually hexosylceramides (or HexCer, with one or more sugars moieties), lactosylceramides (LacCer), gangliosides and sulfatides. The study evaluates three key

variables: (1) mass spectrometry analyzers (QTOF vs. Orbitrap), (2) lipid extraction method (Bligh & Dyer vs. MTBE), and (3) biological contexts (PKD2 knockout vs. wild-type mice fed with high-fat diet). No derivatization strategy was used.

Preliminary results indicate that although the Orbitrap analyzer showed lower sensitivity than the QTOF, its higher mass resolution and increased fragmentation cycles facilitated improved structural exploration of GSLs. Consequently, the number of GSLs detected with the Orbitrap was substantially higher than with the QTOF, which detected only three species. Among the confirmed lipid species with Orbitrap, 96 showed differences depending on the extraction method, highlighting the strong influence of sample preparation on glycolipid detection. Regarding biological comparisons, only 10 lipid species were statistically significant between PKD2 knockout and wild-type mice using Bligh & Dyer extraction, whereas 41 were statistically reliable using MTBE extraction, suggesting that MTBE extraction is more suitable for revealing biologically relevant differences in this model.

Overall, this workflow advances liver lipidome characterization by enabling detection of low-abundance glycolipids that are typically difficult to observe by mass spectrometry. Ongoing efforts integrate solidphase extraction to deplete phospholipids/triacylglycerols, curbing ion suppression of GSLs for MS/MSlevel characterization and biomarker discovery.

References

1. P. Rada, E. Carceller-López, A. B. Hitos, B. Gómez-Santos, C. Fernández-Hernández, E. Rey, J. Pose-Utrilla, C. García-Monzón, Á. González-Rodríguez, G. Sabio, A. García, P. Aspichueta, T. Iglesias, Á. M. Valverde Mol. Metab. (2024), 90, 102045.P. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.
2. R. C. Barrientos, Q. Zhang Anal. Chim. Acta (2020), 1132, 134–155.

PP-17

Ecotoxicity study of artemisinin derivative drugs in earthworm

F. Gashe[1,2], S. Suleman S[2], B. Dume[3], B. De Spiegeleer[1], E. Wynendaele[1]

1. Ghent University, Faculty of Pharmaceutical Sciences, Drug Quality and Registration (DruQuaR) group, Harelbekestraat 72, B-9000 Ghent.
2. Jimma University, Laboratory of Drug Quality (JuLaDQ) and School of Pharmacy, PO Box 378, Jimma
3. Jimma University, College of Agriculture and Veterinary Medicine, PO Box 378, Jimma.

According to the WHO, 4.5 billion artemisinin-based combination therapy (ACT) treatment courses have been delivered over the past ten years, mostly to Sub-Saharan African countries.[1] The extensive use of these drugs, particularly in these regions, may therefore pose risks to the ecosystem. Consequently, this study aimed to evaluate the reproductive and biochemical effects of these drugs in *Estenia fetida*. The study was thus carried out on *E. fetida* using artificial soil spiked with artesunate, artemether and dihydroartemsinin at concentrations ranging from 3.12 to 400 mg/kg (dry weight), following OECD guideline.[2] Cocoon production was evaluated on day 28, while hatched juveniles were assessed on day 56. In addition, homogenized tissues were analysed on day 14 to assess the effects of the drugs on selected biomarkers using UV-Vis spectrophotometry. A significant weight reduction was observed for artesunate at a relatively lower concentration of 12.50 mg/kg soil ($p < 0.05$). The drugs also significantly inhibited hatching success at concentrations of 3.12 - 6.25 mg/kg soil (dry weight) compared to the control group ($p < 0.05$). A significantly decrease in superoxide dismutase (SOD) and catalase (CAT) activity was observed at 100 mg/kg, while lower values were also determined at the lowest tested concentration (3.12 mg/kg), although these were not statistically significant ($p > 0.05$). Peroxidase (POD) activity was significantly reduced for all drugs at every tested concentration compared to the control group ($p < 0.05$). Additionally, malondialdehyde (MDA) levels, an indicator of membrane lipid peroxidation, were elevated at all tested drug concentrations. In conclusion, the studied drugs could induce reproductive toxicity and alter biochemical activities in *E. fetida* at concentrations potentially below 3.12 mg/kg soil. Hence, these findings suggest that artemisinin derivatives may pose ecotoxic risks to terrestrial organisms.

Acknowledgements

The authors are thankful to Ghent University (BOF DOS 01W04820) and Jimma University for funding this study.

References

1. WHO. World malaria report. (2024).
2. OECD. Test No. 222. (2004).

PP-18

Wastewater-based epidemiology to monitor gabapentinoid consumption in Belgium: method validation and proof-of-concept

Y. Gridda[1] , A. Muratuly[1] , T. Boogaerts[1] , A. Covaci[1] , A. van Nuijs[1] and C. Gys[1]

1 University of Antwerp , Toxicological Centre, 2610 – Antwerp

Gabapentin and pregabalin, collectively known as gabapentinoids, are anticonvulsant medications prescribed for the treatment of epilepsy and neuropathic pain. [1, 2] Beyond their clinical use, misuse has reportedly increased due to their anxiolytic and sedative properties, and at higher doses, their euphoric effects.[3] Misuse likely occurs in polysubstance contexts where gabapentinoids are combined with opioids, benzodiazepines or alcohol, severely increasing the risk of respiratory depression and fatal overdoses.[4, 5, 6] However, despite increased awareness of this problem, comprehensive data on misuse patterns in Belgium are limited. This project aims to develop and validate an analytical workflow for the detection of gabapentinoids in influent wastewater (IWW) as a first step towards estimating population-level consumption in Belgium.

Instrumental analysis was conducted using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) operating in multiple reaction monitoring (MRM) mode. Chromatographic separation and mass spectrometric parameters were optimized to ensure analytical selectivity and sensitivity. Three transitions per analyte were monitored.

IWW samples will be collected from selected wastewater treatment plants across Belgium in collaboration with Aquafin. Given the complexity of the IWW matrix, dedicated analytical method optimisation was undertaken. Sample preparation was optimized using solid-phase extraction (SPE) evaluating SPE cartridge, pH adjustment, conditioning, washing protocols and elution solvents. Mixedmode cation exchange sorbents demonstrated favourable recovery for both analytes.

A proof-of-concept study on the application of WBE to monitor gabapentinoid consumption across Belgium was performed. IWW samples were collected from five wastewater treatment plants (WWTPs) (Brussel-Noord, Antwerpen-Zuid, Gent, Leuven, Liège Oupeye) representing geographically distinct regions and population catchments in Belgium. 24-h composite sampling was applied to enhance representativeness.

Pregabalin and gabapentin were successfully detected in all these IWW samples, confirming the applicability of the developed analytical method for their identification in a complex matrix. At this stage, the detection represents a qualitative approach, indicating widespread presence of these compounds in IWW across Belgium. As a next step, the study

will proceed with quantitative analysis, enabling the determination of concentration levels and allowing further assessment of spatial and temporal consumption patterns at population level.

The analytical method will be validated in accordance with the ICH guideline M10 on bioanalytical method validation. This validation encompasses evaluating selectivity, linearity (including the limit of quantification (LOQ)), carryover effects, accuracy, precision, different stability conditions, recovery and process efficiency. Following successful validation, the method will be applied to wastewater samples from several geographically distributed locations to investigate spatial patterns in gabapentinoid consumption. Additionally, repeated sampling already conducted at selected sites will allow an assessment of temporal trends in consumption using a wastewater-based epidemiology approach.

References

1.S. Mathieson et al. BMJ (2020), 369, m1315.
2.U. Bonnet, N. Scherbaum. Eur. Neuropsychopharmacol. (2017), 27, 1185-1215.
3.M. Hofmann, M. Besson. Psychiatry Res. (2021), 305, 114193.
4.FDA. Drug Safety Communications (2019).
5.FAGG. Flash VIG-news: Gabapentine (2024).
6.R.V. Smith et al. Addiction (2016), 111, 1160-1174.

PP-19

Spotting Signals: Detection of Quorum Sensing Peptides from Dried Matrix Samples

Hannah Hirmz[1], Petar Naumovski[1,2], Anton De Spiegeleer[2,3], Bart De Spiegeleer[1,2] and Evelien Wynendaele[1,2]

1 Drug Quality and Registration (DruQuaR) Group, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium.
2 Translational Research in Immunosenescence, Gerontology and Geriatrics (TRIGG) group, Ghent University, 9000 Ghent, Belgium.
3 Department of Geriatrics, Faculty of Medicine and Health Sciences, Ghent University, 9000 Ghent, Belgium.

In recent years, dried matrix sampling (DMS) of biological fluids such as saliva, urine and blood has gained increasing popularity in the fields of therapeutic drug monitoring and disease diagnosis. This is due to several advantages, including non-invasive sampling, lower required sample volumes, simplified transport and storage, and reduced cost.[1]

Within our own Saliva and Muscle Study (SaMu), the associations between the salivary microbiome and accelerated muscle ageing are being investigated. Saliva and blood samples are collected from 200 elderly individuals. From the saliva samples, the microbiome composition is determined, and salivary proteomics, metabolomics, and targeted quorum sensing peptidomics are performed.[2] However, as saliva collection in an elderly population can be challenging, the potential of dried matrix spotting was explored in the context of salivary quorum sensing peptidomics.

During method development, a total of 14 selected QSPs were succesfully retrieved from dried saliva spots (DSS), and 16 selected QSPs from dried plasma spots (DPS), highlighting their potential applicability in targeted quorum sensing peptidomics.

References

1. Y. Han, X. Li, M. Zhang, J. Wang, S. Zeng, J. Min. Journal of Pharmaceutical Analysis. (2022), 12, 815823.
2. L. Crombez, A. Descamps, H. Hirmz, M. Lambert, J. Calewaert, D. Siluk, M. Markuszewski, M. Biesemans, G. Petrella, D. Cicero, S. Cesaroni, T. Stokowy, G.K. Gerber, C. Tataru, P. Naumovski, D. Elewaut, C. Van De Looverbosch, P. Calders, N. Van Den Noortgate, B. De Spiegeleer, A. De Spiegeleer. The Journal of Frailty & Aging. (2024), 13, 331340.

PP-20

A stable, user-friendly *Quercus infectoria* extract-loaded phytosome dry powder with potential for pharmaceutical and nutraceutical applications

Chanawee Jakkawanpitak[1,2], Rungphailin Phattana[1,2], Suttiwan Wunnoo[3] and Supayang Piyawan Voravuthikunchai[3]

1 Center of Excellence for Biochemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
2 Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.
3 Center of Antimicrobial Biomaterial Innovation-Southeast Asia, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

Quercus infectoria Olivier (Fagaceae) galls have long been used in Asian traditional medicine and are a rich source of polyphenolic tannins with potent antioxidant and anti-inflammatory activities.[1],[2] These galls have been widely applied in the treatment of wounds and gastrointestinal disorders due to their astringent and protective properties.[3] However, translating these bioactive compounds into stable and user-friendly formulations remains a significant challenge. This study aimed to develop a phytosome-based dry powder delivery system for *Q. infectoria* extract to enhance physicochemical stability and handling convenience while preserving its biological activity. The phytosome formulation was prepared using a phospholipid-based encapsulation method and subsequently converted into a dry powder via freeze-drying. The resulting system was characterized in terms of particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (%EE), and drug loading (%DL). The phytosome exhibited a mean particle size of 688.9 ± 14.2 nm with a low PDI (0.35 ± 0.007) and a zeta potential of -32.51 ± 1.82 mV, suggesting a uniform particle distribution and good colloidal stability. In addition, the system showed high encapsulation efficiency (81.67 ± 1.05%) and drug loading (15.55 ± 0.10%), confirming efficient incorporation of bioactive compounds. To further elucidate molecular interactions, Fourier-transform infrared spectroscopy (FTIR) was performed. The FTIR spectra showed peak shifting, broadening, and changes in intensity without the appearance of new peaks, suggesting predominantly non-covalent interactions between the extract and phospholipid and confirming successful phytosome complex formation. The freeze-dried powder demonstrated good redispersibility and maintained its physicochemical properties upon reconstitution. Antioxidant activity, as evaluated by DPPH and ABTS assays, was effectively preserved after freeze-drying (IC_{50} = 103.07 ± 11.18 and 86.53 ± 5.64 µg sample, respectively), with no significant difference compared to the fresh formulation (IC_{50} = 94.73 ± 6.99 and 75.07 ± 10.32 µg sample, respectively; $p > 0.05$). Notably, the antioxidant capacity remained stable after 30 days of storage at room temperature, demonstrating excellent formulation stability and suitability for long-term storage. In cytotoxicity studies using RAW264.7 macrophages, at an equivalent extract

concentration of 200 µg/mL, the phytosome formulation exhibited significantly higher cell viability (100.35 ± 6.83%) than the free extract (75.50 ± 1.64%), indicating improved cellular biocompatibility. Meanwhile, anti-inflammatory activity, as assessed by inhibition of nitric oxide production in LPS-stimulated RAW264.7 cells, remained comparable between the phytosome (IC_{50} = 141.25 ± 8.20 µg/mL, extract equivalent) and the free extract (IC_{50} = 122.14 ± 12.58 µg/mL, extract equivalent). Taken together, these findings demonstrate that the phytosome-based dry powder formulation provides a stable and user-friendly delivery system for *Q. infectoria* extract with enhanced biocompatibility and preserved bioactivity, supporting its potential as a practical and translatable platform for pharmaceutical and nutraceutical applications.

Figure 1. Preparation and reconstitution of *Q. infectoria* gall (QIG)-phytosome powder. The freshly prepared QIG-phytosome dispersion (left) was subjected to freeze-drying to obtain a dry powder form (middle). Upon rehydration with distilled water, the powder was successfully reconstituted into a homogeneous phytosome dispersion (right), demonstrating the stability and reversibility of the formulation after freeze-drying.

References

1. G. Kaur, H. Hamid, A. Ali, M. S. Alam, M. Athar. J. Ethnopharmacol. (2004), 90, 285–292.
2. S. Wunnoo, D. Sermwittayawong, R. Praparatana, S. P. Voravuthikunchai, C. Jakkawanpitak. Antioxidants. (2024), 13, 1094.
3. A. Elham, M. Arken, G. Kalimanjan, A. Arkin, M. Iminjan. J. Ethnopharmacol. (2021), 273, 113592.

PP-21

A pressure-driven automatic ligand fishing platform for affinitive compounds screening

J. Jian[1], K. Wolfs[1], H. Tian[1,2], Z. Jiang[2], E. Adams[1], A. Van Schepdael[1]

1. Pharmaceutical Analysis, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, O&N2, PB 923, Herestraat 49, 3000 Leuven, Belgium
2. Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, 510632 Guangzhou, China

Many affinity-based screening methods have been developed for rapid and accurate discovery of bioactive compounds from complex natural products. Among these, ligand fishing using ultrafiltration and magnetic beads has been widely applied due to its simple operation and convenient preparation. However, most of the ligand fishing procedures are usually performed manually, which limits efficiency.

In this study, a pressure-driven automatic ligand fishing platform was developed to perform ultrafiltration- and bead-based screening from complex matrices. Customized components were fabricated using 3D printing to assemble the device, which was integrated with a syringe pump. The pressure-driven workflow was established and optimized for both ultrafiltration and micro-silica bead modes. The entire system was automated using a Raspberry Pi to control the syringe pump and stepper motors.

As a proof of concept, the platform successfully performed ligand fishing in both modes for screening known ligands targeting bovine serum albumin. The platform was further applied to screen affinitive compounds targeting pancreatic lipase from *Scutellaria baicalensis Georgi* extracts. This system can be readily adapted for screening ligands to other biological targets.

PP-22

Validation of GC-MS and LC-MS /MS analytical methods for the quantification of phthalates and bisphenols in commercial drinking water from Burkina Faso, West Africa

Jean Luc Téégnimbé KABORE [1,2], Abdoul Karim SAKIRA[3], Basile TINDANO[1]; Axelle Bourez[2]; Valentin Dubois[2], Moumouni BANDE[3]; Nathalie OUEDRAOGO[1,2]; Pierre Van ANTWERPEN[2]; BAYALA Balé[1], Cédric DELPORTE[2]

1 Laboratory of Animal Physiology, Doctoral School of Science and Technology, Joseph KI-ZERBO University, Ouagadougou, Burkina Faso

2 RD3, Pharmacognosy, Bioanalysis and Drug Discovery Unit and Analytical Platform of the Faculty of Pharmacy, Faculty of Pharmacy-ULB, Bld Triomphe, Campus Plaine, CP 205/5, 1050, Brussels, Belgium

3 Laboratory of Toxicology, Environment, and Health, Doctoral School of Health Sciences, Joseph KI-ZERBO University, Ouagadougou, Burkina Faso

The consumption of drinking water sold in plastic containers, including plastic bags, is steadily increasing across both urban and rural regions of Burkina Faso. However, certain chemical compounds used in the manufacture of these plastic materials are suspected of functioning as endocrine disruptors. These substances may migrate into the water, raising concerns about potential health risks for humans. Additionally, high levels of endocrine disruptors particularly bisphenols have frequently been reported in bagged water in plastic packaging in Burkina Faso.[1]

This study aimed to analyze drinking water packaged in plastic bags to identify and quantify potential heavy metals and endocrine disruptors that may contaminate it. Three commercial brands of plastic-bagged drinking water (EC11, EC9, and EC14) were purchased from production units in Ouagadougou. Heavy metal concentrations were determined by atomic absorption spectrophotometry using routine validated methods. For endocrine disruptors, analytical techniques such as GC-MS and LC-MS/MS, combined with the QuEChERS extraction method, were verified and are currently undergoing full validation. Standard solutions of bisphenols (BP) and phthalates (PAE) were prepared in acetonitrile from certified reference materials. Calibration curves were established at nine concentration levels (0.5–1000 µg/L) for BP and six levels (25–800 µg/L) for PAE.

The analysis revealed that the EC11, EC9, and EC14 brands exhibited lead concentrations ranging from 13.5 to 64.1 µg/L and cadmium concentrations ranging from 0.03 to 4.90 µg/L. Lead levels in all samples exceeded the World Health Organization (WHO) guideline limit of 10 µg/L. Furthermore, preliminary findings showed that calibration curves for PAE were linear under optimized conditions. Limits of detection (LOD) ranged from 7.0 to 49.8 µg/L, while limits of quantification (LOQ) ranged from 21.3 to 150.8 µg/L. Intra-day coefficients of variation (n = 3) ranged from 9.0% to 18.3%, demonstrating good analytical repeatability.

For BP, the calibration curve fit a quadratic model. Upcoming analyses of water samples pretreated using the QuEChERS method will provide additional validation parameters to further assess the robustness and reliability of these analytical approaches and to monitor endocrine disruptors in water in plastic packaging in Burkina Faso.

In conclusion, the increasing number of water brands, combined with inadequate storage and preservation conditions, may contribute to the contamination of drinking water by endocrine disruptors released from plastic packaging. Exposure to these contaminants may lead to reproductive disorders in both men and women. The analytical method developed in this study can be proposed to decision-makers as a reliable tool for assessing population exposure to contaminants originating from plastic packaging in Burkina Faso.

Reference

1. Sawadogo B., Konaté F. O., et al, Int. J. Environ. Res. Public Health. (2023), 20(20) :6908

PP-23

Development and validation of an LC–MS/MS method for the quantification of ruxolitinib and its metabolites in plasma: clinical application in patients with graft-versus-host disease

M. Karaźniewicz-Łada[1], J. Kerner[1,2], J. Trąbka[1]

1 Poznan University of Medical Sciences, Department of Physical Pharmacy and Pharmacokinetics, Rokietnicka 3, 60-806 Poznan, Poland
2 Poznan University of Medical Sciences, Doctoral School, Bukowska 70, 60-812 Poznan, Poland

Ruxolitinib is a tyrosine kinase inhibitor that selectively targets Janus kinase (JAK1 and JAK2) which, with signal transducer and activator of transcription, regulates hematopoiesis and modulates the immune system.[1] The drug is used for the treatment of disorders, such as steroid-refractory graft-versus-host disease (GvHD), characterized by a dysregulated JAK/STAT signal transduction pathway and an increased concentration of pro-inflammatory cytokines.[2] Inter-individual variability in response to ruxolitinib treatment may result from altered absorption and metabolism, particularly in patients with GvHD involving the gut and liver.[1] Therefore, monitoring the concentrations of the drug and its biologically active metabolites, M18 and M27, may be useful for predicting treatment response and identifying the risk of treatment failure or intolerance.

The aim of the study was to develop and validate an LC-MS/MS method for determining the concentrations of ruxolitinib and its two main metabolites, M18 and M27, in blood plasma.

Chromatographic separation was systematically optimized, including the selection of the stationary phase, column temperature, and sample preparation solvent. Based on these studies, a Luna Phenyl-Hexyl column was selected, and the mobile phase consisted of 0.1% formic acid in water and acetonitrile applied in a gradient elution mode. Separation was carried out at 40°C. Sample preparation involved protein precipitation with methanol followed by filtration. Analyte detection was performed using a triple quadrupole mass spectrometer following electrospray ionization. The method validation was performed in accordance with ICH M10 guidelines. Calibration curves demonstrated linearity over the concentration ranges of 0.2–200 ng/mL for ruxolitinib and 0.2–20 ng/mL for metabolites M18 and M27, with correlation coefficients (r) ≥ 0.997. The method exhibited acceptable accuracy (89–112%) and precision (CV<13%), with no evidence of carry-over. Ruxolitinib, M18 and M27 were stable in plasma samples for up to 3 h at room temperature, and for 14 days at −80°C, as well as after three freeze–thaw cycles and in the autosampler for up to 24 h. The developed method was successfully applied for analysis of the drug and its metabolites in plasma samples collected from paediatric patients with GvHD treated with ruxolitinib.

The validated LC-MS/MS method is the first analytical assay allowing to determine simultaneously ruxolitinib and its main metabolites M18 and M27 in clinical samples. The method fulfils the validation requirements for quantitative analysis of drugs and their metabolites in biological samples. It is specific, repeatable, reproducible, adequately accurate and precise, and provides a reliable and effictive tool for optimizing dosing to reduce toxicity and improve efficacy.

Acknowledgements: The study was performed as a result of the research project no. 2024/53/B/NZ7/01110 financed by the National Science Centre in Poland.

References

1. T.Y.J. Appeldoorn, T.H.O. Munnink, L.M. Morsink, M.N.L. de Hooge, D.J. Touw. Clin. Pharmacokinet. (2023), 62, 559.
2. V. Escamilla Gómez, V. García-Gutiérrez, L. López Corral, I. García Cadenas, A. Pérez Martínez. Bone Marrow Transplant. (2020), 55, 641.

PP-24

Non-Invasive Assessment of Steroid Metabolism in the Göttingen Minipig Model Exemplified by Ecdysterone

L. Klöppner[1] , P. Fliegans[2] , J.R. Aschenbach[2] , F. Botré[3] , X. de la Torre[3] , A. M. Keiler[4] and M.K. Parr[1]

1 Freie Universität Berlin, Institute of Pharmacy, 14195 Berlin, Germany
2 Freie Universität Berlin, Institute of Veterinary Physiology, 14163 Berlin, Germany
3 Laboratorio Antidoping FMSI, 00169 Rome, Italy
4 Technische Universität Dresden, Faculty of Biology, Environmental Monitoring & Endocrinology, 01217 Dresden, Germany

Objective: Göttingen Minipigs are a specialised pig breed that has been proposed as a model for humans in preclinical trials in various research areas[1] . Minipigs were used in administration trials, employing non-invasive sampling techniques to minimise harm to the animals, thereby aligning with the refinement based on the 3R rule (Replacement, Reduction, Refinement) as defined by Russell and Burch[2] . We aim to establish both a non-invasive sampling procedure and appropriate sample preparation techniques to support the model in future preclinical trials.

Methods: Göttingen Minipigs (n = 6, male) were administered a single oral dose of 50 mg/100 kg body weight of the phytosteroid ecdysterone. Aliquots of the urine samples were collected continuously for the first 12 h, then twice a day until day 5, and once daily in the morning until day 14. The samples were analysed by LC-MS/MS. The parent compound and the expected phase-I metabolites were identified, compared with human data, and the similarities of elimination between the two species were evaluated.

Results: A successful, harm-reduced sample-collection procedure was established to obtain urine samples from the minipigs: Urostomy bags were utilised to ensure a stress-reduced collection. The urine samples were prepared using a dilute-and-inject protocol, as established for the human data[3] and analysed on the LC-MS/MS system. The phase-I metabolism in minipigs resembled that in humans. More precisely, the parent compound, as well as the metabolites poststerone, 14-deoxyecdysterone, and 14-deoxypoststerone, were detected in porcine urine samples. Additionally, the parent compound had a similar detection window, with the maximum concentration detected at 2-6 h post-administration, similar to the 2.8-8.5 h observed in humans [3] .

Conclusion: These findings suggest that the model is practical and feasible for implementation in future clinical trials investigating drug metabolism. Therefore, the potential to enhance the accuracy and efficacy of translational research was expanded.

References

1. Forster, R., G. Bode, L. Ellegaard, and J.W. van der Laan, The RETHINK project: Minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment. Journal of Pharmacological and Toxicological Methods, 2010. 62(3): p. 158-159.

2. Russell WMS, B.R., The principlesof humane experimental technique. 1959 (as reprinted 1992): Wheathampstead (UK): Universities Federation for Animal Welfare.

3. Ambrosio, G., et al., Urinary Elimination of Ecdysterone and Its Metabolites Following a Single-Dose Administration in Humans. Metabolites, 2021. 11(6).

PP-25

Comparison of physicochemical parameters determined for albumin nanoparticles encapsulated with various anticancer ligands

K. Kulig[1], B. Morak-Młodawska[2], M. Jeleń[2], M. Ziąbka[3] A Owczarzy[1], W. Rogóż[1], M. Maciążek-Jurczyk[1]

[1]Medical University of Silesia in Katowice, Faculty of Pharmaceutical Sciences in Sosnowiec, Department of Physical Pharmacy, 41-200 Sosnowiec, Poland
[2]Medical University of Silesia in Katowice, Faculty of Pharmaceutical Sciences in Sosnowiec, Department of Organic Chemistry, 41-200 Sosnowiec, Poland
[3]AGH University of Krakow, Faculty of Materials Science and Ceramics, Department of Ceramics and Refractories, 30-059 Kraków, Poland

Among the natural polymers used for the synthesis of nanoparticles (NPs), proteins are the most commonly employed. One of the most widely used proteins as a potential carrier for therapeutic agents is albumin. Albumin is the most abundant protein in serum. It is characterized by biocompatibility, biodegradability, and the ability to bind exogenous substances. Albumins are a group of globular proteins responsible for binding and transporting endogenous and exogenous molecules in the bloodstream. The most commonly used forms for pharmaceutical applications are human serum albumin (HSA) and bovine serum albumin (BSA) [1-3].

The aim of the project was to develop a formulation of albumin (HSA and BSA) NPs modified by a desolvation method, with glutaraldehyde as a crosslinking factor, encapsulated with various anticancer ligands. The ligands encapsulated in albumin were drugs approved for use in the European Union (chlorambucil (CLB), 5-fluorouracil (5-FU)), as well as substances with potential anticancer activity (10H-2,7-diazafenothiazine (2,7-DAPT), 10-(2′-pyrimidyl)-3,6-diazaphenothiazine (10-Pyr-3,6-DAPT), and 6-acetylaminobutyl-9-chloroquino[3,2-b]benzo[1,4]thiazine (QBT)).

Quantitative analyses were performed using a spectrofluorometer (JASCO FP-6500) and a UV-Vis spectrophotometer (JASCO V-760). The size and morphology of the resulting nanoparticles were measured using scanning electron microscopy (SEM) (Nova Nano SEM 200 and Apreo 2 S). Circular dichroism (CD) (JASCO J-1500) spectra of NPs as well as native HSA and BSA were recorded using a JASCO J-1500 spectropolarimeter.

With the exception of 2,7-DAPT (<80%), the encapsulation efficiency of ligands in albumin NPs was over 90%. Albumin NPs in the presence of ligands were larger than their counterparts in the absence of ligands. The largest sizes were in the following order: CLB-NPs, 2,7-DAPT-NPs, QBT-NPs and the sizes of the carrier controls for these systems were similar. Based on the SEM analysis it has been concluded that the samples underwent aggregation and degradation under the influence of the electron beam. All samples

exhibited a gradual release of the ligand over time. The low values of the cumulative percentage (%) of released ligand at the first time points were significantly lower than at each subsequent time point. At time points from 24 to 48 hours, a further increase in the amount of released ligand was observed; however, none of the samples released 100% of the ligand. The desolvation method resulted in a reduction in the number of secondary structures, and this phenomenon was also influenced by the high concentration of the encapsulated drug.

Using a modified desolvation method according to a proprietary protocol allows the formation of nanoscale structures capable of releasing ligands. Further studies, including *in vitro* studies on cell lines, are necessary to determine dosage and treatment response.

Funding: This study was supported by BNW-2-028/K/5/F, BNW-2-027/K/5/F and BNW-1-027/K/5/F from the Medical University of Silesia, Poland.

References

1. K. Kulig, M. Ziąbka, K. Pilarczyk, A. Owczarzy, W. Rogóż, M. Maciążek-Jurczyk. Processes. (2022), 10(6), 1170.
2. K. Kulig, B. Morak-Młodawska, M. Jeleń, M. Ziąbka, A. Owczarzy, W. Rogóż, M. Maciążek-Jurczyk. J Clust Sci. (2024), 35, 2353-2362.
3. K. Kulig, P. Sarkowicz, M. Jeleń, B. Morak-Młodawska, M. Ziąbka, A. Owczarzy, W. Rogóż, M. Maciążek-Jurczyk. Colloid Polym Sci. (2026), 304, 617–627.

PP-26

Comparison of two chiral derivatization reagents for the enantioselective analysis of amino acids through UHPLC-MS/MS

C. Lella[1], U. Hennecke[2], Y. Vander Heyden[3] and A. Van Eeckhaut[1]

1 Vrije Universiteit Brussel (VUB), Department of Pharmaceutical and Pharmacological Sciences, Experimental Pharmacology Research Group (EFAR), Center for Neurosciences (C4N), 1090 - Brussels, Belgium
2 Vrije Universiteit Brussel (VUB), Departments of Chemistry and Bioengineering Sciences, Organic Chemistry Research Group (ORGC), 1050 - Brussels, Belgium
3 Vrije Universiteit Brussel (VUB), Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling (FABI), 1090 - Brussels, Belgium

D-amino acids have emerged as potential biomarkers for a wide range of neurological and metabolic disorders. Thus investigating their distribution is crucial for understanding both physiological processes and pathological conditions. However, the quantification of these molecules in mammalian tissues and fluids remains an analytical challenge, because of their trace concentrations and the need for robust enantioselective discrimination for each D/L pair.[1]

In this context, we evaluated two chiral reagents, diacetyl-L-tartaric anhydride (DATAN) and its dibenzoyl derivative (DBTAN) for their ability to separate the amino acid enantiomers on a C18 stationary phase, using ultra-high-performance liquid chromatography - electrospray ionization - tandem mass spectrometry (UHPLC-ESI-MS/MS). Our approach is based on a pre-column derivatization step with an enantiopure chiral reagent to convert the enantiomers into diastereomers, enabling their separation on an achiral stationary phase, while enhancing both sensitivity and metabolite stability. Moreover, the influence of the mobile phase composition on the chiral separations was investigated.

The derivatization was carried out on standard solutions of amino acids, evaporated to dryness, and then incubated with (+)-DATAN or (+)-DBTAN solution at 75°C for two hours. The derivatized solutions were again subjected to evaporation, and then solubilization and dilution in water with 0.1% of formic acid.[2] The analysis was performed on a UHPLC system coupled to a Xevo TQ-MS (Waters) in positive ESI mode. We used an Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 µm, 130 Å) at a temperature of 60°C and a flow rate of 0.3 mL/min. Various mobile phase combinations were investigated to optimize chiral resolution. The aqueous phase was composed of either 0.1% formic acid (A1) or 10 mM ammonium formate with 0.06% formic acid (A2); the organic phase of acetonitrile (B1) or methanol (B2), both with 0.1% formic acid. Instrumental parameters, including capillary voltage, cone voltage, collision energy, and MRM transitions, were individually optimized for each analyte under

each tested chromatographic condition. The results show that the A2B1 mobile phase combination yielded the best performance in terms of chiral resolution. Comparing the two reagents, DBTAN provided better chromatographic retention, enabling the use of a higher organic content for the elution, which leads to an improvement in droplet desolvation and ionization efficiency in the ESI source. Among the tested amino acids, ten provided successful baseline separation, seven were partially resolved, whereas three exhibited co-elution of the enantiomers.

To further improve the chiral separation of the amino acids, alternative stationary phase chemistries are studied.

References

1. C. Lella, L. Nestor, D. De Bundel, Y. Vander Heyden, A. Van Eeckhaut Int. J. Mol. Sci. (2024), 25, 22.
2. R. Pandey, M. Collins, X. Lu, S.R. Sweeney, J. Chou, A. Lodi, S. Tiziani Anal Chem. (2021), 93, 14.

PP-27

Engineered Subcellular Transmembrane Protein Biomimetic Platform for in situ Drug Discovery

Yue Liu [1] and Xiaofei Chen [1]

Naval Medical University, Department of Pharmacy, 200433 –Shanghai

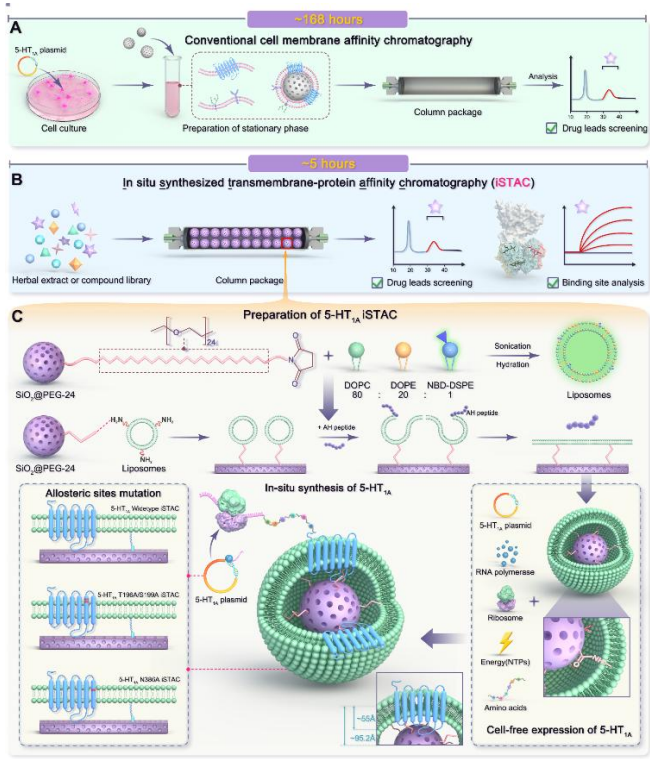

Figure 1. General overview of the ISTAC workflow.

Subcellular membrane environments pose major challenges to target-oriented drug discovery due to the structural complexity and dynamic conformational transitions of transmembrane proteins. Conventional affinity chromatography techniques are limited by prolonged preparation, lack of binding-site resolution, and interference from endogenous

membrane proteins. This study introduces in situ synthesized transmembrane-protein affinity chromatography (iSTAC), a biomimetic platform integrating cell-free protein synthesis, engineered silica-phase modification, and tethered lipid bilayers to reconstitute functional multipass transmembrane receptors under native-like conditions. iSTAC enables rapid column preparation within 5 h (a 33.6-fold reduction in time), while preserving structural integrity and conformational dynamics. This facilitates high-specificity drug screening and binding-site analysis. We use the 5 hydroxytryptamine receptor 1A (5-HT1A) as a model to identify two natural agonists, crocin I and crocin II, from herbal extracts and validated their anti-insomnia and neuroprotective effects in vivo. Mutational iSTAC analysis precisely maps their binding interactions at the residues N386, T196, and S199, demonstrating that both compounds activate 5-HT1A via canonical signaling pathways. iSTAC provides a versatile, high-specificity platform for in situ drug screening and binding-site analysis, with broad applicability to structurally complex transmembrane proteins.

PP-28

Functionalized biomimetic materials for capillary electrophoresis: modulating electroosmotic flow and protein interactions for nanobody analysis

Tristan Corbalan[1] , Margaux Clavié[1] , Catherine Perrin[1] , Mehdi Ahmad[2] , Quentin Lorenzi[1] , Pierre André Lafon [3] , Philippe Rondard[3] , Gilles Subra[1] , Yoann Ladner[1]

1 IBMM, Univ. Montpellier, CNRS, ENSCM, 34090 Montpellier
2 ICGM, Univ. Montpellier, CNRS, ENSCM, 34090 Montpellier
3 IGF, Univ. Montpellier, CNRS, Inserm, 34090 Montpellier

The quantification of molecules in biological media presents substantial analytical challenges due to the complexity of the matrices involved. Target analytes are often present at extremely low concentrations, requiring highly sensitive analytical methods, a constraint that also applies to nanobodies, which are typically available in limited amounts in such samples while remaining of strong interest for their therapeutic potential. Interferences arising from various matrix components may also introduce quantification errors, making the use of specific and reliable methods essential.

A major challenge also lies in the analysis of small-volume samples, particularly in the case of biological fluids such as cerebrospinal fluid, where the amount of available sample can be limited. In this context, there is growing interest in the development of molecularly imprinted polymers (MIPs) within the fields of analytical sciences and diagnostics.

Recently, our team developed a biomimetic method based on silylated amino acid derivatives designed to mimic the specific recognition occurring in biological environments by forming cavities complementary in shape and composition to the template analyte while preserving its native conformation. The polymerization of these silylated amino acids proceeds chemoselectively under non denaturing conditions for the protein, using, leucine, arginine, serine, and tyrosine, which are highly represented on antibody paratopes surfaces [1,2].

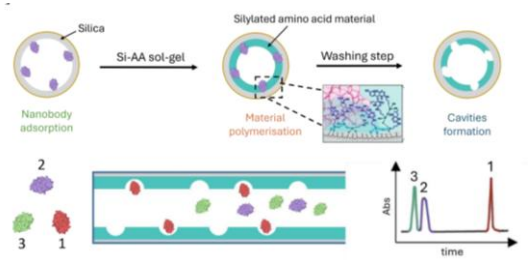

Figure 1: (A) Synthesis of the molecularly imprinted material inside a capillary, (B) Separation of proteins via specific capture of the target

The resulting hybrid imprinted materials, synthesized in 60min within capillaries of 30 µm internal diameter, were evaluated by capillary electrochromatography after injection of 5 nL of sample. Initial analyses were performed on model proteins in a phosphate buffer solution at pH 7, demonstrating excellent specificity of the MIP for the targeted molecules. Subsequent stages of the project confirmed the effectiveness of this material toward a therapeutically relevant molecule, the nanobody Nb-DN1, following optimization of the analytical method such as adsorption and polymerization times or the speed of the analyte inside the capillary, leading to an imprinting factor of 5. These results can be put into perspective with those reported in literature, that show imprinting factor of 1.9 for the capture of Lysozyme [3].

References

1. Gouyon J, Clavié M, Raquel GC et al. A bioinspired approach for the modulation of electroosmotic flow and protein–surface interactions in capillary electrophoresis using silylated amino-amides blocks and covalent grafting. Electrophoresis 2024;45(5–6):557–72.
2. Gutiérrez-Climente R, Ngo G, Clavié M et al. Sol-gel polymerization of silylated amino acids around a protein template yields selective biomimetic imprints. Materials Today Chemistry 2023;27:101317.
3. Lin Z, Lin Y, Sun X et al. One-pot preparation of a molecularly imprinted hybrid monolithic capillary column for selective recognition and capture of lysozyme. Journal of Chromatography A 2013;1284:8–16.

PP-29

A sensitive and selective immunoaffinity LC-MS/MS assay for a therapeutic antibody in monkey serum

T. Sano[1], T. Ishii[1], and Y. Mano[2,3]

1 Sunplanet Co., Ltd., DMPK & Bioanalysis Unit, 300-2635 – Tsukuba, Ibaraki, Japan
2 Eisai Co., Ltd., Global DMPK, 300-2635 – Tsukuba, Ibaraki, Japan
3 University of Tsukuba, Graduate School of Comprehensive Human Sciences, 305-8575 – Tsukuba, Ibaraki, Japan

The development of therapeutic antibodies has been steadily increasing, highlighting the need for robust analytical assays to characterize their pharmacological and toxicological properties based on pharmacokinetic (PK) data. Traditionally, ligand binding assays (LBAs) have been employed for this purpose; however, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has recently been utilized as an alternative assay platform. When applying LC-MS/MS to protein therapeutics, challenges related to selectivity and sensitivity often arise.

We previously developed PK assays for E6011, a novel human anti-fractalkine antibody, in monkey serum [1, 2] and encountered both of these challenges. To address them, we established and optimized an immunoaffinity-based LC-MS/MS (IA-LC-MS/MS) method.

In this assay, E6011 in monkey serum was captured in a 96-well streptavidin plate using either the biotin-labelled target antigen or an anti-drug antibody (ADA). After washing the wells with acid to release E6011, the sample was reduced with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin to generate a signature peptide specific to E6011. The peptide was then detected using UPLC-MS/MS with multiple reaction monitoring under positive electrospray ionization mode.

To evaluate potential interference from endogenous substances and to explore the feasibility of achieving higher sensitivity, various volumes of monkey serum were tested. Minimal interference was observed regardless of whether the target antigen or ADA was used as the capture reagent. The analyte's peak area increased linearly with sufficient signal-to-noise ratios, and the lower limit of quantification was established at ≤ 0.02 µg/mL. The method was qualified through a reproducibility study assessing accuracy and precision of quality control samples. The IA-LC-MS/MS assay is applied to determine E6011 concentrations in monkeys following intravenous administration. The results are compared with those obtained using the Gyrolab-LBA platform. These findings will be presented at the meeting.

References

1. K. Kita, H. Ono, T. Kojima and Y. Mano. J Pharm Biomed Anal. (2025), 255, 116659.
2. K. Kita and Y. Mano. J Pharm Biomed Anal. (2026), 271, 117334.

PP-30

Quality Control Laboratories in the Democratic Republic of Congo: Challenges and Opportunities

D. Kabamb[1,2*], M. Muhona[1,*], S. Kalala[2], P. Mwamba[3], V. Bashige[1], JB. Kalonji[3], H. Mania[1], B. Masereel[4], M. Borive[5], J. Mbinze[6], F. Musuamba[1,4], R. Marini[7], P. Okusa[1]

1 Laboratoire de Chimie thérapeutique, Department of Pharmacology, Faculty of Pharmacy, University of Lubumbashi-Lubumbashi, DR Congo
2 Autorité Congolaise de Réglementation Pharmaceutique (ACOREP), DR Congo
3 Department of Galenic and Drug Analysis, Faculty of Pharmacy, University of Lubumbashi, Lubumbashi, DR Congo
4 Unité de Recherche en Pharmacologie et Toxicologie Clinique, Department of Biomédical and Pharmaceutical Sciences, Faculty of Medicine, University of Namur, Namur, Belgium
5 Laboratory of Drugs Analysis, Faculty of Pharmacy, University of Kisangani, Kisangani, DR Congo
6 Faculty of Pharmacy, University of Kinshasa, Kinshasa, DR Congo
7 Departement of Pharmacy, Faculty of Medicine, University of Liège, Liège, Belgium
*Both authors have contributed equally to this work

Quality control (QC) laboratories are essential components of pharmaceutical regulation and public health systems, ensuring the safety, efficacy, and quality of medical products. In low- and middle-income countries, weaknesses in QC systems contribute to the circulation of substandard and falsified medicines, posing significant health risks [1]. In the Democratic Republic of Congo (DRC), despite a rapidly expanding pharmaceutical market and the establishment of a national regulatory authority, QC laboratory capacity remains limited, fragmented, and insufficiently standardized.

This study aimed to assess the current status of QC laboratories in DRC, analyze the regulatory environment, and identify strategic approaches for strengthening national quality control systems. A narrative review combined with a regulatory policy analysis was conducted using national regulatory data from ACOREP (Autorité Congolaise de Réglementation Pharmaceutique), institutional reports, official records of licensed and certified pharmaceutical establishments, and relevant international standards and scientific literature.

The findings reveal critical gaps between licensing and certification practices. While pharmaceutical establishments are formally authorized to operate, only 3 out of 32 manufacturers (9%) and 6 out of 196 wholesalers (3%) are certified, and no pharmaceutical quality control laboratory is currently accredited according to ISO/IEC 17025. Multiple systemic challenges were identified, including inadequate infrastructure, limited analytical equipment, human resource constraints, and weak regulatory oversight, consistent with observations in other sub-Saharan African settings [2].

A pragmatic and phased approach is proposed, using WHO Good Practices for Pharmaceutical Quality Control Laboratories (GPPQCL) as an entry-level framework, with progressive alignment toward ISO/IEC 17025 and integration of relevant Good Manufacturing Practice (GMP) principles. In parallel, strengthening opportunities such as capacity building, regional regulatory integration, digitalization of laboratory systems, and public private partnerships could further accelerate the development of a sustainable and effective QC system. This approach provides actionable policy guidance for strengthening QC systems in DRC and offers transferable insights for other resource-limited settings.

References

1.WHO. Substandard and falsified medical products. Geneva: WHO; 2024.

2.M. Ndomondo-sigonda, J. Miot, S. Naidoo, Medicines Regulation in Africa : Current State and Opportunities, Pharmaceut. Med. 31 (2017) 383–397.

PP-31

Availability and Quality Assessment of Antimalarial Medicines Marketed in Lubumbashi, DR of Congo

M. Muhona[1,2], T. Kalasa[1], R. Kilela[1,2], P. Mwamba[3], V. Bashige[1], H. Mania[1], JB. Kalonji[1], B. Masereel[2], F. Musuamba[1,2], P. Okusa[1]

1 Université de Lubumbashi, Faculté des Sciences pharmaceutiques, Laboratoire de Chimie thérapeutique, RDC
2 Université de Namur, Unité de Recherche en Pharmacologie et Toxicologie Clinique
3 Université de Lubumbashi, Faculté des Sciences Pharmaceutiques, Laboratoire de Galénique et Analyse des médicaments

Ensuring the quality of antimalarial medicines is essential for effective malaria control, particularly in low-resource settings where substandard and falsified products may circulate [1]. Rapid and accessible analytical approaches are needed to support pharmaceutical surveillance [2].

A cross-sectional survey was conducted in 36 pharmaceutical wholesale establishments in Lubumbashi (DRC), where 82 antimalarial products were recorded and classified according to their international nonproprietary names (INN), pharmaceutical forms, and origins. Five artemether–lumefantrine samples were selected for quality evaluation. Pharmaco-technical tests, including visual inspection, weight uniformity, friability, and disintegration, were performed according to standard procedures. Qualitative identification was carried out using colorimetric reactions, while quantitative determination of lumefantrine was performed using UV–Visible spectrophotometry at 240 nm.

Artemether–lumefantrine was the most represented antimalarial (n = 30; 36.6%), followed by injectable artesunate (n = 27; 32.9%). Non-recommended products, including artemisinin monotherapies, were identified. All tested samples complied with pharmacopeial specifications for mass uniformity and friability. The assay of lumefantrine showed content ranging from 92.4% to 98.3% of the labeled claim. However, variability in disintegration times was observed between samples.

The study demonstrates that basic pharmaco-technical tests combined with UV–Visible spectrophotometry can provide a rapid and cost-effective approach for the quality assessment of antimalarial medicines. While overall compliance was observed, the presence of non-recommended products and variations in galenic performance highlight the need for strengthened pharmaceutical surveillance in resource-limited settings.

References

1 S.S. Nundu, S. V. Simpson, H. Arima, J.-J. Muyembe, T. Mita, S. Ahuka, T. Yamamoto, It Is Time to Strengthen the Malaria Control Policy of the Democratic Republic of Congo and Include Schools and School-Age Children in Malaria Control Measures, Pathogens 11 (2022) 729. https://doi.org/10.3390/pathogens11070729.

2 M.D. Green, D.M. Hostetler, H. Nettey, I. Swamidoss, N. Ranieri, P.N. Newton, Integration of Novel Low-Cost Colorimetric , Laser Photometric , and Visual Fluorescent Techniques for Rapid Identification of Falsified Medicines in Resource-Poor Areas : Application to Artemether – Lumefantrine, Am. J. Trop. Med. Hyg. 92 (2015) 8–16. https://doi.org/10.4269/ajtmh.14-0832.

PP-32

Broadening Accessibility: a RP-LC-UV Method for PNGase F Activity Characterization

W. Mensah[1] , M. Naldi[1] , S. Peljhan[2] , and M. Bartolini[1]

1 Department of Pharmacy and Biotechnology, University of Bologna, 40126- Bologna (Italy)
2 Sartorius BIA Separations d.o.o, Mirce 21,5270- Ajdovščina (Slovenia)

Enzyme immobilization (IMER) has become a powerful tool in pharmaceutical bioanalysis, enabling improved enzyme stability, reusability, and compatibility with automated workflows. In this framework, robust and quantitative methods for enzyme activity assessment are essential to ensure reliable IMER development, including accurate determination of immobilization yields and process optimization. [1]

PNGase F (Peptide-N-Glycosidase F), an asparagine amidase widely used for N-glycan release, plays a central role in glycoprotein characterization in biopharmaceutical and clinical settings. The implementation of PNGase F-based IMERs offers significant advantages in terms of enzyme consumption and workflow efficiency. However, the lack of accessible, reproducible activity assays remains a critical bottleneck. [2]

State-of-the-art PNGase F activity assays are based on the deglycosylation of Ribonuclease B (RNase B), typically monitored by PAGE or mass spectrometry (MS). While PAGE suffers from limited resolution and reproducibility, MS provides high sensitivity and selectivity but requires advanced instrumentation and specialized expertise, limiting its routine applicability. [3] [4]

Here, we report a robust and accessible method for PNGase F activity determination based on reversephase high-performance liquid chromatography with UV detection (RP-HPLC-UV). The use of a widepore HALO C4 column (1000 Å) with superficially porous particle technology, combined with an optimized gradient, enabled efficient separation of enzyme, substrate, and deglycosylated product, overcoming the typical on/off elution behaviour of proteins.

Two complementary analytical workflows were developed: (i) an in-solution assay using denatured RNase B for enzyme activity determination and immobilization yield evaluation; and (ii) an IMERoriented assay using denatured and alkylated RNase B, allowing the assessment of catalytic performance and the estimation of kinetic parameters (Vmax and Km). Experimental conditions were systematically optimized, including substrate pre-treatment and reaction quenching.

Method performance was cross-validated against an MS-based reference assay, showing comparable results in terms of activity quantification.

Overall, this RP-HPLC–UV method provides a reliable, cost-effective, and widely accessible alternative for PNGase F activity assessment, supporting both soluble enzyme studies and IMER characterization. The proposed approach expands analytical capabilities for laboratories and industrial environments where MS-based methods are not readily

available, facilitating broader implementation of IMER technologies in pharmaceutical bioanalysis.

References

1.G. Zhang, Enz Eng. (2024), 13,
2. 2.S. Hirani et al, Anal. Biochem. (1987), 162, 2.
3.W.R. Alley Jr et al, Chem, Rev. (2013), 113, 4.
4.C. Hosfield et al, Promega Notes (2013), 124.

PP-33

Rational design of a hybrid polymethacrylate sorbent for dispersive solid-phase microextraction of alpelisib from human plasma

Zvonimir Mlinarić [1], Lu Turković [1], Miranda Sertić [1]

University of Zagreb Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Analysis, Ante Kovačića 1, 10000 Zagreb, Croatia

The increasing demand for reliable bioanalytical methods in therapeutic drug monitoring has highlighted the critical role of sample preparation, particularly for complex biological matrices such as human plasma. Conventional approaches, including protein precipitation or solid-phase extraction, often suffer from insufficient matrix clean-up or high operational costs. In this context, dispersive solid-phase microextraction (DSPME) has emerged as a promising alternative due to its simplicity, rapid kinetics, and low sorbent consumption [1]. However, its performance is governed by the design and properties of the sorbent.

Here, a novel hybrid polymethacrylate-based sorbent was rationally designed, synthesised, and optimised for the extraction of alpelisib (ALP), a clinically relevant PI3Kα inhibitor, from human plasma. The sorbent was prepared via precipitation polymerisation using methacrylic acid (MAA) and 2-hydroxyethyl methacrylate (HEMA) as functional monomers and ethylene glycol dimethacrylate (EGDMA) as a cross-linker. The selected composition (HEMA:MAA = 1:4) and high cross-linking density (3:10 monomer-to-cross-linker ratio) were chosen to achieve a balance between hydrophilicity, structural rigidity, and interaction capability toward ionisable analytes [2]. Initial attempts to develop a molecularly imprinted polymer showed negligible imprinting effects due to strong non-specific interactions. Consequently, a non-imprinted polymethacrylate sorbent was adopted, offering comparable binding efficiency with reduced complexity and improved reproducibility.

Structural characterisation by FTIR confirmed successful polymer formation, while dynamic light scattering revealed a homogeneous submicron particle size distribution (950.4 ± 47.8 nm), ensuring efficient dispersion and enhanced analyte–sorbent interactions. Adsorption studies demonstrated excellent binding efficiency across clinically relevant concentration ranges, with up to 94.6% analyte retention. Kinetic analysis indicated a pseudo-second-order model, suggesting chemisorption-driven interactions. The sorbent exhibited a binding capacity exceeding the expected plasma concentration, confirming its suitability for bioanalytical applications.

The DSPME procedure was systematically optimised. A sorbent mass of 2 mg was selected as a compromise between extraction efficiency and practicality. Washing conditions were tuned to reduce phospholipid-related interferences, identifying ultrapure water as the optimal solvent, achieving substantial matrix clean-up with minimal analyte loss (1.5%). Elution was optimised using three sequential acetonitrile steps, yielding recoveries of 81.6-87.7% with RSD values ≤ 3.8%. The synthesis protocol demonstrated excellent inter-batch reproducibility (RSD 3.5%), highlighting robustness. Given the negligible production cost, the sorbent is suitable for single-use applications without regeneration.

Overall, the developed polymethacrylate sorbent represents a simple, cost-effective, and reproducible material tailored for DSPME, offering efficient analyte retention and improved matrix cleanliness. Its application provides a strong foundation for robust bioanalytical workflows for ionisable drugs in complex biological systems.

This research has been fully supported by the Croatian Science Foundation (grant numbers UIP-2019-04-8461 and IP-2025-02-1882) and the European Regional Development Fund through the project FarmInova (grant number KK.01.1.1.02.0021).

References

1. M. Ghorbani et al. Trends in Analytical Chemistry (2019), 118:793–809.
2. B. Dirion B et al. J Am Chem Soc (2003), 125:15101–15109.

PP-34

Greenness and Efficacy Comparison of LC and SFC Chiral Method Development Strategies for Pharmaceutical Analysis

A. Mohr[1,2], M. Steppe[2], D. Mangelings[1] and Y. Vander Heyden[1]

1 Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Research Group of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, 1090 Jette, Belgium
2 Federal University of Rio Grande do Sul (UFRGS), Faculty of Pharmacy, Pharmaceutical Quality Control Laboratory, 90610-000 Porto Alegre, Brazil

The separation of chiral compounds is essential in the pharmaceutical industry, as enantiomers often exhibit distinct pharmacological activities. However, achieving efficient chiral separations remains challenging. To support the method development process and minimize time and resource consumption, generic screening strategies are recommended. At the same time, it is important that these strategies align with the principles of Green Analytical Chemistry, in addition to focusing on analytical performance. This study aimed to compare the environmental impact and separation efficacy of generic chiral method development strategies in High-Performance Liquid Chromatography (HPLC) and Supercritical Fluid Chromatography (SFC). To achieve this, a diverse test set of 22 pharmaceutical racemates was applied in two earlier defined LC and SFC strategies. [1,2] The HPLC strategy consisted of a sequential screening with an acidic and a basic mobile phase on three polysaccharide-based chiral stationary phases. The acidic mobile phase was composed of 100 mM potassium hexafluorophosphate in 50 mM phosphate buffer, pH 2.0/acetonitrile (60:40, v/v), and the basic mobile phase was 20 mM borate buffer, pH 9.0/acetonitrile (60:40, v/v). The SFC strategy screened four polysaccharide-based chiral stationary phases with mobile phases of either CO2/methanol (80:20, v/v) or CO2/2-propanol (80:20, v/v), both containing 0.1% (v/v) of isopropylamine and 0.1% (v/v) of trifluoroacetic acid as additives. In both strategies, the screening was subsequently followed by a specific optimization step. The environmental impact of the techniques was assessed using the Analytical GREEnness calculator (AGREE). This widely used metric illustrates the environmental performance through a 12-part clock pictogram (each corresponding to one principle of Green Analytical Chemistry), with the final score displayed in the center. The results highlighted differences in the separation efficiency of the two techniques. The SFC screening strategy successfully separated 19 out of 22 compounds, compared to 16 of 22 using the HPLC strategy. In the AGREE assessment, the HPLC strategy received a score of 0.49, and the SFC strategy achieved 0.56 (where 1.0 represents the ideal green method). The main differences between the scores were situated at solvent toxicity and waste generation. Although SFC is inherently a greener technique, its score was penalized by the use of isopropylamine and trifluoroacetic acid. Overall, the SFC strategy demonstrated a higher success rate and a more favorable balance

between performance and sustainability. Future improvements for both strategies are being explored by substituting more toxic solvents (e.g. acetonitrile, isopropylamine, trifluoroacetic acid) with sustainable alternatives, such as ethanol or greener additives, to further enhance their environmental compatibility.

References

1. A.A. Younes, D. Mangelings, Y. Vander Heyden J. Chromatogr. A (2012), 1269, 154-167.
2. K. De Klerck, Y. Vander Heyden, D. Mangelings J. Chromatogr. A (2014), 1363, 311-322.

PP-35

Development and optimization of a UPLC-HRMS (QTOF) method for multi-residue analysis of pharmaceuticals in wastewater

Adibia Umoroye Nathan[1], Manon Masschelein[1], Karel De Schamphelaere [2], Diederik Rousseau [3] and Evelien Wynendaele[1]

1 Drug Quality and Registration (DruQuar) Group, Pharmaceutical analysis, Ghent University 9000 – Ghent, Belgium
2 Environmental Toxicology Group, Animal Science and Aquatic Ecology, Ghent University 9000- Ghent, Belgium
3 Laboratory for Industrial Water and EcoTechnology Group, Green Chemistry and Technology, Ghent University 8500-Kortrijk, Belgium

Pharmaceutical residues are increasingly recognized as contaminants of emerging concern (CECs) due to their continuous release into aquatic environments and incomplete removal during wastewater treatment. Their persistence, biological activity, and potential contribution to antimicrobial resistance pose significant risks to aquatic ecosystems. Reliable analytical methods are therefore essential for the accurate detection, quantification and removal evaluation of these contaminants in complex environmental water matrices [1].

This study focuses on the development and optimization of a multi-residue analytical method targeting selected contaminants of emerging concern, including pharmaceuticals such as macrolide antibiotics, anti-inflammatory drugs, anticonvulsants, endocrine-disrupting compounds (EDCs), and selected pesticides. Method development was performed using ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF). Comparative evaluation of different stationary phases (columns) and different mobile phases were tested to achieve the best optimal conditions for the identification of the selected compounds.

Based on this optimization, HSS T3 column provided superior overall analytical performance, with improved peak shape, better analyte retention, and reduced background noise. Comparative mobile phase assessment showed that acetonitrile combined with aqueous 5 mM ammonium formate and 0.1% formic acid provided improved signal intensity and peak symmetry compared to methanol-based conditions. Comparative assessment of mass spectrometry (MS) and mass spectrometry with elevated energy (MSE) acquisition modes on the high-resolution mass spectrometry (HRMS) showed that MS mode provided greater signal stability and more reliable quantification for macrolide compounds, while MSE resulted in reduced precursor ion intensity under the tested conditions. After method development and optimization, the different compounds were successfully detected,

demonstrating baseline chromatographic separation, strong linearity ($R^2 > 0.99$), and acceptable precision (%RSD < 15%) across the tested concentration ranges [2].

References

1. S. Afsa, K. Hamden, P. A. Lara Martin, and H. Ben Mansour, "Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination," Environmental Science and Pollution Research, vol. 27, no. 2, pp. 1941–1955, Jan. 2020, doi: 10.1007/s11356-019-06866-5.. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.
2. EMA, "Committee for Medicinal Products for Human Use ICH Q2(R2) Guideline on validation of analytical procedures," 2023. [Online]. Available: www.ema.europa.eu/contact. Scott, V. Adels J. Appl. Crystallogr. (2007), 35, 24.

PP-36

Chemical analysis of Amoxicillin and Ciprofloxacin finished products beyond their labelled expiry dates

Goodluck G. Nyondo [1,2], Eliangiringa A. Kaale [2], Erwin Adams [1]

[1] KU Leuven, Department of Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, Leuven, Belgium

[2] Muhimbili University of Health and Allied Sciences, School of Pharmacy, Dar es Salaam, Tanzania

Shelf lives are used to signify the period during which pharmaceutical products are expected to remain within acceptable physicochemical and biological properties when properly stored [1]. Expiry dates are assigned to each batch of pharmaceutical products to show the end of their shelf life [2]. Expired products, which constitute the largest proportion of pharmaceutical waste, remain a significant public health challenge due to financial losses and environmental damage associated with the current disposal practices [3]. Increasing evidence shows that medicines can remain stable beyond their labelled expiry dates, making shelf-life extension a suitable approach to minimise wastage from premature disposal [4,5].

In this work, assay and related substances analyses were conducted on two commonly used antibiotics available in Tanzania from different global manufacturers with the aim of assessing their suitability for shelf-life extension.

Samples were analysed using liquid chromatographic methods according to the respective monographs in the British and European Pharmacopoeia. Seven samples of amoxicillin capsules, two samples of amoxicillin dispersible tablets, and eight samples of ciprofloxacin tablets were analysed. Expiry dates of amoxicillin samples ranged from March 2018 to January 2024, while those of ciprofloxacin samples ranged from May 2019 to August 2025. Assay values above 90% [6] of the label claim were considered acceptable for shelf-life purposes.

Eight amoxicillin samples passed the assay test, with values between 91.8% and 98.5% of the label claim. One sample failed with an assay of 88%. The assay results for the ciprofloxacin samples ranged from 92.3% to 98.9%, which were within the acceptance limits. Related substances exceeded limits in two amoxicillin samples, while they were within acceptable limits for all ciprofloxacin samples. So, seven expired amoxicillin samples were considered compliant with the performed tests, and all eight ciprofloxacin tablet samples were compliant. However, the change in assay or impurity levels did not show a relationship with time since expiry.

It can be concluded that many samples (15/17) remained within acceptable levels of assay and related substances up to eight years beyond their labelled expiry dates. These findings suggest that labelled expiry dates may be conservative for certain products and that the majority of these samples can qualify for shelf-life extension. The findings support the need for evidence-based shelf-life extension programmes to minimize pharmaceutical waste and increase access to medicines, particularly in resource-limited settings. Further tests to evaluate the physical properties of the individual formulations are recommended to enable regulatory bodies to make informed decisions about extending shelf lives.

References

1. ICH Q1A(R2) guidelines (2003).
2. WHO TRS No. 1044, Annex 8 (2022).
3. Alnahas F, Yeboah P. Int J Environ Res Public Health (2020), 17, 787.
4. Zilker M, Sörgel F, Holzgrabe U. J Pharm Biomed Anal. (2019), 166, 222.
5. Lyon RC, Taylor JS. J Pharm Sci. (2006), 95, 1549.
6. Bajaj S, Singla D. J Appl Pharm Sci. (2012), 2, 129.

PP-37

Evaluation of an in-house 2D-LC setup for encapsulation efficiency determination of pDNA-LNPs

S. Planckaert[1], Wendi A. Hale[2] M. De Beer[1], J. Voorspoels[1], J. Vercruysse[1]

1 Eurofins CDMO, Drug Product Unit, 9052 – Zwijnaarde, Belgium
2 Agilent Technologies – Lexington, MA 02421 USA

Two dimensional liquid chromatography (2D LC) is emerging as a powerful approach to characterize lipid nanoparticles (LNPs) and their nucleic acid payloads, enabling separation of particle level and payload level attributes within a single workflow. Compared with dye-based assays (e.g., PicoGreen), LC-based EE (i) separates intact pDNA from fragments via IP-RP instead of reporting only total dsDNA, (ii) minimizes topology bias by resolving supercoiled, open-circular, and linear plasmid forms before quantification [1], and (iii) is less affected by surfactant- (e.g., Triton X-100), excipient- or time-dependent fluorescence artefacts.

Proprietary 2D LC systems have demonstrated simultaneous readouts for encapsulation efficiency (EE), nucleic acid integrity, and particle size; however, these platforms often rely on specific column combinations and hardware not universally available. Here, we present an in-house 2D-LC set-up (Agilent 1290 II) to quantify EE of plasmid DNA (pDNA) LNPs. This 2D-LC set-up also allowed to compare alternative first-dimension (1D) mechanisms and column orders in function of analytical performance.

We evaluate multiple 1D strategies to separate LNPs from free pDNA prior to payload analysis: (i) hydrophobic interaction chromatography (HIC) to retain LNPs at high salt strength, (ii) weak/strong anion-exchange (IEX) bio-monoliths to bind free pDNA with LNPs in the flow-through, and (iii) size-exclusion chromatography (SEC). The second dimension uses an ion-pair reversed-phase (IP-RP) approach for nucleic-acid separation. Study variables include column order and stationary phase, mobile-phase composition, inter-dimension solvent compatibility and control of sample-diluent/mobile-phase mismatch.

This study outlines a flexible, stand-alone 2D-LC framework for EE determination of pDNA-LNPs using broadly available columns and methods, and it is readily extendable to mRNA-LNPs by adapting the payload separation to RNA. By systematically screening column chemistries and column order, we aim to provide a practical roadmap for labs seeking an open, patent-neutral alternative for LNP EE analytics suitable for research and early development.

References

1. C.-H. Lin, Y.-C. Chen, T.-M. Pan PLoS ONE (2011), 6, e29101.

PP-38

Analysis of Etomidate Analogues and Controlled Drugs in Etomidate-Positive Pods by LC-HRMS

Yi Ling Quek[1], Yun Zeng[1], Xiaowei Ge[1], Yimin Xu[1] and Min Yong Low[1]

Pharmaceutical Division, Applied Sciences Group, Health Sciences Authority, Singapore

Etomidate is a short-acting intravenous general anaesthetic drug traditionally administered in controlled medical settings for rapid induction of anaesthesia. It is not approved for recreational or outpatient use. Following reports of illicit pods being adulterated with etomidate, etomidate and its analogues were classified as Class C controlled drugs under the Misuse of Drugs Act from 1 September 2025 in Singapore. However, no sensitive method was available for the simultaneous analysis of etomidate analogues and controlled drugs in E-liquids in the presence of etomidate. Hence, there was a need to develop a sensitive Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) screening method that could divert etomidate out, while simultaneously analysing trace levels of etomidate analogues and controlled drugs in etomidate-positive pods.

A sensitive LC-HRMS screening method was developed for the simultaneous analysis of 10 etomidate analogues and 11 controlled drugs in etomidate-positive pods. Extracted E-liquids were diluted 10 times with acetonitrile and further diluted 100 times with acetonitrile/deionised water (1:1, v/v) before injection into Thermo Q Exactive Plus Hybrid Quadrupole-Orbitrap MS coupled with UHPLC. A divert valve was set at 9.4 – 10.1 min to divert etomidate away and prevent contamination. The column used was a Kinetex Biphenyl column (150 × 2.1 mm, 2.6 µm). Mobile phases A and B were 0.1% formic acid in deionised water and 0.1% formic acid in acetonitrile, respectively, with gradient elution. Analysis was performed in positive electrospray ionisation (ESI) mode at a flow rate of 0.5 mL/min, with column temperature set at 45 ºC and injection volume of 5 µL. The method was validated with low limits of detection (LOD) of 0.5 – 2 µg/mL for the 21 analytes.

Analysis of 39 etomidate-positive pods using LC-HRMS detected metomidate in all 39 pods, propoxate in 32 pods, iso-propoxate in 23 pods, and both butomidate and iso-butomidate in 21 pods. Among the controlled drugs, only methamphetamine was detected in 8 pods and ketamine in 2 pods. This analytical testing capability provided intelligence information on the etomidate analogues and controlled drugs detected at trace-level in E-liquids. The findings revealed that the analogues and controlled drugs detected so far were at impurity/contamination levels.

PP-39

CZE optimization for the separation of varying-size DNA fragments

E. Renard[1], C. Davoine[1], M. Fillet[1]

1 Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liège, Belgium

Nucleic acid–based therapeutics are rapidly expanding on the market and in clinical pipelines, with structures ranging from short oligonucleotides such as ASOs or microRNAs to long and structurally complex modalities like lncRNAs and mRNA constructs. Their increasing commercial presence, combined with this broad structural diversity, significantly increases analytical challenges, particularly the risk of analyte adsorption and poor detectability, which can compromise method robustness. This highlights the need for analytical strategies capable of accommodating such heterogeneity while limiting these issues. In this context, capillary zone electrophoresis (CZE) represents a highly suitable technique thanks to its low sample consumption, high separation efficiency, short analysis times and potential for MS coupling

This work aims to establish CZE conditions suitable for analyzing double-stranded DNA fragments covering a broad size range (10 - 20 000 bp), selected as stable model analytes prior to extending the method to DNA/RNA hybrids and RNA-based molecules. To explore conditions that could accommodate such size variability, different capillary surfaces (coated and uncoated) and multiple BGE compositions were screened across varying ionic strengths, pH values and voltages. Peak shape, migration behavior, adsorption and sensitivity were assessed, and preliminary considerations regarding the compatibility of selected BGEs with MS detection were also included.

Particular attention was paid to adsorption, a known challenge when working with nucleic acids and a potential source of signal loss or inconsistent detection, especially for larger fragments. These investigations aim to provide a better understanding of the parameters shaping the separation and to support the identification of CZE conditions that minimise adsorption-related effects while remaining applicable across the tested size range.

P-40

High-Resolution Mass Spectrometry for Accelerated Stability Modeling and Shelf-Life Prediction of Lipid-Modified Oligonucleotides

Kevin Roeleveld[1], Laura Corveleyn[1], Birger Zutterman[1] and Geert Van Raemdonck[1]

1 AnaBioTec, 9940 – Evergem, Belgium

Lipid-conjugated antisense oligonucleotides (ASOs) improve pharmacokinetics and tissue uptake but present complex stability challenges. Understanding thermally driven degradation pathways is critical for shelf-life prediction and formulation design. This study evaluated accelerated stability of a gapmer ASO bearing a 5′-palmitate conjugate and 2′-O-methoxyethyl (MOE) termini. Heat-stress data were generated and modeled using Accelerated Stability Assessment Program (ASAP®) software, with degradation pathways and kinetic markers characterized by high-resolution LC-MS/MS. The objective was to identify shelf-life-limiting degradation mechanisms and assess heat-only accelerated stability modeling for lipid-modified ASOs.

A lipid-conjugated 16-mer phosphorothioate gapmer ASO standard (Waters, PN 186010747) was heat-stressed at 37, 50, 60, and 70°C for up to 168 hours. Samples were analyzed on a Vanquish Flex UHPLC system coupled to an Orbitrap Exploris 240 mass spectrometer operated in negative ESI mode. Separation was achieved on a Waters Premier BEH C18 Oligonucleotide column (2.1 × 50 mm, 1.7µm 130Å) using ion-pair reversed-phase liquid chromatography (IP-RPLC). High-resolution full MS and data-dependent MS/MS spectra were acquired. Data processing, quantitation, and Arrhenius-based shelf-life modeling were performed using BioPharma Finder and ASAP®prime software.

Heat stress produced a simple, ordered degradation profile well suited for Arrhenius modeling. The dominant early degradation pathway was hydrolytic loss of the 5′-palmitate conjugate. This transformation increased with temperature and time and was the primary kinetic driver in ASAPprime modeling. With extended heat exposure, a shortmer ladder emerged, initiated predominantly from the 3′-terminus, sequentially losing MOE-modified nucleotides before entering the DNA gap. Early shortmers (n−1 and n−2) retained the lipid conjugate, whereas longer stress durations produced delipidated shortmers. Minor levels of phosphorothioate desulfurization (−16 Da variants) were detected at higher temperatures but remained too low to impact kinetic modeling. Notably, lipid chain oxidation or nucleobase oxidation were not observed under heat-only conditions, confirming that degradation chemistry remained hydrolytic and Arrhenius-consistent. ASAPprime modeling predicted a refrigerated shelf-life of more than 1 year and 6-12 months at room temperature, with palmitate linker hydrolysis identified as the shelf-life-

limiting pathway. These results demonstrate HR-MS and ASAP modelling as powerful tools for shelf-life prediction of lipid-conjugated gapmer ASOs.

PP-41

Thermal Emissivity as a Novel Parameter in the Quality Assessment of Solid Oral Dosage Forms

Michał Meisner [1], Natalia Szarek [2], Beata Szulc-Musioł [3], Beata Sarecka-Hujar [2]

1 Faculty of Pharmaceutical Sciences in Sosnowiec, Doctoral School of the Medical University of Silesia in Katowice, 41-200 Sosnowiec, Poland
2 Department of Basic Biomedical Science, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, 8b Jedności Str, 41-200 Sosnowiec, Poland;
3 Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, 8b Jedności Str, 41-200 Sosnowiec, Poland; corresponding address: bsarecka-hujar@sum.edu.pl

Background: In recent years, the importance of modern, non-destructive analytical methods in assessing the quality or durability of solid pharmaceutical dosage forms has been growing. Emissivity (ε) is one of the new parameters that can be used to assess the thermal and optical properties of active pharmaceutical ingredients (APIs), e.g., to monitor API degradation under UV radiation. ε reflects the material's radiative properties and may change as the medicinal product ages, and it is strongly correlated with the surface characteristics of the tested object. It can be defined as the ratio of radiation emitted by the surface to the radiation emitted by a perfectly black body at the same temperature. The study aimed to analyse and compare the thermal emissivity of selected commercial tablets.

Methods: The study utilized an ET 100 emissometer (Surface Optics Corporation, USA) operating in the infrared range (1.5–21 μm). Unexpired extended-release tablets containing metformin hydrochloride (from two manufacturers), film-coated tablets containing ibuprofen, and chewable tablets containing sodium aluminium dihydroxycarbonate were tested. Directional thermal emissivity determined at beam angles of 20° (DTE20) and directional thermal emissivity determined at beam angles of 60° (DTE60), and hemispherical thermal emissivity (HTE) were measured as a function of temperature at four simulating temperatures: 300 K, 500 K, 800 K, and 1200 K to evaluate the radiative properties under varying thermal loading conditions. For each sample, measurements were performed in triplicate. The study was conducted under the BNW-1-037/N/5/F project.

Results: At 300 K, both analysed preparations containing metformin hydrochloride exhibited comparable DTE20, DTE60, and HTE values. On the other hand, the DTE20 values for the chewable preparation with sodium aluminium dihydroxycarbonate were significantly higher than those for the I and II extended-release preparations (0.971 vs 0.956 and 0.971 vs 0.958, respectively; $p<0.001$ for both) and for coated tablets with ibuprofen (0.971 vs 0.931; $p<0.001$). In turn, coated tablets showed the highest DTE60

values compared to other preparations (0.941 vs 0.931 for chewable tablets, $p<0.001$; 0.941 vs 0.929 for I extended-release tablets, $p<0.001$; and 0.941 vs 0.931 for II extended-release preparation, $p=0.001$, respectively). The HTE parameter was again significantly higher for chewable tablets ($p<0.001$ for comparisons with other preparations). In addition, the values of DTE20, DTE60, and HTE decreased with increasing temperature across all preparations, reaching their lowest values at 1200 K.

Conclusions: The selected pharmaceutical preparations with different release profiles differ in the values of the DTE20, DTE60, and HTE emission parameters, indicating their potential use in the quality assessment.

PP-42

Development and validation of a UHPLC–MS/MS method coupled with DSPME for quantification of alpelisib in human plasma

Miranda Sertić[1], Zvonimir Mlinarić[1], Lu Turković[1]

1 University of Zagreb Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Analysis, Ante Kovačića 1, 10000 Zagreb, Croatia

Alpelisib (ALP), a selective PI3Kα inhibitor used in the treatment of PIK3CA-mutated breast cancer, exhibits substantial interindividual pharmacokinetic variability and a high incidence of dose-limiting adverse effects. These characteristics necessitate accurate and reliable bioanalytical methods for its quantification in human plasma to support therapeutic drug monitoring [1]. While liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the method of choice due to its sensitivity and selectivity, its performance is highly dependent on efficient sample preparation that minimises matrix effects.

In this study, a rapid, sensitive, and robust ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) method was developed and validated in combination with a previously developed dispersive solid-phase microextraction (DSPME) workflow employing a novel polymethacrylate-based sorbent. Chromatographic separation was achieved on a Poroshell 120 EC-C18 column using gradient elution with water and acetonitrile containing 0.1% formic acid, with a total runtime of 5 minutes, enabling high-throughput analysis. Detection was performed in positive electrospray ionisation mode using multiple reaction monitoring (MRM), ensuring high selectivity. In addition to ALP, representative phospholipid transitions were monitored to evaluate the effectiveness of matrix clean-up.

The method was validated in accordance with ICH M10 guidelines over a concentration range of 50-2000 ng/mL, covering clinically relevant levels. Excellent linearity was achieved, with correlation coefficients ranging from 0.9969 to 0.9986 and low variability of calibration slopes (RSD 1.7%). Accuracy and precision were systematically assessed across multiple concentration levels, demonstrating intra- and inter-day deviations within ±15% and RSD values below 5%, confirming the reliability of the method. Matrix effects were rigorously evaluated using six independent plasma sources, including hemolysed and lipemic samples. Although a moderate degree of signal suppression (47.5%) was observed, it was highly reproducible across all matrices (RSD 4.7%). This consistency ensured reliable quantification despite the absence of an internal standard, highlighting the effectiveness of the DSPME procedure in controlling matrix variability.

The total analytical workflow, including sample preparation and chromatographic analysis, is simple, rapid, and compatible with routine laboratory operation. The applicability of the method was confirmed through the analysis of real patient plasma samples, yielding ALP concentrations consistent with reported clinical data. This demonstrates its suitability for therapeutic drug monitoring in clinical settings.

In conclusion, the developed DSPME–UHPLC–MS/MS method provides a reliable, cost-effective, and high-throughput approach for the quantification of ALP in human plasma. Its robustness, reproducibility, and efficient matrix clean-up make it a strong alternative to conventional sample preparation techniques and a valuable tool for routine bioanalysis.

This research has been fully supported by the Croatian Science Foundation (grant numbers UIP-2019-04-8461 and IP-2025-02-1882) and the European Regional Development Fund through the project FarmInova (grant number KK.01.1.1.02.0021).

References

1. Y.M.M. Cheung et al. Breast Cancer Res Treat (2022), 192:303–311.

PP-43

Acylcarnitines ionization process optimization with the use of Quality by Design approach

J. Jacyna-Gębala, W. Struck-Lewicka, M. Waszczuk-Jankowska, R. Wawrzyniak, J. Raczak-Gutknecht, M. Biesemans, M.J. Markuszewski and D. Siluk

Department of Biopharmaceutics and Pharmacodynamics, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland, danuta.siluk@gumed.edu.pl

Carnitines, known to play a key role in cellular energy metabolism, have been identified as potential indicators of cardiovascular and cancer diseases. Simultaneous quantification of several carnitines extracted from diagnostic fluids would be of high value for validation of those hypotheses. The aim of the study was development of selected acylcarnitines in human plasma with the use of HPLC-ESI-QqQ-MS/MS system (Agilent Technologies, G6475A).

One of the aspects of quantitative method development is finding optimal ionization parameters' settings in order to obtain the highest possible method's sensitivity. To achieve this goal in the most efficient way Quality by Design approach was proposed, which allowed for selecting the parameters' optimal settings enabling highest possible signals intensities while maintaining the highest repeatability of analyses. 19 carnitines were included in the study. Mass spectrometry parameters optimized for carnitines' quantification method were selected on the basis of the literature review and capabilities of the analytical instrument used in the study: capillary voltage, gas flow, gas temperature, nebulizer pressure, sheath gas flow, sheath gas temperature, nozzle voltage. Box-Behnken design was chosen due to reliable determination of the response surface for studied design space along with optimal settings, with relatively low experimental workload and low reagents' consumption.

Based on multivariate regression analysis separate models for each acylcarnitine were built, which allowed for prediction of influence of tested parameters on individual response. Models built for individual metabolites allowed to conclude that all studied compounds would benefit from similar conditions. With the use of maximize desirability function a compromise parameter' setting was achieved. The suggested settings were as follows: capillary voltage 3900 V, gas flow 11 L/min, gas temperature 325 C, nebulizer pressure 50 psi, sheath gas flow 11 L/min, sheath gas temperature 350 C, nozzle voltage: 150 V.

Acknowledgements

The project was founded by the Medical Research Agency under the National Plan for Rehabilitation and Enhancement of Immunity Component D Efficiency, accessibility and quality of the health care system Investment D3.1.1 Comprehensive development of research in medical and health sciences (no 2024/ABM/03/KPO/KPOD.07.07-IW.07-0075/24-00) as well as NAWA-PROM FERS.01.05-IP.08-0218/23.

PP-44

Quantification of urinary nucleosides and deoxynucleosides by LC-MS/MS in urogenital tract cancer patients

W. Struck-Lewicka[1], J. Jacyna[1], M. Artymowicz[1], P. Wiczling[1], M. Markuszewski[2], P. Gutknecht[3], M.J. Markuszewski[1], D. Siluk[1]

1 Department of Biopharmaceutics and Pharmacodynamics, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland, wiktoria.struck-lewicka@gumed.edu.pl
2 Department of Urology, Medical University of Gdańsk, Faculty of Medicine, Gdańsk, Poland
3 Division of Family Medicine, Medical University of Gdańsk, Faculty of Medicine, Gdańsk, Poland

Quantitative metabolomics is commonly used for verification of importance of selected promising candidates obtained from untargeted phenotyping. After untargeted metabolomics we selected 11 nucleosides and deoxynucleosides and measured their concentration in urine from urogenital tract cancer patients and healthy controls using LC-ESI-QqQ/MS determination technique.

In the present study urine samples were collected from bladder (=90), prostate (89) and renal cancer (n=80) patients as well as healthy controls (n=171). The sample preparation included the following steps: centrifugation, dilution with MeOH:H_2O (1:1, *v/v*), evaporation to dryness and sample reconstitution. Quantification of eleven nucleosides was conducted using liquid chromatography coupled with triple quadrupole mass spectrometry detection based on the developed and validated determination method [1]. The obtained data sets were normalized and statistically analyzed with the use of univariate (t-test, U Mann-Whitney test) and multivariate statistical methods (logistic regression, OPLS-DA) using Matlab and SIMCA software. As a result of t-test, nine out of eleven nucleosides were found to be statistically significand in bladder cancer patients versus healthy controls ($p<0.05$). In prostate and renal cancer patients 2 and 4 out of 11 nucleosides were statistically significant ($p<0.05$), respectively. The fold change was between 0.54 to 1.52. The multivariate models were achieved with promising results in terms of sensitivity, specificity and accuracy. The obtained results support the promise of nucleosides and deoxynucleosides as urinary markers in urogenital tract cancer.

Acknowledgements
The project was founded by the Medical Research Agency under the National Plan for Rehabilitation and Enhancement of Immunity Component D Efficiency, accessibility and quality of the health care system Investment D3.1.1 Comprehensive development of research in medical and health sciences (no 2024/ABM/03/KPO/KPOD.07.07-IW.07-0075/24-00) as well as PROM Programme – short-term academic exchange – is financed by the European Union under the project titled "Short-term academic exchange as a way to improve the quality of education in higher education and research institutions", project number FERS.01.05-IP.08-0218/23 (FERS Projects).

References

[1] M. Artymowicz, W. Struck-Lewicka, P. Wiczling, M. Markuszewski, M.J. Markuszewski, D. Siluk. Targeted quantitative metabolomics with a linear mixed-effect model for analysis of urinary nucleosides and deoxynucleosides from bladder cancer patients before and after tumor resection. *Anal Bioanal Chem.* 415 (2023) 5511-5528.

PP-45

Gabapentin cream for vulvodynia: development and rollout of a robust, stable formulation

J. Vanmeerhaeghe[1], M. Van Steenkiste[2], D. Vandendooren[3], N. Mokadem[3], K. Wauters[2], K. De Paepe[3], J. Verrax[1]

1 Association of Pharmacists Belgium, SCM-DGO - Brussels
2 Association of Pharmacists Belgium - Brussels
3 Vrije Universiteit Brussel (VUB), In Vitro Toxicology and Dermato-cosmetology - Health Campus Jette

Background:

Topical gabapentin cream is frequently prepared by community pharmacists due to its off-label use in the management of vulvodynia, a condition affecting approximately 1 in 12 women. Despite its growing clinical relevance, pharmacists frequently report formulation-related problems, including crystallization of gabapentin and discoloration of the cream. Questions arise regarding the choice of base, co-solvents, stability, and appropriate storage conditions. [1,2,3,4]

Purpose:

This study aimed to investigate the physicochemical stability of gabapentin cream, focusing on understanding and mitigating discoloration and crystallization. We evaluated commonly used pharmaceutical bases, co-solvents, and buffering strategies to develop an accessible, stable, and pharmacy-feasible formulation.

Method:

Research investigated multiple gabapentin cream formulations, assessing key variables including:

- starting material: gabapentin raw material or pulverized commercial medicine;
- concentration;
- use of co-solvent: glycerol, propylene glycol and Tween 80;
- type of cream base for vaginal use: buffered vs. non-buffered cetomacrogol cream and modern commercial bases;
- storage conditions: room temperature vs. refrigeration;
- buffering: acetate buffer pH 4;
- preservation: sorbic acid/potassium sorbate vs. aqua conservans;
- intended shelf life.

Stability testing included an analytical evaluation of the active content by LC-UV/VIS and pH measurement, as well as a visual assessment (i.e. color, absence of crystals).

Results:

Analytical stability testing by LC-UV/VIS demonstrated a clear preference for using raw material rather than a pulverized commercial medicine. Furthermore, the results indicated a maximum concentration of 10% gabapentin proved feasible without negatively affecting stability and storage at room temperature was preferable.

Discoloration was linked to an interaction with the preservative sorbic acid/potassium sorbate, commonly used in ready-to-use commercial cream bases. The use of aqua conservans as a preservative did not cause discoloration, but it required the preparation of a self-compounded base.

Crystallization resulted from gabapentin's low solubility and zwitterionic nature. The co-solvents (glycerol, propylene glycol, Tween 80) showed minimal benefit. By adding an acetate buffer at pH 4, the solubility of gabapentin increases, and crystal formation is effectively prevented, while maintaining stable active content as confirmed by LC-UV/VIS analysis.

Modern commercial bases offer no advantage over a self-compounded cetomacrogol base.

Conclusion:

Gabapentin cream presents formulation challenges due to preservative interactions and insufficient buffering capacity of commercial bases. A self-compounded cetomacrogol cream buffered with 1 M acetate buffer at pH 4, preserved with aqua conservans, provided a clear advantage and remained stable for at least one month. A limitation of this formulation is the presence of an acetic odor.

Ongoing research is focused on alternative formulation strategies, including the use of polyethylene glycol 400 as a co-solvent, an approach that is internationally applied in gabapentin formulations.

References

1. B. Vergauwe, S. Weyers , H. Verstraelen. Gunaïkeia. (2021), 26, 3.
2. J. M. Schlaeger, J. E. Glayzer, M. Villegas-Downs, H. Li, E. J. Glayzer, Y. He, M. Takayama, H. Yajima, N. Takakura, W. H. Kobak, B. L. McFarlin. J Midwifery Womens Health. (2023), 68, 9-34.
3. M. Ergisi, A. Law, N. Chaudhari, S. Tsatsari, K. Lawson, C. Jenner. Front Pain Res. (2023) 3, 4.
4. Federatie Medisch Specialisten. Richtlijn: vulvodynie. 2019. Available from: https://richtlijnendatabase.nl/richtlijn/vulvodynie. Accessed 2026 Mar 17.

PP-46

Identifying active fractions of toad venom using CPC-guided bioassays and synergy analysis

M. Wells[1], A. Hervé[2], A. Tiflit[2], D. Konare[2], D. Beukens[1], V. Lefebvre[1], A. Genbauffe[3], F. Journé[3], S. Zubrzycki[2], L. Fougère[2], B. Blankert[1] and E. Destandau[2]

1 University of Mons, Laboratory of Pharmaceutical Analysis, 7000 – Mons
2 University of Orléans, Institute of Organic and Analytic Chemistry, 45067 – Orléans
3 University of Mons, Laboratory of Human Biology and Toxicology, 7000 – Mons

Toad venoms, such as the one of *Rhinella marina*, constitute a chemically diverse source of bioactive compounds, including bufadienolides and more polar constituents such as small peptides, alkaloids and biogenic amines.[1] While bufadienolides are frequently considered major contributors to biological activity, their role within complex crude extracts remains not fully elucidated. This study combines method development using Centrifugal Partition Chromatography (CPC) with biological evaluation of extracts and fractions to investigate the contribution of different chemical classes across different *in vitro* models.

Crude extracts were obtained by methanolic ultrasound-assisted extraction and subsequently fractionated using CPC. Several solvent systems were evaluated. A triphasic system in ascending mode led to the best phase retention and compound resolution, enabling the separation of bufadienolide-rich fractions from more polar constituents. Chemical profiling of samples was performed by HPLC-DAD-MS, leading to the identification of major bufadienolides, including resibufogenin (RBG).

Fractions and crude extracts were evaluated for both antiplasmodial (3D7 strain) and anti-melanoma (MM074; MM074-R) activities. In antiplasmodial assays, fractions enriched in polar constituents tended to exhibit a higher level of activity, whereas bufadienolide-rich fractions showed limited or no significant effects at the tested concentrations. Although RBG displayed activity when tested individually, this was observed only at concentrations exceeding those expected in the crude extract, suggesting limited contribution under extract-relevant conditions. In contrast, in anti-melanoma assays, bufadienolide-rich fractions demonstrated significant cytotoxic effects.

Interestingly, in antiplasmodial assays, the crude extract ($IC_{50\,(\mu g/mL)} = 16 \pm 3$) showed lower activity than the most active polar fraction ($IC_{50\ (\mu g/mL)} = 8 \pm 4$). This suggests that interactions between co-occurring compounds may influence the overall bioactivity of the extract, with non-polar constituents potentially modulating or attenuating the activity of polar active compounds. Ongoing studies aim to investigate these effects through reconstitution and combination experiments, including evaluation using the Bliss Independence model.

In conclusion, CPC proved to be an effective tool for the fractionation of *R. marina* venom extract and enabled the identification of distinct activity profiles across fractions. The results indicate that the polar constituents are major contributors to the antiplasmodial activity, whereas bufadienolides appear to play a more limited role under the tested conditions, despite the activity of RBG at higher concentrations. Moreover, while bio-guided fractionation is often time-consuming and does not systematically lead to enhanced activity [2], the increased potency observed for the polar fraction in this study highlights its potential to reveal key active components within complex extracts.

References

1. C. Rodriguez, L. Rollins-Smith, R. Ibáñez et al., J. Ethnopharmacol. (2017), 198.
2. L.K. Caesar, N.B. Cech, Nat Prod Rep. (2019), 36, 6.

PP-47

Microwave-assisted natural deep eutectic solvent extraction of *Rhodomyrtus tomentosa*: phytochemical characterization and biological evaluation of formulation-ready extracts

Suttiwan Wunnoo[1,2], Damiyah Charoenrit[1,2], Chanawee Jakkawanpitak[3,4], and Supayang Piyawan voravuthikunchai[1]

1 Center of Antimicrobial Biomaterial Innovation-Southeast Asia, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
2 Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
3 Center of Excellence for Biochemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand
4 Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, Thailand

Rhodomyrtus tomentosa (Aiton) Hassk. is a rich source of rhodomyrtone, a bioactive acylphloroglucinol with promising therapeutic potential and a novel mechanism of action.[1] However, conventional extraction methods, such as maceration, often result in low yields, limiting large-scale production, while synthetic rhodomyrtone is costly and scarcely available. To maximize the utilization of limited plant material, alternative extraction strategies are required. Natural deep eutectic solvents (NaDES), combined with microwave-assisted extraction, represent a green and efficient approach to enhance the recovery of bioactive constituents, enabling the direct production of formulation-ready extracts suitable for pharmaceutical applications. This study aimed to optimize microwave-assisted NaDES extraction of *R. tomentosa* leaves and to characterize the phytochemical profile and biological activities of the resulting extract. NaDES formulations (lactic acid:glycerol:water) were optimized using a single-factor design. Phytochemical characterization included the determination of total phenolic (TPC) and total flavonoid (TFC) contents, while the presence of rhodomyrtone was confirmed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Biological activities were evaluated using ABTS and DPPH radical scavenging assays, anti-tyrosinase activity, antibacterial activity against *Staphylococcus aureus* (broth microdilution), cytotoxicity in fibroblast cells, and nitric oxide inhibition in LPS-stimulated cells. The extract exhibited TPC of 147.99 ± 5.72 mg GAE/10 g and TFC of 134.97 ± 1.70 mg QE/g. TLC and HPLC analyses confirmed the presence of rhodomyrtone.

The extract demonstrated strong antioxidant activity, with IC_{50} values of 0.55% ± 0.01 (ABTS) and 1.29% ± 0.34 (DPPH), as well as anti-tyrosinase activity (IC_{50} = 2.23% ± 0.04). Antibacterial activity against *S. aureus* showed a minimum inhibitory concentration of 0.78% v/v and a minimum bactericidal concentration (MBC) of 3.12% v/v, while NaDES alone exhibited no bactericidal activity (MBC > 50% v/v). The extract showed no cytotoxicity toward fibroblast cells (cell viability > 80%) and significantly reduced nitric oxide

production (32.17 μM) compared with LPS-stimulated cells (43.54 μM, $p < 0.05$), with effects comparable to indomethacin (50 μg/mL; 33.07 μM). In conclusion, microwave-assisted NaDES extraction represents an effective and sustainable green approach for obtaining bioactive-rich extracts from *R. tomentosa*. The resulting extract demonstrates strong biological activities and potential as a formulation-ready system for pharmaceutical and cosmeceutical applications.

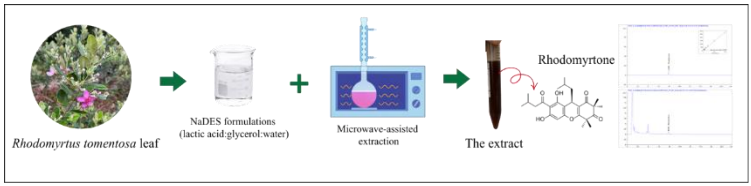

Figure 1. Microwave-assisted NaDES extraction of *Rhodomyrtus tomentosa* leaves for generating formulation-ready extracts.

References

1. D. Saeloh, V. Tipmanee, K.K. Jim, M.P. Dekker, W. Bitter, S.P. Voravuthikunchai, M. Wenzel, L.W. Hamoen. PLoS Pathog. (2018), 14, e1006876.

PP-48

FT-IR laser imaging for amyloidosis infected tissues analysis

Céline Duchateau[a] , Robin Zanelli[a] , Corentin Rossez[a, b], Camille Zany[c] , Laëtitia Herzog[c] , Vincent Beringue[c] , Human Rezaei[c] , Vincent Raussens[b] , Jehan Waeytens[a]

a Université libre de Bruxelles –Faculté de Pharmacie – RD3 PBM – Campus de la Plaine - Boulevard du Triomphe – 1050 Ixelles – Belgium
b Université libre de Bruxelles – Faculté des Sciences – SFMB – Campus de la Plaine - Boulevard du Triomphe – 1050 Ixelles – Belgium
c Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, MAP², Jouy-En-Josas, France

Vibrational spectroscopy is a powerful technique for the characterization of biological samples. It is possible to identify multiple components like lipids, proteins, and glycans. It is also possible to acquire infrared imaging with a hybrid system combining the benefits of imaging and vibrational spectroscopic techniques. This powerful tool has grown for a large variety of applications including medical field [1]. Recently, the development of quantum cascade laser (QCL) in the mid-infrared region improves the speed of data acquisition by a factor 5 (a full spectral imaging takes 3 min/cm² instead of 16 min/cm²).
In amyloidosis, proteins aggregate in the brain and their b-sheet content increase during this aggregation. Those aggregated protein form plaques in the brain. Vibrational spectroscopy has been used as a method for studying brain tissue for a long time due to the high infrared (IR) spectral contrast between gray and white matter. This has facilitated the development of spectroscopy imaging methods on tissues [2]. Spectroscopic imaging gives a spectral signature for each pixel and allows us to identify various pathological conditions and, more specifically, the formation of amyloid plaques. We will discuss the results obtained by the new QCL based infrared microspectroscopy in the Alzheimer and Prions diseases.

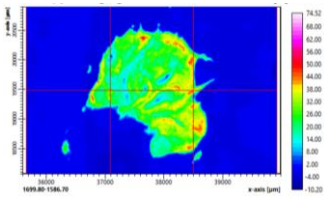

Figure 1: IR transmission map of a cerebellar section from a non-infected mouse brain organotypic slice after 21 days of incubation

References
[1] Bhargava A. et al Hyperspectral imaging and its applications: a review, Heliyon, 2024. [2] Kretlow A. et al FTIR-microspectroscopy of prion-infected nervous tissue, BBA, 2006.