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ABSTRACT BOOK

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PBA 2024 Abstract Book, Table of Contents

List of Plenary Lectures Page 02
List of Keynote Lectures Page 02
List of Oral Presentations Page 03
List of Short Oral Presentations Page 06
List of Poster Page 07

Oral Presentations, abstracts Page 14
Short Oral Presentations, abstracts Page 56
Posters, abstracts Page 64

LIST OF PLENARY LECTURES

PL-01 Can one truly understand by counting?
Hugo Duminil-Copin

PL-02 Performance Characteristics and Applications of Next-Generation Micro-Pillar Array Columns
Gert Desmet

PL-03 Bio- and bio-mimetic molecules as powerful tools for target analysis of compounds in biological fluids and complex molecule characterization
Valérie Pichon

PL-04 Information Quality: The Analytical Chemistry Challenge in Metabolomics
Coral Barbas

PL-05 Translating UHPSFC/MS method into real clinical practice: Pancreatic cancer screening based on plasma lipidome
Michal Holcapek

PL-06 A journey into pharmaceutical and biomedical analysis: from metabolite biomarker discovery to characterization of protein and nucleic acid based medicines
Koen Sandra

LIST OF KEYNOTE LECTURES

KN-01 Molecularly Imprinted Polymers (MIPs) in Sensors for pharmaceutical and biomedical assay- New Sensing Technologies
Sibel Ozkan

KN-02 Characterization of extracellular vesicles, challenges, opportunities and prospects in therapeutics
Marianne Fillet

KN-03 Confident MS-based Metabolite Annotation in Natural Product Metabolomics : New opportunities in the digital age.
Jean-Luc Wolfender

KN-04 Mass spectrometry-based multi-omics analysis for molecular pharmacology
Jingwu Kang

KN-05 From linear peptides to supramolecular mimotope peptide nanofibers: novel technologies for fast, efficient and high-throughput bioseparation of antibody drugs
Zhengjin Jiang

KN-06 Metabolomics in gastrointestinal stromal tumor studies
Michal Markuszewski

KN-07 Translational exhalomics for diagnosis purposes in Clinics
Pablo Sinues

KN-08 Capillary electrophoresis-based strategies to characterize or predict in-vivo behaviour of nanomedicines
Myriam Taverna

KN-09 Untargeted metabolomics in routine analysis
Nicola Zamboni

KN-10 Exploring Emerging Contaminants: Analytical Strategies in food safety and the Exposome Connection
Ana Garcia-Campaña

KN-11 Analytical Quality by Design and Quality control: from small molecules to biopharmaceuticals.
Sandra Furlanetto

KN-12 Multi-dimensional chiral HPLC analysis of amino acids and related compounds for drug discovery and diagnosis
Kenji Hamase

KN-13 Hydrophilic interaction liquid chromatography (HILIC) for intact proteins analysis: a journey into new glycoconjugated vaccines.
Gabriella Massolini

KN-14 Old and new directions for small molecule (bio-) analytical applications with ion mobility-mass spectrometry
Tim Causon

LIST OF ORAL PRESENTATIONS

OP1 - A1 New frontier for cells characterization Spectralomics: the Raman Spectroscopy
*Rizzarda Maffezzoni, Francesca Ravera, Hugh Byrne, Graziella Pellegrini, **Maria Cristina Gamberini***

OP1 - A2 Potential of lectin- and aptamer-based sorbents for extraction and/or fractionation of the human chorionic gonadotropin glycoforms prior to analysis at the intact level by LC-HRMS
***Nathalie Delaunay**, Sophia Smires, Anastasia Goumenou, Audrey Combès, Christophe Chendo, Valérie Pichon*

OP1 - A3 2D MXene/Surfactant nanocomposite preparation and its electrochemical performance towards the identification of Vandetanib level in human urine sample
*Mehmet Gülcan, **Hasret Subak**, Pınar Talay Pınar*

OP1 - B1 Imaged capillary isoelectric focusing associated with multivariate analysis to detect degradation of therapeutic monoclonal antibodies
*Cecile Tardif, Emmanuel Jaccoulet, Frederic Halgand, Myriam Taverna, **Claire Smadja***

OP1 - B2 Unveiling the dynamics of enzymatic activity in crowded environments: insights from hyaluronidase kinetics and interactions in extracellular matrix mimics
***Zahia Bouchelaghem**, Rouba Nasreddine, Alexandra Launay, Magdalena Andelini, Bérengère Claude, Claudia Bich, Josef Hamacek, Francesco Piazza, Catherine Perrin, Reine Nehmé*

OP1 - B3 Portable capillary electrophoresis: a novel device for determining the date-rape drug gamma-hydroxybutyric acid (GHB) at the point of need.
***Giacomo Musile**, Marc-Auréle Boillat, Götz Schlotterbeck, Peter C. Hauser*

OP2 - A1 Two orthogonal techniques for natural products analysis - SFC and CE, a practical comparison
Markus Ganzera, Michael Zwerger

OP2 - A2 Off-line CE search for PTP1B - inhibiting Traditional Chinese Medicines
***Ann Van Schepdael**, Juan Gao, Zhengjin Jiang, Erwin Adams*

OP2 - A3 Phytochemical, antioxidant, and androgenic effects of multi-solvent extracts of Sclerocarya birrea on mouse testicular TM3 Leydig cells
***Sogolo Lebelo**, Nelisiwe Masuku*

OP2 - B1 An analytical view on outpatient parenteral antimicrobial therapy
***Erwin Adams**, Tam Nguyen, Ann Van Schepdael, Isabel Spriet, Charlotte Quintens*

OP2 - B2 Implementation, Use and Outlook of Vendor-Agnostic Data Management Tools in Chemical and Analytical Development
*Adrian Clarke, **Martin Thomas Gaugg***

OP2 - B3 Simultaneous detection of unspecific trace N-nitrosamine impurities by LC-MS/MS in a pharmaceutical formulation
***Yue Zhang**, Sabah Houari, Thomas Van Laethem, Philippe Hubert, Cédric Hubert*

OP3-A1 Critical role of proline hydroxylation in collagen functionality: analytical characterization of 3- and 4-hydroxyproline isomers at amino acid and peptide levels

*Martina Lioi, Sara Tengattini, Valentina D'Atri, Gabriella Massolini, Simona Daly, Davy Guillarme, **<u>Caterina Temporini</u>***

OP3-A2 Expedited method development and characterization of therapeutic peptides and oligonucleotides
<u>Alexandre Goyon</u>

OP3-A3 Analytical peptidomics: future challenges
***<u>Evelien Wynendaele</u>**, Amélie Descamps, Hannah Hirmz, Seppe Ghijs, Bart De Spiegeleer*

OP3-B1 Exploring seminal liquid as a matrix for early detection of testicular cancer metabolism
***<u>Víctor González-Ruiz</u>**, Maricruz Mamami-Huanca, Constanza Fernández-Hernández, Luz Alonso-Dasques, Sandra Camunas, Ángeles López-Gonzálvez, Nina Mørup, Francisco J. Rupérez, Antonia García, Ana Gradillas, Serge Rudaz, Serge Nef, Ameer Y. Taha, Kristian Alsmtrup, Coral Barbas*

OP3-B2 Chemical, Metabolic and Plasma stability studies of different glucotrifluoroborates as potential agents for Boron Neutron Capture Therapy (BNCT)
*Salvatore Villani, Daniela Imperio, Silvia Fallarini, Silvio Aprile, Luigi Panza, **<u>Erika Del Grosso</u>***

OP3-B2 LC-MRM3, a high potential technique for pandemic preparedness: a case study of SARS-CoV-2
***<u>Nicolas Drouin</u>**, Hyung L. Elfrink, Stefan A. Boers, Sam van Hugten, Els Wessels, Jutte J.C. de Vries, Geert H. Groeneveld, Paul Miggiels, Bart van Puyvelde, Maarten Dhaenens, Andries E. Budding, Leonie Ran, Roy Masius, Zoltan Takats, Arjen Boogaerds, Markus Bulters, Wouter Muurlink, Paul Oostvogel, Amy C. Harms, Mariken van der Lubben, Thomas Hankemeier*

OP4-A1 MS-based metabolomics and lipidomics for differential diagnosis of Parkinson's disease and atypical Parkinsonisms
*Erika Esposito, Nicolò Interino, Giovanna Calandra Buonaura, Pietro Cortelli, Manuela Contin, Giovanna Lopane, Francisco Javier Rupérez, Coral Barbas, **<u>Jessica Fiori</u>***

OP4-A2 Characterization of IgG N-glycans in COVID-19, sepsis and HIV infection.
*Yosra Helali, Pierre Van Antwerpen, Michael Piagnerelli, Axelle Bourez, Yvan Vander Heyden, Karim Zouaoui Boudjeltia, Arnaud Marchant, **<u>Cedric Delporte</u>***

OP4-A3 Monitoring by LC-MS/MS of MPO-dependent oxidation of LDL and HDL in serum
***<u>Pierre Van Antwerpen</u>**, Axelle Bourez, Catherine Coremans, Cedric Delporte, Karim Zouaoui Boudjeltia*

OP4-B1 Charge to move forward in Volume-restricted Metabolomics
<u>Rawi Ramautar</u>

OP4-B2 Ligand-protein interaction's monitoring by CE-MS in the context of drug development: BGE and capillary coating optimizations
***<u>Clara Davoine</u>**, Marianne Fillet*

OP4-B3 Simultaneous quantification of therapeutic monoclonal antibodies and neutralizing anti-drug antibodies in patient serums using capillary electrophoresis coupled to tandem mass spectrometry
*Tessa Reinert, Pascal Houzé, Nathalie Mignet, Aynur Naghizade, Lola Alez-Martin, Matthieu Allez, Yannis François, **<u>Rabah Gahoual</u>***

OP5-A1 Molecular networking approach for clinical analysis: Benefits of creating in-silico databases of all drugs marketed in France as well as centrally authorized drugs approved by the European Medicines Agency for their identification
*Sacha Guilhaumou, Romain Magny, **<u>Emmanuel Bourgogne</u>***

OP5-A2 Integrating large metabolomics dataset from chemodiverse natural extracts and biological screenings into knowledge graphs to enhance bioactive natural product discovery.
***<u>Luis-Manuel Quiros-Guerrero</u>**[1,2], Frederic Burdet[3], Olivier Kirchhoffer[1,2], Paola Haemmerli[1,2], Louis-Felix Nothias[4], Pierre-Marie Allard[5], Arnaud Gaudry[1,2], Jahn Nitschke[6], Nabil Hanna[6], Florence Mehl[3], Antonio Grondin[7], Bruno David[7], Chunyan Wu[8], Thierry Soldati[6], Wolfrum Christian[8], Marco Pagni[3], Jean-Luc Wolfender[1,2]*

OP5-A3 Chemometrically estimated repeatability in UHPLC equipped with a noise filter and the evaluation of their statistical reliability
Akira Kotani[1], *Koichi Machida*[1], *Ryo Watanabe*[1], *Hideki Hakamata*[1]

OP5-B1 Metabolomics insights into solvent neurotoxicity using 3D brain organoids.
Mathieu Galmiche[1,2,3], *Isabel Meister*[1,2,3], *David Lopez-Rodriguez*[3,4], *David Pamies*[3,4], *Tatjana Sajic*[5,6], *Aurélien Thomas*[5,6], *Marie-Gabrielle Zurich*[3,4], *Julien Boccard*[1,2,3], *Serge Rudaz*[1,2,3]

OP5-B2 Development of a workflow targeted on phase II biomarkers by on-line SPE-LC-(ESI-)-HRMS in human urine for exposomic study purposes.
Emmanuelle Bichon[1], *Tatiana Metlej*[1], *Laurena Rutin*[1], *Anne-Lise Royer*[1], *Bruno Le Bizec*[1], *German Cano-Sancho*[1]

OP5-B3 Metabolomic profiling of mouse hippocampus to unravel the effects of chemogenetic modulation of astrocytes in a model for temporal lobe epilepsy
Liam Nestor[1], *Yvan Vander Heyden*[2], *Ann Van Eeckhaut*[1], *Dimitri De Bundel*[1], *Ilse Smolders*[1]

OP6-A1 Analytical Quality by Design: only probability matters !
Eric Rozet[1]

OP6-A2 Robustness and Validation of a Size-Exclusion Chromatography Method for Monoclonal Antibody Mass Variants Analysis: An AQbD-Inspired Approach
Arnaud Delobel

OP6-A3 Using the Analytical Target Profile (ATP) for Efficient Procedure Lifecycle Management
Amanda Guiraldelli Mahr, *Pierre Lebrun*, *Jean-Marc Roussel*

OP6-A4 Understanding links between Total Analytical Error, Measurement Uncertainty, Analytical Target Profile and Quality Target Product Profile.
Bruno Boulanger[1]

OP6-B1 Overcoming matrices inter-individual differences and in-source isobaric fragments for LC-MS native lactic acid enantiomeric quantification in pediatric human feces
Gioele Visconti, *Adrien Frézal*, *Yohan Grzywinski*, *Olivier Ciclet*

OP6-B2 RETENTION AND ENANTIOSELECTIVITY MODELLING OF STRUCTURALLY DIVERSE PHARMACEUTICALS ANALYZED ON POLYSACCHARIDE-BASED STATIONARY PHASES
Pieter De Gauquier, *Jordy Peeters*, *Fardine Ameli*, *Kenno Vanommeslaeghe*, *Yvan Vander Heyden*, *Debby Mangelings*

OP6-B3 Isotope effect in high-performance liquid chromatography: Good or bad for bioanalysis?
Giorgia Sprega, *Giorgi Kobidze*, *Alfredo Fabrizio Lo Faro*, *Tivadar Farkas*, *Francesco Paolo Busardo*, ***Bezhan Chankvetadze***

OP6-B4 Exploring chiral supercritical fluid chromatography for preparative scale.
Emmanuelle Lipka

OP7-A1 Interest of pI determination in the quality control of virus-like particles of human papillomavirus
Aurore Boclinville, *Marylène Vandevenne*, *Nicolas Thelen*, *Marc Thiry*, *Nathalie Jacobs*, *Alain Brans*, *Marianne Fillet*, ***Anne-Catherine Servais***

OP7-A2 Development of an enantio- and chemo-selective screening method for asparagine/aspartic acid and glutamine/glutamic acid residues in proteins
CHIHARU ISHII, *KANAKO TAKESHIMA*, *TAKEYUKI AKITA*, *MASASHI MITA*, *YUSUKE MURAKAMI*, *TADASHI UEDA*, *KENJI HAMASE*

OP7-B1 Charge Variant Separation and Characterization of Various Antibodies Using CZE-MS, cIEF-MS, and IEX-MS on Intact and Subunit Level
Jasmin Schairer, *Jennifer Römer*, *Christian Neusüß*

OP7-B2 Novel approach for the isolation of β-estradiol metabolites from human serum samples employing a 3D-printed device
Anna Kaliszewska, *Mariusz Belka*, *Tomasz Bączek*, *Lucyna Konieczna*

OP8-A1 A comparative study of analytical methods for assessing mRNA integrity in lipid nanoparticles: spotlight on mRNA-lipid adducts, fragments, and oligomers.
Camille Malburet, *Marco Morani, Perrine Lardellier, Thibaut Willemin, Stéphanie Fertier-Prizzon, Marc Francois-Heude*

OP8-A2 Characterization and relative quantification of oxidation in a monoclonal antibody by a multi-level approach based on mass spectrometry.
Sarah Nguyen, *Armelle Martelet, Séverine Clavier, Sarah Cianféran[2], Oscar Hernandez-Alba*

OP8-A3 Endotoxin Quantification by a Chemical Instrumental Analytical (U)HPLC-Assay
Anika Hoffmann, *Blanka Bucsella, Mathieu Zollinger, Kevin Pacios, Franka Kalman*

OP8-B1 The Effect of Column Length and Gradient Steepness on the Separation of Oligonucleotides in Ion Pair Reversed Phase Chromatography
Szabolcs Fekete, *Mateusz Imiolek, Matthew Lauber*

OP8-B2 Rapid isomer-specific identification and relative quantitation of new psychoactive substances in street samples
Govert Somsen, *Hany Majeed, Tijmen Bos, Ruben Kranenburg, Arian van Asten, Dr Isabelle Kohler*

OP8-B3 Microflow liquid chromatography coupled to nanoelectrospray ionization as a strategic combination for high-throughput bioanalytical methods with challenging sensitivity requirements
Sergey Girel, *Mathieu Galmiche, Mathis Fiault, Cedric Schelling, Isabel Meister, Serge Rudaz*

LIST OF SHORT ORAL PRESENTATIONS

SO-1 Endogenous steroid analysis by supercritical fluid chromatography-mass spectrometry: super possibilities and critical aspects
Tobias Langer, *Raul Nicoli, Davy Guillarme, Serge Rudaz, Tiia Kuuranne, Alessandro Musenga*

SO-2 Ultra-miniaturized weak affinity chromatography: towards a screening strategy of native membrane proteins in fragment Based Drug Discovery: Adenosine Receptor as a case-study.
Adrien Deloche, *Vincent Dugas, Renaud Wagner, Claire Demesmay*

SO-3 Advancements in neoglycoprotein synthesis and monitoring strategies
Isabella Senini, *Sara Tengattini, Francesca Rinaldi, Simone Nicolardi, Luciano Piubelli, Gabriella Massolini, Marco Terreni, Caterina Temporini*

SO-4 Navigating Experimental Metabolomics Knowledge Graphs to Highlight Anti-Infective Natural Products Within a Large Collection of Plant Extracts
Olivier Kirchhoffer, *Luis Quiros-Guerrero, Jahn Nitschke, Louis-Félix Nothias, Frédéric Burdet, Laurence Marcourt, Nabil Hanna, Florence Mehl, Antonio Grondin, Emerson Ferreira Queiroz, Marco Pagni, Thierry Soldati, Jean-Luc Wolfender*

SO-5 Characterization of Human Papillomavirus virus-like particles using iCIEF
Aurore Boclinville, *Marylène Vandevenne, Nicolas Thelen, Marc Thiry, Nathalie Jacobs, Alain Brans, Marianne Fillet, Anne-Catherine Servais*

SO-6 Detailed characterization of monoclonal antibodies charge variants using capillary electrophoresis and tandem mass spectrometry
Lola Alez-Martin, *Pascal Houzé, Rania Joomun, Nathalie Mignet, Yannis-Nicolas François, Rabah Gahoual*

SO-7 Towards automated decision support in the analytical reliability assessment of biomarker candidates: a multicriteria optimization framework.
Elfried M. B. Salanon, *Julien BOCCARD, Melanie PETERA, Stephanie DURAND, BLANDINE COMTE, Estelle PUJOS-GUILLOT*

SO-8 Phage therapy: quality control challenges and advances
Marie Willocx, *Mathieu de Jode, Flore Laurent, Laure Cuignet, Loïc Debehault, Maya Merabishvili, Jean-Paul Pirnay, Rob Lavigne, Pieter-Jan Ceyssens, Celine Vanhee*

LIST OF POSTERS

PS01-01 A simple LC-MS/MS assay of E6011, a novel anti-fractalkine human monoclonal antibody, in monkey serum - comparison with ligand binding assay -
__Yuji Mano__, Kenji Kita, Haruna Ono, Tomoko Kojima

PS01-02 Analytical developments towards new metrological references for the quantification of testosterone, aldosterone and 17β-estradiol in human serum.
__Chloé Duret__, Hélène Vaneeckhoutte, Vincent Delatour, Amandine Boeuf, Valérie Pichon

PS01-03 Affinity selection- mass spectrometry for Identifying Ligands of Acetylcholinesterase from Topsentia ophiraphidites and Docking Studies for the Dereplicated Ligands
Larissa R. G. da Silva, Chrystine B. de Sá, Nelilma C. Romeiro, __Quezia Cass__, Alessandra Valverde

PS01-04 mRNA/LNP Multiattribute Quantitation of Payload(s), Size and Heterogeneity With Size Exclusion Chromatography Coupled to Multiangle Light Scatteringps
__D Mateusz Imiolek__, Lavelay Kizekai, Bala Addepalli, Szabolcs Fekete, Matthew Lauber

PS01-05 Vizualisation of the bivariate dispersion structure for the robust assessment of the repeatability and reproducibility of analytical measurements.
__Elfried M. B. Salanon__, Blandine Comte, Delphine Centeno, Stephanie Durand, Estelle Pujos-Guillot, Julien Boccard

PS01-06 Enhancement of LC-MS analysis of intact glycoprotein
__Pierre Besson-Magdelain__, Christophe Chendo, Audrey Combès, Valérie Pichon, Nathalie Delaunay

PS01-07 Development and validation of a High-Performance Liquid Chromatography coupled Mass Spectrometry (HPLC-MS/MS) method for ABT-263 quantification in two different biological matrices
__Maxime Murphy__, Louiza Mahrouche, Valérie Gaelle Roullin, Grégoire Leclair

PS01-08 Electrochemical Biosensors: Practical Solutions for Microorganism Identification
__Seda Nur Topkaya__

PS01-09 Transacylation and hydrolysis of the acyl glucuronides of ibuprofen and its α-methyl-substituted analogues investigated by 1H NMR spectroscopy and computational chemistry: Implications for drug design.
__Selena E. Richards__, Peter R. Bradshaw, Caroline H. Johnson, Andrew V. Stachulski, Toby J. Athersuch, Jeremy K. Nicholson, John C. Lindon, Ian D. Wilson

PS01-10 Absolute quantification and structural characterization of therapeutic monoclonal antibodies after administration to patients using capillary electrophoresis-tandem mass spectrometry
Tessa Reinert, Pascal Houzé, Nathalie Mignet, Alexandre Kulus, Matthieu Allez, Yannis François, __Rabah Gahoual__

PS01-11 Qualitative and quantitative evaluation of the metabolism of various drugs for five types of CYP2D6 SNPs
__Seon Min Lee__, Hyejin Kim, Heejin Jeong, Jongwook Song, Jonghwan Kim, Jeong-Doo Heo

PS01-12 The investigation of structural characteristics of biologically active natural polymers using solid-state NMR experiments
__Ketevan Lomsadze__, Isabelle Lengers, Vakhtang Barbakadze, Jens Köhler, Michael Ryan Hansen, Sascha Lebioda, Bodo Saake, Joachim Jose

PS01-13 Exploring Chiral Recognition: Docking Simulations and Experimental Validation on chiral Polysaccharide-based Stationary Phases
Anca-Elena DASCALU, Alina GHINET, Christophe FURMAN, __Emmanuelle Lipka__

PS01-14 Electrochemical biosensor designing for investigation of the interaction between DNA and Ciprofloxacin
__Hasret Subak__

PS01-15 Enhancing microflow LC-MS/MS analysis of neuromedin U through reduced aspecific adsorption
Linus Donvil, *Jana Bongaerts, Maria Bjerke, Debby Mangelings, Yvan Vander Heyden, Ann Van Eeckhaut*

PS01-16 Study on pesticides contamination in Chinese Proprietary Medicine (CPM) products retailed in Singapore
Cuilian Sun

PS01-17 Clinical Calcium Ion Analysis with Isomeric Calcium Ionophores in Ion-Selective Electrodes
Yupu Zhang, *Junyu Zhou, Tianbao Yang, Zejian Huang, Gabriel J. Mattos, Nikolai Yu Tiuftiakov, Yaotian Wu, Jinxu Gao, Yu Qin, Eric Bakker*

PS01-18 Unveiling the potential of ion-pairing RPLC for mRNA characterization
Jonathan Maurer, *Camille Malburet, Marc François-Heude, Davy Guillarme*

PS01-19 LC-MS/MS determination and pharmacokinetic study of selected breast cancer drugs in clinical practice
Lu Turković, *Zvonimir Mlinarić, Dubravka Glasovac, Sven Komljenović, Mila Lovrić, Tajana Silovski, Marija Križić, Miranda Sertić*

PS01-20 Potential for drug-drug interactions of breast cancer drug abemaciclib – an in vitro metabolism study
Martin Kondža, ***Lu Turković***, *Hrvoje Rimac, Guadalupe Maria Gutiérrez Acosta, Zvonimir Mlinarić, Miranda Sertić*

PS01-21 Chiral targeted brain metabolomics in volume-limited biological samples
Cinzia Lella, *Liam Nestor, Yvan Vander Heyden, Ann Van Eeckhaut*

PS01-22 An automated CZE-MS and Genedata-RefinerMS based analytical workflow for plate-based milligram scale siRNA synthesis.
Jürgen Kühnöl, *Sven Zapf, Dominik, Hanna Hunziker, Francois, Corinne, Jörg Hunziker, Juan Zhang*

PS01-23 Fluorescent compounds as pH tracing standards for capillary isoelectric focusing analysis of labeled proteins and their glycoforms using laser-induced fluorescence detection
Filip Duša, *Pavlína Dadajová, Richard Čmelík, Karel Šlais, Jana Lavická*

PS01-24 Measurement of exogenous and endogenous steroids in dried blood spots by LC-MS/MS for application to pharmacokinetic studies
Greta Galante, *Valentina Bissi, Federico Mao, Gianmarco Pizza, Francesca Donnarumma, Guido Di Dalmazi, Uberto Pagotto, Flaminia Fanelli*

PS01-25 Circadian rhythm profiling of amino acids and biogenic amines in chronic hypercortisolism states: utility of dried blood spots
Greta Galante, *Valentina Bissi, Lorenzo Tucci, Ilaria Improta, Alessandro Perrone, Kimberly Coscia, Giacomo Colombin, Valentina Vicennati, Uberto Pagotto, Guido Di Dalmazi, D Flaminia Fanelli*

PS01-26 Beyond conventional: evaluating the potential of HILIC for collagen peptide mapping analysis
Martina Lioi, *Sara Tengattini, Valentina D'Atri, Gabriella Massolini, Simona Daly, Caterina Temporini, Davy Guillarme*

PS01-27 Biomonitoring of phthalates in the Swiss population
Joelle Houriet, *Alexandra Jaus, Baptiste Clerc, Gisela Umbricht, Beat J. Brüschweiler*

PS01-28 Determination of organic acids and phenolic compounds by capillary electrophoresis
Olivier Vorlet, *Sara Driad*

PS01-29 Therapeutic drug monitoring of asparaginase in acute lymphoblastic leukemia (ALL) patients Swiss-wide implementation and international cross-validation.
Dalila Alessi, Geraldine Sauvain, Amarande Murisier, Eric Grouzmann, Tony Teav, Julijana Ivanisevic, Francesco Ceppi, Massimo Zucchetti, Manfred Fobker, Antoine Pierrot, Catia Marzolini, François Girardin, Laurent Arthur Decosterd, ***Eva Choong***

PS01-30 A multiplex LC-MS/MS assay for the Therapeutic Drug Monitoring of ivacaftor, lumacaftor, tezacaftor, and elexacaftor and their active metabolites, in Cystic Fibrosis.
Fabrizio Corrado, Thomas Mercier, François Versace, Amarande Murisier, Susana Alves Saldanha, Sylvain Blanchon, Zisis Balmpouzis, Angela Koutsokera, François Girardin, Catia Marzolini, Alain Sauty, Laurent Arthur Decosterd, ***Eva Choong***

PS01-31 Quantification of recombinant human serum albumin (rHSA) in serum-free preservation medium for human donor corneal tissues, by liquid chromatography-tandem mass spectrometry (LC-MS/MS)
Amarande Murisier*, Vincent Desfontaine, Marie-Claude Amoureux, François R. Girardin, Eva Choong, Laurent A. Decosterd*

PS01-32 Quantification of NNAL and anabasine with consecutive analysis of nicotine, cotinine and 3'-hydroxycotinine in urine using LC-MS/MS to determine tobacco smoking status and nicotine use from nicotine-containing alternatives for smoking cessation studies
Vera van der Velpen, ***Kristina Žajdlíková****, Mats B. Hirt, Evangelia Liakoni, Manuel Haschke*

PS01-33 Caffeine metabolic ratios from diet to estimate CYP1A2 activity: association with olanzapine plasma concentrations
Nicolas Ansermot*, Frederik Vandenberghe, Nermine Laaboub, Harish Vathanarasa, Setareh Ranjbar, Séverine Crettol, Franziska, Kerstin Jessica Plessen, Armin von Gunten, Philippe Conus, Chin Bin Eap*

PS01-34 Methodological Support for Clinical Analysis of Patients in Critical Condition
Alisa Pautova

PS01-35 Association of type 2 diabetes mellitus to arsenic metabolism?
Maïwenn Perrais*, Marie Rohrbacher, Grégory Plateel, Carmine Schipani, Julien Vaucher, Aurélien Thomas*

PS01-36 Determination of sildenafil by capillary electrophoresis
Océane Roulin

PS01-37 DEVELOPMENT AND VALIDATION OF A HPLC-MS/MS METHOD FOR DETERMINATION OF BUDESONIDE IN NEWBORNS AFTER INHALATION OF THE DRUG
Marta Karaźniewicz-Łada*, Maria Pietraszak, Kamila Paschke, Franciszek Główka, Jan Mazela*

PS01-38 A prick is enough! Therapeutic drug monitoring of antiseizure medication through capillary microsampling devices
Chiara Cancellerini, ***Alice Caravelli****, Erika Esposito, Laura Maria Beatrice Belotti, Martina Soldà, Luca Vignatelli, Barbara Mostacci, Jessica Fiori, Francesca Bisulli, Laura Licchetta*

PS01-39 Potential Identification of An Undeclared Compound in Herbal Supplements; Beta-Methylethephenylamine
Thamer Alghamdi*, Anwar Al-Suhaibani, Fahad Aldawsari*

PS01-40 Comparative Analysis of Antibodies and Molecularly Imprinted Polymers for Electrochemical Detection of Dopamine
Meltem Okan*, Zeynep Çağlayan Arslan, Ender Yıldırım, Haluk Külah*

PS01-41 Developing HPLC-UV Method for Multi-Analyte Detection of Caffeine, Nicotine, Clozapine, and Their Metabolites in Plasma Samples
Kenan Can Tok*, Goksin Kara, Sinan Suzen, Ezel Boyaci, Mehmet*

PS01-42 Electrochemical and Spectroscopic Methods Provide Insight Into The Interaction between Nabumetone and double-stranded DNA.
Bengi Uslu*, Ruqia Khan, Cigdem Kanbes-Dindar, Arzu Karayel*

PS01-44 Comparative Study of Molecularly Imprinted Polymer Immobilization Techniques on Microfluidic Electrochemical Chips for Dopamine Biosensing
Zeynep Çağlayan Arslan*, Meltem Okan, Ender Yıldırım, Haluk Külah*

PS01-45 Subunit glycoprofiling by HILIC and FcγRIIIA affinity chromatography to address the glycoengineering of Rituximab from Nicotiana benthamiana

Sara Tengattini, *Francesca Rinaldi, Aurora Tini, Isabella Senini, Carla Marusic, Claudio Pisano, Marcello Donini, Caterina Temporini*

PS01-46 Oligonucleotide Characterization: Combining Anion Exchange Chromatography with In-line Multi-Angle Light-Scattering Detection
Andrea Krumm, *Elena Kumm*

PS01-47 Effect of Analytical Conditions on Oligonucleotide Adsorption in Ion-Pairing Reversed Phase and Ion Exchange Chromatographyps
Simon Schumacher, *Daniel Esser, Sae Miyagishi, Chiaki Matsumura, Ken Tsutsui, Noriko Shoji*

PS01-48 Native anion exchange chromatography coupled to mass spectrometry for the analysis of charge variants of IgG4-based monoclonal antibodies
Simon Schumacher, *Kirstin Arend, Ann Marie Rojahn, Daniel Eßer*

PS01-49 Microwave-assisted extraction and characterization of fatty acids: a sustainable approach to fatty acid production from wheat bran
Enrica Calleri, *Gloria Brusotti, Giulia De Soricellis*

PS02-01 Application of inert biphenyl stationary phase in the analysis of biologically relevant metabolites
Mathieu Galmiche, Marie-Anaïs Monat, ***Cyrille Lamboley***, *Diego A. Lopez, Paul Connolly, Serge Rudaz*

PS02-02 Improvements in body odor sampling and analysis for medical diagnosis applications using canine olfaction and GC-MS
Laetitia Maidodou, *Damien Steyer, Eric Marchioni, Igor Clarot*

PS02-03 Blockchain and machine learning based framework for managing EMRs: Security, Privacy, Interoperability, and Clinical Decision Support
Abdelaziz ElFazziki, ***Hasna Elalaoui Elabdallaoui***, *Youssef Mourdi*

PS02-05 Capillary electrophoresis as a green alternative to liquid chromatography for determination of alpelisib in pharmaceutical dosage forms
Zvonimir Mlinarić, *Lu Turković, Ivor Babić, Tajana Silovski, Nina Kočevar Glavač, Miranda Sertić*

PS02-06 On going procedure performance verification of chromatographic assay methods
Jean-Marc Roussel, *Michel Righezza*

PS02-08 Full analytical characterization of an innovative nanomedicine targeting human islet amyloid polypeptide for type 2 diabetes mellitus treatment
Mathilde Jégo, *Julia Kaffy, Lynda Benrabah, Julie Mougin, Claire Smadja, Myriam Taverna*

PS02-09 Analysis of enantiomeric purity of some fluoroquinolones antibacterials pharmaceutical formulation on different chiral stationary phases
Mohamed Nadjib Rebizi

PS02-10 Untargeted Metabolomics of PAC-containing Dietary Supplements by LC-MS: Data Fusion Approach For authentication and Detection of Adulteration in Botanical Materials
Amanda Guiraldelli Mahr, *Maria Monagas, Gabriel Giancaspro, Cuying Ma*

PS02-11 Chemical derivatization for targeted brain metabolomics of volume-limited samples using LC-MS/MS
Liam Nestor, *Cinzia Lella, Dimitri De Bundel, Ilse Smolders, Yvan Vander Heyden, Ann Van Eeckhaut*

PS02-12 Monitoring of Leachable Compounds in Hospital Pharmacy-Compounded Drug Products by UHPLC-HRMS
Julian Pezzatti, *William Bello, Laurent Carrez, Serge Rudaz, Sadeghipour Farshid*

PS02-13 SPME as a novel extraction approach for in vivo analysis of phytocannabinoids in Cannabis spp. plants
Katarzyna Woźniczka, *Vaclav Trojan, Krzysztof Urbanowicz, Patrik Schreiber, Julia Zadrożna, Tomasz Bączek, Ryszard Tomasz Smoleński, Anna Roszkowska*

PS02-14 LC-MS/MS targeted-metabolomics of kynurenines in cell supernatants: a reliable readout for assessing the in vitro potency of selective hIDO1 and TDO inhibitors
Salvatore Villani, *Silvia, Sarah Jane Rezzi, Rita Maria Concetta Di Martino, Silvio Aprile, Erika Del Grosso*

PS02-15 Hunting for oxidized lipids in complex biological matrices
Beatrice Zoanni, *Vincen Wu, Nicola Zamboni, Marina Carini*

PS02-16 Early Biopharmaceutical Assessment and Formulation Development in Drug Discovery
Anaëlle Monfort, *Maxime Murphy, Louiza Mahrouche, Isabelle St-Jean, Martin Jutras, Mihaela Friciu, Valérie Gaëlle Roullin, Grégoire Leclair*

PS02-17 Development and optimization of an SFC-MS method for the simultaneous analysis of anticancer drugs with a wide range of physicochemical properties.
Nathalie Nguyen, *Davy Guillarrme, Serge Rudaz, Pascal Bonnabry, Sandrine Fleury-Souverain*

PS02-18 Comparison of hemispherical directional reflectance and thermal emittance of metformin extended-release tablets exposed to sunlight
Beata Sarecka-Hujar

PS02-19 Non-Invasive Quantification of Protein Glycation in Drug Development: A Time Domain NMR Approach
Hani Alam, Özlem Gezici Koç, ***Gözde Özeşme Taylan***, *Cem Yamalı, Mecit Öztop*

PS02-20 Enhanced Insulin Encapsulation Using Core-Shell Natural Polysaccharide-Based Aerogels for Oral Delivery
Gözde Özeşme Taylan, *Carlos Illanes-Bordomás, Özge Güven, Carlos A. García-González, Mecit Halil Öztop*

PS02-21 Microsampling Meets Microextraction: Bridging the Gap for More Efficient Drug Analysis
Adam Reguli, *Hana Bavlovič Piskáčková, Olga Lenčová, Petra Kollárová-Brázdová, Martin Štěrba, Petra Štěrbová-Kovaříková*

PS02-22 Development of an in vitro BBB model to study the passage of magnesium taurate and related molecules in the brain compartment.
Anna Laurent, *Julie Horne, Cédric Hubert, Marianne Fillet*

PS02-23 High Throughput FAMS – A Fatty Acid Mass Spectrometry Method for Monitoring Polysorbate Hydrolysis in QC
Anja Bathke, *Jan Wendler, Sina Hoelterhoff*

PS02-24 First steps in 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
Kevser Kemik, *Charlotte De Bleye, Pierre-Yves Sacré, Philippe Hubert, Eric Ziemons*

PS02-25 Online and automated sample handling – The future of fast and efficient mAb characterization and quantification
Sina Hölterhoff, *Anja Bathke, Saban Özipek*

PS02-26 Pick your method - A Toolbox for LC-MS Peptide Mapping
Sina Hölterhoff, *Anja Bathke, Saban Özipek*

PS02-27 Design of experiments-based sample-preparation optimization of plasma samples for targeted metabolic profiling of neurologically relevant amino acids
Phaedra Verding, *Yvan Vander Heyden, Ann Van Eeckhaut, Debby Mangelings*

PS02-28 Expediting Online Liquid Chromatography for Real-Time Monitoring of Product Attributes to Advance Process Analytical Technology in Downstream Processing of Biopharmaceuticals
Lukas Naumann, *Tobias Graf, Lea Bonnington, Jakob Heckel, Bernhard Spensberger, Sascha Klein, Christoph Brey, Ronnie Nachtigall, Maximilian Mroz, Thomas Hogg, Christopher McHardy, Andrés Martinez, Reinhard Braaz, Michael Leiss*

PS02-29 Optimizing solvent dilution for enhanced performance in online comprehensive two-dimensional liquid chromatography: theoretical and practical guidelines

***Megane Aebischer**, Soraya Chapel, Davy Guillarme, Sabine Heinisch*

PS02-30 Determination of Ethylene Oxide Residuals in Sterilized Active Pharmaceutical Ingredients via Full Evaporation Headspace - Gas Chromatography
***Ihtesham Rehman**, Kris Wolfs, Erik Haghedooren, Erwin Adams*

PS02-31 Analytical Characterization of Plasmid DNA Topological Forms
***Alexandre Goyon**, Daniel Nguyen*

PS02-32 Negligible depletion in-vivo SPME for determining free and total endocannabinoid concentrations in rat brain
***Igor Gustavo Carvalho Oliveira**, Maria Eugênia Costa Queiroz, Janusz Pawliszyn*

PS02-33 A rarely advertised approach to sensitize LC-sQMS methods
***Gabriella Szabovik**, Zoltán Béni*

PS02-34 Instant and non-destructive analysis of medicines using cloud-based portable NIR technology and machine learning
***Hervé Rais**, Pierre Esseiva, Olivier Delémont*

PS02-35 Is Transmission surface-enhanced Raman spectroscopy a good alternative to separative techniques for the nicotine dosage in e-liquid boosters?
***Charlotte De Bleye**, Pierre Beckers, Julie Horne, Kevser Kemik, Pierre-Yves Sacré, Eric Caudron, Philippe Hubert, Eric Ziemons*

PS02-36 Untargeted metabolomic analysis of hawthorn-based food supplements: study of circulating molecules for rationalizing phytotherapy practices
*Astrid De Radigues de Chennevière, Nausicaa Noret, Cécile Vanhaverbeke, **Florence Souard***

PS02-37 Metaboscan AI – the ML-based platform for full-Cycle management of metabolomic data
***Kseniia Shestakova**, Andrey Boldin, Pavel Rezvanov, Svetlana Appolonova*

PS02-38 An attempt to explain the mechanism of statin-induced muscle pain using a multiplatform metabolomics approach
***Ewa Paszkowska**, Steen Larsen, Paulina Choinska, Karolina Pietrowska, Michal Ciborowski*

PS02-39 From untargeted to targeted HRMS-based proteomics: study of a biotechnological drug in plasma and CSF for Lafora disease
***Erika Esposito**, Alice Caravelli, Chiara Cancellerini, Eleonora Pizzi, Raffaella Minardi, Lorenzo Muccioli, Maria Tappatà, Roberto Michelucci, Francesca Bisulli, Jessica Fiori*

PS02-40 Strategy for Multiplexing Absolute Quantification in Metabolomics with SILs as internal calibrant: one to quantify them all !
***Oriane Strassel**, Julien Boccard, Serge Rudaz*

PS02-41 Development of an optimised icIEF method for harmonising Quality Control of Monoclonal Antibodies by using an AQbD approach
***Virginia Ghizzani**, Alessandro Ascione, Serena Orlandini, Sandra Furlanetto, Gabriella Massolini, Francesca Luciani*

PS02-42 Targeted metabolomics as a tool for therapy efficacy monitoring of autoimmune and hematooncological diseases
***Sabina Baskhanova**, Valeria Varzieva, Natalia Moskaleva, Pavel Markin, Larisa Musaeva, Irina Menshikova, Irina Ilgisonis, Svetlana Appolonova*

PS02-43 Lipidomics and metabolomics to investigate synergistic effects of erlotinib-HCl and tubacin anti-cancer drugs within 3D spheroid cell model of kidney cancer
***Mathis Fiault**, Valentin Mieville, Cédric Schelling, Serge Rudaz, Patrycja Nowak-Sliwinska, Isabel Meister*

PS02-44 Gas Chromatography-Tandem Mass Spectrometry Method for the Quantitative Determination of Ethylene Glycol and Diethylene Glycol in Pediatric Syrups
***Monerah A. Altamimy**, Yahya M. Alshehry, Fahad S. Aldawsari, Norah H. Altalyan, Sultan K. AlShmmari, Shaikah F. Alzaid, Naif A. Al-Qahtani, Turki M. Alsagoor, Mobarak S. Al Shahrani*

PS02-45 A single LC-MS/MS method to quantify 8 cannabinoids in blood, breast milk and meconium

Anaëlle Monfort, *Louiza Mahrouche, Martin Jutras, Wissal Ben Jmaa, Gregory Lodygensky, Ema Ferreira, Grégoire Leclair*

PS02-46 DEVELOPMENT OF HPLC METHOD FOR THE DETERMINATION OF ABEMACICLIB FROM LIPID-POLYMER HYBRID NANODELIVERY SYSTEMS

Mehmet Gumustas, *Ceren H. Bozmaoglu, Gamze Guney Eskiler, Ceyda Tuba Sengel Turk*

PS02-47 INVESTİGATİON OF THE MOLECULAR İNTERACTİON BETWEEN APROCLİNİDİN, AN α2-ADRENERGİC RECEPTOR AGONİST, AND BOVİNE SERUM ALBUMİN WİTH FLUORESCENCE AND MOLECULAR DOCKİNG TECHNİQUES

Ipek Kucuk, *Oyku Buket Kucuksahin, Merve Yıldırım, Zahirul Kabir, Hülya Silah, İsmail Çelik, Doctor Bengi Uslu*

PS02-48 Design and Applications of Electrochemical Nanozyme Platform Consisting of Magnetic Nanomaterials and Inorganic Nanomaterials

Selenay Sadak, *Cigdem Kanbes-Dindar, Emine Yağmur, Bengi Uslu*

PS02-49 APTAMER-BASED HYBRID MONOLITHIC SORBENT FOR SELECTIVE EXTRACTION OF HOMOCYSTEINE IN BIOLOGICAL SAMPLES

Caroline Frenandez Grecco, Maria Eugênia Costa Queiroz, Audrey Combès, **Valérie Pichon**

PS02-50 Drug quality control in Senegal using capillary electrophoresis: retrospect on 12 years of work

Thierno Mouhamed Wane, *Elhadji Assane DIOP, Serge RUDAZ, Yerim Mbagnick DIOP, Serigne Omar SAARR*

PS02-51 Developpement and validation of a capillary electrophoresis method for the control of salbutamol sulfate

Thierno Mouhamed Wane, *Papa Elhadji THIAM, Elhadji Assane DIOP, Serge RUDAZ, Yerim Mbagnick DIOP, Serigne Omar SARR*

OP1 - A1

New frontier for cells characterization Spectralomics: the Raman Spectroscopy

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Raman spectroscopy is in fact an attractive technique for non-destructive and label-free imaging, while maintaining chemical selectivity, it can be combined with optical microscopy enabling powerful approaches for noninvasive chemical imaging. Due to the superior resolution of Raman microscopy (1 µm) and its applicability in aqueous environments, confocal Raman microscopy is today the prevalent technique for cellular visualization. Despite few exemptions, cells are commonly cultured submerse in aqueous medium. However, the aqueous environment does not impede Raman microscopy analysis. Cells can be analyzed using Micro Raman spectroscopy, by comparing the spectral profiles, or "fingerprints," of treated and untreated cells. This unique Raman fingerprinting approach enables the detection of chemical alterations within cellular chemical composition. It provides a means of visualizing and characterizing cells beyond the limitations of examining isolated markers or metabolic pathways. By analyzing these spectral signatures and comparing them, valuable insights into the molecular composition of cells can be obtained, including the relative proportions of various biomolecules. Variations in spectral signatures between different cell types and states reflect differences in molecule distribution and concentration, offering significant information on cellular identity, differentiation, and functional attributes. In this study a Raman spectral signature of differentiation has been conducted and a preliminary PCA analysis is discussed. This fingerprints enabled differentiation between different cell types or conditions based on variations in their spectral signatures as well as revealing valuable information about cellular identity, differentiation, and functional attributes. This approach, often referred to as "spectralomics," captures the entire cell state instantaneously, offering insights into metabolism and phenotype in a straightforward manner. Compared to scrutinizing individual markers, spectralomics offers a more resilient approach, in particular Raman analysis offers a holistic and comprehensive perspective on cellular attributes, mechanisms, and functions. It represents a transformative way of visualizing and characterizing cells, providing valuable insights into cellular biology allows for the simultaneous interpretation of modifications across entire cell phenotypes.

OP1 - A2

Potential of lectin- and aptamer-based sorbents for extraction and/or fractionation of the human chorionic gonadotropin glycoforms prior to analysis at the intact level by LC-HRMS

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The human chorionic gonadotropin (hCG) is the hormone specific to human pregnancy, essential for the development of placenta and foetus. It is a dimeric protein with two subunits, hCGα and hCGβ, having in total 4 N- and 4 O-glycosylation sites, leading to a high number of glycoforms. A method based on reversed phase liquid chromatography (LC) at the nano format hyphenated to high resolution mass spectrometry (HRMS) (Orbitrap), was developed for the analysis of the hCG glycoforms at the intact level and dozens of hCGα and hCGβ were detected in hCG-based drugs [1].

Affinity supports based on immobilized lectins were used in solid phase extraction (SPE) to evaluate their potential for fractionation of hCG glycoforms according to their glycosylation state. Home-made Concanavalin A (Con A) sorbents were prepared by immobilizing lectin on Sepharose with a mean grafting yield of 98.2% (RSD=3.5%, n=15) [2]. A capacity of about 100 µg of purified urinary hCG (uhCG) per ml of sorbent, grafted with a density of 10 mg of Con A per ml, was estimated. Average extraction yields of around 60% for both hCGα and hCGβ glycoforms were obtained after optimization of the extraction protocol. Intra- and inter-assay evaluation led to average RSD values of around 10%, indicating a repeatable extraction procedure. Con A-SPE led to the fractionation of some glycoforms between the retained and non-retained portion of uhCG, which allowed the detection of hCGα with two tetra-antennary N-glycans that couldn't be detected by direct analysis in LC-HRMS without Con A-SPE. Regarding a recombinant hCG, a fractionation was also observed leading to the detection of unretained hCGα glycoforms with tri-antennary N-glycans. A similar study was next performed with sorbents functionalized with Jacalin. Therefore, the combination of lectin-SPE with intact protein analysis by LC-HRMS can contribute to a more detailed glycosylation characterization of the hCG protein.

Regarding the development of a selective extraction step of hCG glycoforms from serum of pregnant women, i.e. complex biological samples rather than concentrated and pure hCG-based drugs, the development of SPE with aptamer-based sorbents is currently under study. One oligonucleotide aptamer and one peptide aptamer specific of hCG were selected after reviewing literature. They were grafted on CNBr- and thiol-activated Sepharose, respectively. Evaluation of the retention of hCG glycoforms on the resulting sorbents is in progress and the most relevant results will be presented.

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OP1 - A3

2D MXene/Surfactant nanocomposite preparation and its electrochemical performance towards the identification of Vandetanib level in human urine sample

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In this study, the electrochemical analysis of Vandetanib was carried out using 2D Mxene-SDS/GCE obtained by the deposition of Ti3C2Tx (MXene) suspended in polyvinyl fluoride with anionic surfactant (SDS) on the surface of GCE at a specific potential (0.6 V). Surface characterization of MXene-SDS/GCE was performed using electrochemical techniques (electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV)), morphological analysis (scanning electron microscopy (SEM)), and spectroscopic methods (Fourier transform infrared spectroscopy (FT-IR)). The proposed electrochemical sensor exhibited excellent selectivity, accuracy, precision, and reproducibility. Under optimized conditions, it showed excellent linearity in the concentration range of 0.05–8.0 µg mL-1 for Vandetanib (VAN) and effectively detected VAN in biological and pharmaceutical preparations.

OP1 - B1

Imaged capillary isoelectric focusing associated with multivariate analysis to detect degradation of therapeutic monoclonal antibodies

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Therapeutic monoclonal antibodies (mAbs) are increasingly used in hospitals. However, mAbs treatments are associated with high immunological risks due to protein denaturation and aggregation issues that may occur during manipulations at the hospital. We aim to develop a fast, straightforward method to provide information on mAbs states before patient administration. Therefore, a novel approach has been developed based on imaged capillary isoelectric focusing (icIEF) combined with Principal Component Analysis (PCA). This approach has been applied to mAbs submitted to mild stress. First, a generic method based on icIEF has been developed to analyze native and degraded mAbs. A signal processing has been designed to extract relevant data from the electropherograms (mAbs icIEF profiles), independently of pI scaling. After mAbs submission to mild stresses, analysis, and signal preprocessing, PCA score plots allowed discrimination between stressed and non-stressed mAbs for Infliximab, Pembrolizumab, and Pertuzumab. In addition, distinct clusters (score 79%) allowing differentiation of stress sources (thermal, UV, agitation) were formed for Infliximab. The clusters obtained were correlated to time exposure for infliximab submitted to UV. To assess the results obtained by iCIEF-PCA analysis, stressed samples were analyzed by SEC combined with ion mobility-mass spectrometry (IM-MS). Results obtained highlight conformational modifications of mAbs submitted to mild stress, thereby assessing the potential of the icIEF combined with Principal Component Analysis (PCA).

For the first time, we highlight the high potential of the combination of icIEF and PCA to discriminate forced degraded mAb samples from native ones. This new approach could provide a fast and very efficient strategy to control the degradation states of therapeutic mAbs routinely used in hospitals. iCIEF is also a promising tool for evaluating the impact of stresses on mAbs' interactions with their biological targets.

OP1 - B2

Unveiling the dynamics of enzymatic activity in crowded environments: insights from hyaluronidase kinetics and interactions in extracellular matrix mimics

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The extracellular matrix (ECM) is dominated in-vivo by macromolecular crowding and resultant excluded volume effects [1]. It is composed of a large quantity of various macromolecules which fill the interstitial space within cells forming a hydrated gel [2]. ECM is a highly dynamic structure. It is constantly regenerated, remodeled and degraded to maintain tissue homeostasis, through the action of metalloenzymes such as collagenase, hyaluronidase (Hyal) and elastase [3]. The present investigation is part of a large multidisciplinary project, X-Crowd, aiming to scrutinize the kinetics of these enzymes in a realistic picture. For this purpose, crowded environments mimicking the ECM in-vitro are used. The crowding environment was simulated using dextran at two different molecular weights (40 and 476 kDa), respecting so the ratio between enzyme and crowder size.

We have first studied the activity of Hyal, a glycosidase responsible for the degradation of hyaluronic acid (HA), a large polysaccharide responsible for skin hydration and cartilage lubrication. Capillary electrophoresis (CE), thanks to its miniaturized dimensions, was advantageously used to monitor the enzymatic reaction, after optimizing the injection step, taking into account the media viscosity and complexity. To better understand the effect of dextran on the catalytic activity of Hyal, a small substrate, decasaccharide (10-mers), was firstly used. Results were compared to those obtained with the high molecular weight natural substrate, HA. Moreover, the interaction between Hyal and the Dextran was characterized using microscale thermophoresis (MST), a biophysical miniaturized technique based on fluorescence detection. Hyal was thus labeled with ATTO-647 and studied in the presence of dextran with different buffer compositions and pH conditions. The inhibition study of this enzyme by a referenced inhibitor firstly, and a home-made inhibitor secondly, was also carried out in dilute and crowded media. Finally, ionic mobility spectrometry (IMS) was used to evaluate the effect of crowding on the folding state and conformational dynamics of Hyal. CE combined to IMS and MST allowed to disentangle the impact of crowding on Hyal kinetics, folding state and interactions. Therefore, this reaffirms the significance of conducting biological assays under conditions that closely mimic in vivo environments. The approach we propose ensures the development of efficient and more reliable bioactive compounds during the development process.

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OP1-B3

Portable capillary electrophoresis: a novel device for determining the date-rape drug gamma-hydroxybutyric acid (GHB) at the point of need.

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Background
Gamma-hydroxybutyric acid represents one of the illicit compounds used as date rape drugs because of amnesia and sleep-inducing effects. However, the determination is demanding because of the rapid metabolism (<6h blood, <12h urine), and the low performances characterizing commercial on-site methods both in terms of sensitivity and specificity [1]. On this basis, a separative method for having high performances is needed.

Although chromatography-based methods are the most investigated separation approaches, the portability of equipment is limited by large volumes of mobile phases, derivatization reactions for gas chromatography, and pump systems for liquid chromatography. Conversely, in capillary electrophoresis (CE) the volume of the separation buffer is a few microliters and the high voltage used as driving force of the separation process is easily controllable by electronic boards. Consequently, these technical aspects facilitate the assembly of portable devices.

On this basis, the present work was aimed at developing a portable capillary electrophoresis to be used on-site for determining gamma-hydroxybutyric acid (GHB) in a toxicology context.

Method
An already-validated method was used [2]. The buffer composition was: arginine 20 mM, maleic acid 10 mM, cetyltrimethylammonium-bromide (CTAB) 30 µM and vancomycin 5 mM (pH 7.35). The separation occurred in a fused silica capillary (I.D. 25 µM, effective length 70 cm) applying a voltage of -20 kV and injecting the sample by pressure (0.4 psi x 12.5 sec). The detection was carried out by using a Capacitively-Coupled Contactless Conductivity Detector [excitation frequency 320 kHz, amplitude 240 Vpp (peak-to-peak)].

Results
A feasibility study for allocating the device inside a protector case Peli1450® (37.36x25.96x15.44 cm) has been carried out. The following components have been studied: pneumatic elements, hydraulic components (it integrates a multiport injector - 10 µL sample loop), Capacitively-Coupled Contactless Conductivity Detector, 2 high-voltage modules, and a microcontroller board. The configuration allowed to run 25 analyses, and the performances have been preliminarily evaluated. The method has been optimized for analyzing urine using the dilute-and-shoot approach. After a fourfold dilution, samples are injected without derivatization or extraction procedures. The determination of GHB was linear within 15 - 200 µg/mL, showing a limit of detection of 4 µg/mL (S/N = 3), and a precision of determination better than 6%. On this basis, the approach was able to discriminate between low and high GHB levels, also allowing quantitative results after a proper calibration using spiked real samples. The method has been also tested for resolving gamma-hydroxybutyric acid from the endogenous isomers alfa-hydroxybutyric acid (AHB) and beta-hydroxybutyric acid (BHB), resulting in a resolution higher than 1.8.

Conclusions
The developed technology is an additional tool in cases of drug-facilitated sexual assaults (DFSA) at the point-of-need. The use of a rapid on-site instrumental technique will provide in objective results that will speed up investigative actions. In addition, this new technology can open up further opportunities for on-site analysis for a wide variety of applications, including environmental analysis, custom control, drug falsification and food safety.
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OP2 - A1

Two orthogonal techniques for natural products analysis - SFC and CE, a practical comparison

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Apple leaves are a rich, but currently unexplored source for several dihydrochalcones (DHCs), phenolic compounds with interesting pharmacological potential, including immunomodulatory (trilobatin), antioxidative (phloretin, phloridzin, sieboldin), and antihyperglycemic (phloretin, phloridzin) potential. Their analysis in plant material by (U)HPLC is well established. However, less common approaches like Capillary Electrophoresis (CE) or Supercritical Fluid Chromatography (SFC) have never been considered before, even they provide high separation efficiency combined with orthogonal selectivity.

Besides the novel application, the here presented study is one of the few direct comparisons of CE and SFC till date. Both methods are considered niche techniques and follow different principles, i.e. electrophoretic migration in buffer filled narrow bore glass capillaries or chromatographic separation using a supercritical fluid as mobile phase. Despite of these fundamental differences, both were capable to excellently resolve the five standard compounds within short separation times (CE: 9 min, SFC: 5 min), fulfilling the demands for an environmentally friendly "green" technology. The optimum CE buffer showed to be a 25 mM borax solution with a pH of 8.25, containing 2.5% isopropanol as an additive. For separation by SFC a Torus™ Diol column (1.7 µm) combined with a mobile phase comprising CO_2 and 2.5 mM oxalic acid in methanol was best suited.

After a short introduction to the theoretical background of respective techniques, method development and validation will be described. Both approaches fulfilled ICH validation criteria, yet by trend the method's precision and accuracy were slightly better when using SFC. In terms of LOD (≤ 0.98 µg/mL) and LOQ (≤ 2.94 µg/mL) CE and SFC were comparable. The practical applicability of both approaches was finally confirmed by analyzing the DHC content in diverse Malus species. Significant qualitative and quantitative differences were noticed, with overall DHC concentrations ranging from 5.47 to 17.24%; yet, both techniques yielded in nearly identical results.

OP2-A2

Off-line CE search for PTP1B - inhibiting Traditional Chinese Medicines

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The enzyme protein tyrosine phosphatase 1B (PTP1B) is a target for the therapy of diabetes mellitus type 2, which remains to be explored more deeply. It regulates dephosphorylation and participates in the insulin signaling pathway.

This study made use of capillary electrophoresis (CE) with Ultraviolet detection for the establishment of a fast and efficient method for screening and evaluation of hypoglycemic ingredients of Traditional Chinese Medicine acting on PTP1B.

In a first step, a suitable CE background electrolyte was selected for the separation of p-nitrophenyl phosphate from the enzymatic reaction product p-nitrophenol. Response Surface Methodology was used to this end, resulting in a CE analysis time below 2 min. After subsequent method validation, the conditions for enzymatic incubation were optimized and a kinetic analysis was performed. The IC50 values of two known PTP1B inhibitors, IV and XVIII, were consistent with values reported in the literature. In a next step, extracts were prepared starting from six Traditional Chinese Medicines. Using the developed test procedure, it could be shown that the extracts of Astragalus membranaceus (Fisch) Bunge and Morus alba L. inhibited the activity of PTP1B more strongly than the positive controls.

This method could be a powerful tool for screening PTP1B inhibitors from complex systems. It can also provide an effective basis for lead compound development in drug discovery.

OP2 - A3

Phytochemical, antioxidant, and androgenic effects of multi-solvent extracts of Sclerocarya birrea on mouse testicular TM3 Leydig cells

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Medicinal plants have been widely used to treat and prevent various human ailments especially in developing countries with limited resources and health care facilities. The study was aimed to evaluate the antioxidant content, identification of bioactive compounds, and androgenic activity of Sclerocarya birrea leaf extracts prepared from different solvents (water, methanol, ethanol, and acetone) on TM3 Leydig cells. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS), and ferric-reducing antioxidant power (FRAP) assay, while the UPLC-Q-TOF-MS and Fourier transform infrared (FTIR) spectroscopy were also performed to identify the bioactive metabolites and functional groups of chemical compounds present in the plant extracts. Quantitative phytochemical analysis was performed to measure the contents of the polyphenolic compounds. TM3 cells were cultured, subsequently the cell viability tests, and membrane mitochondria potential assays were performed. Testosterone levels were measured using the ELISA kit. The percentage extraction yield of the water extracts was found to be the highest (14.25%) compared to percentage extraction yield of acetone extract which was the lowest (2.70%). The proanthocyanidin content (PAC) was found to be significantly high in the methanolic extract

(440.41±2.25 mg/CE/g), compared to the water extract (97.48±1.13mg/CE/g). The total flavonoid content (TFC) was found to be significantly high in the methanolic extract (394.39±9.04 µmol/TE/g) compared to the acetone extract content (49.53±1.33 µmol/TE/g). FRAP values for methanolic extract were found to be 1.30 µmol TE/g and the acetone extract was found to be 0.28±0.01 µmol/TE/g. Treatment of TM3 with extract with 5-100 µg/mL exhibited dose-response change on cell viability. IC50 value for acetone extract was found to be 42.42 µg/mL compared to the 21.55 µg/mL for ethanol extract. A total of thirteen (13) bioactive compounds were identified from the extracts. The FTIR analysis showed O-H stretching (hydroxyl groups) for alcoholic and phenolic compounds. There was no increase in testosterone production by TM3 cells in the presence of human chorionic gonadotropin (hCG), when cells were treated with the extracts. The results of the study revealed that S. birrea leaf extracts can be considered a potent antioxidant source for therapeutic purposes.

OP2 - B1

An analytical view on outpatient parenteral antimicrobial therapy

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Outpatient parenteral antimicrobial therapy (OPAT) refers to the administration of parenteral antimicrobial treatment in a minimum of two doses on separate days, without the necessity for hospitalization. OPAT is primarily indicated for patients who need prolonged intravenous (IV) antibiotic therapy. OPAT offers many potential benefits including reduced risk of hospital-acquired infections, cost savings due to reduced bed utilization, and enhanced patient satisfaction. However, it also raises a concern. Home care nurses need to visit the patient's home several times per day to prepare and administer each dose of antibiotics. This can lead to some inconvenience for both healthcare professionals and patients. The use of portable elastomeric infusion pumps (PEIPs), which are non-electric pumps consisting of an elastomeric balloon to release the drug solution at a constant flow, could be an ideal solution when antibiotics are prepared and immediately administered continuously over 24 h. By this way, nurse visits can be reduced to once per day. However, the antibiotics should be chemically stable during the administration duration. This concern is considerable in case of beta-lactam antibiotics such as ceftazidime, piperacillin, and flucloxacillin due to the instability of the beta-lactam ring and the temperature of the PEIP which can reach 33 °C when the patient wears the PEIP against his/her body or puts it under the blankets at night. Structure of the antibiotic, temperature and duration of the storage, combined with the drug concentration, diluents and container composition are the main factors affecting the drug stability.

In some countries like the UK, antibiotic solutions are prepared in the hospital pharmacy and stored in the refrigerator for some days until use. However, in other countries like Belgium, it is common that the nurse prepares the solution just before administration. While the first approach has been studied already, the second has not. So, the general aim of this study was to check whether intravenous solutions of antibiotics can be practically employed for OPAT during 24 h when prepared immediately before use. If the solutions are stable, this would require only a one time per day visit of the home care nurse. This can be ascertained if the concentration of antibiotics in the IV solution after 24 h of administration remains at least 90% of the starting concentration and no precipitate is formed during administration. Furthermore, the concentration of antibiotics in patients' plasma should be higher than the minimum inhibitory concentration (MIC).

To investigate this, stability studies of ceftazidime, piperacillin and flucloxacillin, were conducted. Solutions were prepared in saline or a mixture of water and saline and they were stored in PEIPs at 33

°C for 24 h. Concentration of the antibiotics, as well as pH, color and clarity of the solution at different time points over 24 h, were evaluated. In addition, analytical methods were developed and validated to determine the concentration of the antibiotics in plasma samples of patients receiving antimicrobial therapy over 24 h continuous administration.

OP2 - B2

Implementation, Use and Outlook of Vendor-Agnostic Data Management Tools in Chemical and Analytical Development

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The development of new chemical processes for Active Pharmaceutical Ingredients (API) is an intricate, cross-disciplinary endeavor where end-to-end information flow is critical. Throughout the development process, a plethora of analytical data is being generated in multiple laboratories and groups, to support core activities such as the carryover of impurities, identification of impurities, and Quality by Design process development experiments. These tasks yield a wide array of data types - both qualitative, like HR-MS/MS, 2D-NMR, and XRPD, and quantitative, including HPLC-UV and Assay determinations.

Ineffective management of these data can be a key source of inefficiency and lost opportunities, so novel software solutions are being developed to manage this data at an enterprise scale. A vendor-agnostic database software for analytical raw data built upon Spectrus DataBase, plays a pivotal role in this field. However, transitioning well-established business processes into the digital realm poses considerable challenges, including the upfront costs of digital solutions, a deficit in digital expertise, rigid legacy systems, and resistance from employees. Nevertheless, the critical role of structured high quality data in fostering innovation, operational efficiency, and improved time to market is undeniable. This talk will provide insights into the deployment of this impurity knowledge management software within chemical and analytical development, with an emphasis on our key processes for structure characterization and impurity carryover studies, and also highlight use in reference standard characterization, and degradation and stability studies. We will discuss the obstacles encountered, the proposed solutions and benefits for our existing processes, and elaborate on future strategies to expand the use of Luminata within technical research and development.

OP2 - B3

Simultaneous detection of unspecific trace N-nitrosamine impurities by LC-MS/MS in a pharmaceutical formulation

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N-nitrosamines, a class of organic compounds, can occur naturally or be formed in the environment and food. Many of these compounds are recognized as DNA-reactive mutagens with carcinogenic potential. In past years, significant levels of N-nitrosamine impurities have been found in pharmaceutical products for human use, prompting serious health concerns.

In response to these concerns, we developed a liquid chromatography method in reverse phase mode coupled with tandem mass spectrometry detection (LC-MS/MS) allowing versatile application, as well as high sensitivity and specificity. Our work aimed to validate generic LC-MS/MS methods for the simultaneous determination of various trace impurities of N-nitrosamines. We focused on those unrelated to the drug substance, specifically for the Quality Control of a commercial pharmaceutical product. The generic methods were designed to offer flexibility in application, requiring no modifications in sample preparation or equipment configuration and thus allowing a single-run (sequential) analysis of 17 N-nitrosamines.

Method validation was conducted according to the ICH Q2(R2) guidelines. Upon successful validation, these methods were subsequently used to determine unspecific N-nitrosamine content in the selected pharmaceutical formulation, as mandated by the European Medicines Agency for confirmatory testing.

The confirmatory testing results revealed the absence of certain N-nitrosamines, permitting the omission of their specification limits in routine analysis. However, one N-nitrosamine was detected, and its content was consistently determined below and around (for aged stability samples) the specification limit, necessitating routine quantitative impurity testing.

OP3-A1

Critical role of proline hydroxylation in collagen functionality: analytical characterization of 3- and 4-hydroxyproline isomers at amino acid and peptide levels

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Collagen, the most abundant protein in the human body, is crucial for the structural integrity of tissues and organs, making it of great interest in many biopharmaceutical fields. Its stability and mechanical properties depend on hydroxyproline, specifically 4-hydroxyproline, which is located at the Y position of the typical repeated amino acid pattern (Gly-X-Y)n and is essential for the stability and proper folding of the collagen triple helix. This unique amino acid is enzymatically introduced during collagen biosynthesis by prolyl hydroxylases. Defects in this process can result in non-functional proteins with severe biological consequences. Although less is known about 3-hydroxyproline, it is believed to also play a role in collagen stability.

In the context of recombinant collagen production, which remains an ongoing challenge, a comprehensive assessment of the hydroxyproline pattern becomes critical, as successful expression of functional collagen requires the engineering of the host strain to contain both the collagen and prolyl hydroxylase genes. Monitoring the occurrence of hydroxyproline along the collagen sequence can therefore verify the quality and efficacy of prolyl hydroxylase and reflect the overall success and reliability of recombinant collagen production.

In this work we present the development of two analytical methods for the extensive characterization of hydroxyproline at both the amino acid and peptide levels.

The first one exploits RPLC-UV-MS for the separation and quantification of hydroxyproline as amino acid. Protein gas-phase hydrolysis was carried out to release individual amino acids, followed by their derivatization with L-FDVA to enhance UV detection. Hydroxyproline has two stereocenters, resulting in four possible stereoisomers considering both 3- and 4-hydroxyproline. Harsh sample treatment conditions, such as acid hydrolysis, can also produce small amounts of D-amino acids. Consequently, the analysis of hydroxyproline should consider a total of eight stereoisomeric forms, each with the potential to affect the biophysical properties and overall functionality of collagen. Chromatographic development involved optimizing column selection, mobile phase composition, and elution modes. The integration of MS increased detection sensitivity and provided thorough characterization. This validated method was applied to both native and recombinant collagen to better understand their hydroxyproline composition.

The second study presents a systematic approach aimed at developing a robust HILIC-UV-MS method for collagen peptide mapping analysis. The presence of hydroxylated proline residues in collagen-derived peptides introduces polar functionalities that can interact favorably with the hydrophilic stationary phases used in HILIC. These hydrophilic interactions may enhance the retention and separation of collagen peptides, making HILIC a suitable platform for their analysis. A set of sixteen model peptides derived from in silico predicted tryptic digestion were selected to investigate the selectivity towards different positional isomers of hydroxyproline (3-hydroxyproline and 4-hydroxyproline), or multiple potential hydroxylation sites. The methodology explores both conventional and state-of-the-art HILIC stationary phases with different chromatographic conditions (mobile phase, injection mode). Finally, the optimized method was applied to real collagen digests.

OP3-A2

Expedited method development and characterization of therapeutic peptides and oligonucleotides

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Small molecules and antibodies have dominated the pharmaceutical landscape for decades. However, limitations associated with drugging difficult targets and progress in biology and chemistry have led to the blossoming of alternate modalities such as peptides and oligonucleotides. The increase of molecular and chemistry complexity pose unique analytical challenges. In addition, fast turnaround times are needed to quickly parse and advance drug candidates. A comprehensive characterization of impurities with minimal sample is desirable to enable data-driven decisions early in drug development. Despite their difference in building blocks, i.e. amino acid vs nucleotide, similar strategies can be utilized to characterize therapeutic peptides and oligonucleotides. In particular the purity can be determined under non-denaturing or denaturing conditions to assess different types of impurities. For example, aggregates or unhybridized strands can be determined by size exclusion chromatography (SEC) while impurities resulting from deletion or addition can be separated using RPLC columns. A multi-dimensional LC-MS approach was developed to streamline the determination of multiple quality attributes with limited amount of sample needed. Finally, the current challenges and progress in the fingerprinting of diastereomer and conformer populations are discussed.

OP3-A3

Analytical peptidomics: future challenges

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Analytical peptidomics, i.e. the comprehensive study of peptides within biological systems, faces significant future challenges as it progresses towards deeper, more precise, and functionally relevant insights. A major challenge is the inherent complexity and dynamic range of peptide populations, necessitating highly sensitive and selective analytical techniques. Various parts of the analytical process require novel approaches:

1. Sample collection: ensuring the proper collection and storage of biofluids (plasma/serum, feces, and saliva) is crucial to prevent peptide degradation/metabolization (leading to false negatives) and protein degradation (leading to false positives) before laboratory handling [1].
2. Sample preparation: effective removal of interfering compounds, releasing peptides from adsorbing matrix components, and preconcentrating peptides are essential steps in the sample preparation step.
3. Chromatography: addressing adsorption losses, which affect detection limits, and carry-over issues, in addition to optimizing separation power and orthogonality of complementary systems, is critical [2].
4. MS detection: enhancing both targeted and untargeted MS analyses to improve sensitivity and selectivity of the methods remains a key focus [3].
5. Data treatment: employing appropriate data evaluation methods is necessary to minimize the loss of information, particularly for low-abundance peptides.

Advancing these areas is essential for overcoming the inherent challenges in analytical peptidomics, facilitating more accurate and comprehensive peptide analyses.

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OP3-B1

Exploring seminal liquid as a matrix for early detection of testicular cancer metabolism

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Over the past five decades, there has been an increase in the incidence of testicular cancer, coinciding with a global decline in semen quality. Both trends are associated with industrialized societies and the related changes in lifestyle and environmental exposures. Seminal fluid, in addition to sperm cells, contains different proportions of secretions from the testes, seminal vesicles, prostate, bulbourethral, and periurethral glands. Such a biochemically complex matrix can provide metabolic insights into pathologies affecting these organs, while being a non-invasive and readily obtainable clinical sample. Testicular germ cell tumors (TGCTs) remain the most prevalent malignancy in young adult males, underscoring the clinical importance of developing simple and accurate diagnostic methods to facilitate appropriate patient management. In this study, we employed capillary electrophoresis coupled with mass spectrometry (CE-MS) to characterize the polar metabolomic profile of seminal plasma samples from healthy donors and TGCT patients. By using a liquid chromatography-mass spectrometry (LC-MS) targeted method to monitor a panel of over 80 oxylipins, we also examined the diagnostic role of this family of lipidic mediators.

Seminal fluid samples were obtained from 36 patients with a TGCT and 11 healthy controls. Fifty microliter aliquots of cell-free plasma were subjected to protein precipitation and spiked with effective electrophoretic mobility (μeff) markers prior to filtration on 3 kDa cut-off centrifugal devices. To gain a broad perspective of the seminal fluid metabolome, an untargeted CE-MS approach comprising positive and negative separation and ionization was chosen. Time-of-flight (ToF) data were pre-processed to correct analytical drift, normalize sample content, and filter out unreliable signals before univariate and multivariate analysis. Compound identification was performed by matching accurate mass, in-source fragmentation pattern, and effective electrophoretic mobility value to those obtained from reference standards using the same experimental setup. Oxylipins were extracted by using a protein precipitation step in the presence of BHT. Extracts were analyzed in reverse-phase LC coupled to a triple quadrupole mass spectrometer to perform a targeted analysis of 79 molecules including their calibration curves.

Classical supervised multivariate models (OPLS-DA) failed to reliably distinguish the metabolomic profiles of the control and cancer groups in CE-MS. Therefore, Monte Carlo Uninformative Variable Elimination (MCUVE) was used to remove least meaningful metabolites. The resulting models pointed towards differences between the metabolic profiles of the two groups, characterized by the levels of aspartic acid, glutamine, and other energy metabolism-related molecules. On the side of oxylipins, some of these compounds also indicated changes related to the disease. This suggests that seminal plasma composition could become a useful sample in the diagnosis of testicular cancer by revealing the metabolic rewiring that takes place in malignant germ cells.

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OP3-B2

Chemical, Metabolic and Plasma stability studies of different glucotrifluoroborates as potential agents for Boron Neutron Capture Therapy (BNCT)

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Introduction

Boron neutron capture therapy (BNCT) is a binary radiotherapy based on the selective accumulation of boron carriers in tumour tissues which can give excellent control over malignant tumours not responding to conventional therapies. The BNCT efficacy is based on the fission reactions that occur when the boron delivery agent captures a thermal neutron coming from a neutron flux generated by a proper external source. 4-Boronophenylalanine is commonly used as boron delivery agent in clinical settings; however, its performance is often not optimal. To guarantee great development, new boron delivery agents should act also as theranostics.

Boronated carbohydrate derivatives have received attention in BNCT considering the demonstrated and preferential uptake of glucose and derivatives by tumour cells, stimulating us to design new agents based on monosaccharide scaffolds. Recently, we reported [1] the preparation of a library of monosaccharides that could act as theranostics for BNCT exploiting a trifluoroborate moiety as a source of boron and fluorine, tracer for Positron Emission Tomography.

Aim

Exploring trifluoroborate derivatives as theranostic and BNCT agents has been recently reported as a goal. Understanding their in vivo stability is essential for assessing their potential clinical applications. The aim of this contribute is to provide insights into gluco-trifluoroborate derivatives stability, aiding the research and development of novel BNCT agents and facilitating predictions of their behavior in biological systems, thus promoting their clinical utility.

Experimental

Chemical stability studies were conducted under five experimental conditions: Tris-HCl buffer (50 mM, pH=7.4), HCl (0.05 M), NaOH (0.5 M), and Saline Solution (0.9% NaCl) for 72 hours and H_2O_2 0,3% for 1 h at 15°C. The metabolic and plasma stabilities were performed using mouse liver microsomes (MLM) and murine plasma respectively (1-hour incubation, 37°C).

Results and Discussion

The trifluoroborate moiety undergoes hydrolysis equilibrium with its boronate derivative.[2] This phenomenon has been still confirmed in our study for all the boronate carbohydrates studied, with the formation of the corresponding boronic acid. Furthermore, the degradation kinetics studies pointed out a steady state between the trifluoroborate species and boronate degradants which is influenced by linker chemistry and experimental conditions. Moreover, a trace amount of another degradant relative to the deboronate ammonium carbohydrate was detected. The chemical structures of the degradants were identified by mass and/or NMR analysis. The absence of oxidative degradants steming from Graham's mechanism[3], highlighted a particular oxidative cleavage not yet described with the formation of the corresponding deboronated product. The evaluation of the metabolic stability was made by comparing study versus control samples. The instrumental response decrease obtained in all study samples was related only to the hydrolysis, leading to the formation of the boronic acids, since no metabolites were found. Analogous results were also obtained from the plasma stability studies.

Conclusion

All the compounds analyzed resulted hydrolitically unstable whereas they were stable metabolically and in plasma. Although, the acid-catalyzed degradation only directly affects the theranostic effect's lifetime of the trifluoroborate moiety on the contrary this does not impact their application as BNCT agents.

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OP3-B2

LC-MRM3, a high potential technique for pandemic preparedness: a case study of SARS-CoV-2

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Since its first appearance, SARS-CoV-2 quickly spread around the world and the lack of adequate PCR testing capacities, especially during the early pandemic, led the scientific community to explore new approaches such as mass spectrometry (MS).

We developed a proteomics workflow to target several tryptic peptides of the nucleocapsid protein (NCAP). A highly selective multiple reaction monitoring MRM3 strategy provided a sensitivity increase in comparison to conventional MRM acquisition. Our MRM3 approach was first tested on an Amsterdam public health cohort (alpha-variant, 760 participants) detecting viral NCAP peptides from nasopharyngeal swabs samples presenting a cycle threshold (Ct) value down to 35 with sensitivity and specificity of 94.2% and 100.0%, without immuno-purification. A second iteration of the MS-diagnostic test, able to analyze more than 400 samples per day, was clinically validated on a Leiden-Rijswijk public health cohort (delta-variant, 2536 participants) achieving 99.9% specificity and 93.1% sensitivity for patients with Ct-values up to 35. In this study, we also developed and brought the first proof of the concept of viral variant monitoring in a complex matrix using targeted mass spectrometry.

OP4-A1

MS-based metabolomics and lipidomics for differential diagnosis of Parkinson's disease and atypical Parkinsonisms

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Parkinson's disease (PD), classified as the second most common global neurodegenerative disease, presents a multifaceted clinical profile [1]. It is mainly distinguished by motor symptoms, however, Parkinson's disease extends to a wide range of non-motor manifestations. These include cognitive decline, mood disorders such as depression and anxiety, sleep disorders, and autonomic dysfunction, making the clinical landscape of Parkinson's disease intricate and difficult to navigate. Diagnosis of Parkinson's disease remains a clinical undertaking, relying on the experience of healthcare professionals to recognize specific motor symptoms. This lack of specific diagnostic tests poses significant obstacles, especially during the early stages of the disease, where subtle manifestations can

lead to misdiagnosis, delaying crucial therapeutic interventions. Indeed, distinguishing Parkinson's disease from related neurodegenerative disorders named Atypical Parkinsonism (AP), such as multiple system atrophy (MSA) and progressive supranuclear palsy (PSP), poses a number of complexities.

This investigation aimed, therefore, to identify molecular biomarkers for the differential diagnosis of PD, MSA and PSP by untargeted metabolomics and lipidomics using micro liquid chromatography-high resolution mass spectrometry (microLC-HRMS). 93 patients (53 PD, 18 MSA-P, 22 PSP-P) were enrolled. The study protocol consisted in a neurological exam to evaluate patients' performances by specific rating scales. Venous blood was collected in fasting patients before taking the therapy. Plasma samples were prepared via protein precipitation [2] and treated differently for metabolomics and lipidomics [3]. The extracts were analysed by microLC-HRMS in both Data Independent (DIA) and Dependent (DDA) Acquisition mode, using internal standards or not. Row data were processed by peak picking and alignment, data cleaning (missing data imputation, filtration, etc.), normalization.

Preliminary multivariate statistical analysis (supervised and unsupervised) on untargeted metabolomics data showed disease-specific metabolic alterations. Metabolites involved in tryptophan, purines, and lipids metabolism resulted to be discriminant for PD and AP. To overcome the possible concealment of the analytical signals, which could cause features to go undetected in the completely untargeted metabolomic approach, lipidomics was explored in semi-untargeted and pseudo-targeted modality. By employing the latter analytical pipeline, we found a dozen of lipids that exhibited significant differences among these groups and achieved an acceptable separation through partial least square- differential analysis (PLS-DA).

This metabolomics study provided several important insights both on the complementarity of the results obtained with the different investigation approaches and on clinically relevant information. Hopefully, the creation of a predictive machine learning model, based on the combination of clinical data and metabolome will be achieved, this would be fundamental for the early differential diagnosis between PD and AP and therefore for specific therapeutic interventions from the onset of the disease.

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OP4-A2

Characterization of IgG N-glycans in COVID-19, sepsis and HIV infection.

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N-glycosylation is one of the most prominent post-translational modifications of proteins that plays a crucial role in protein stability and activity. Antibodies are glycoproteins that play a central role in humoral immunity against pathogens, especially immunoglobulin G (IgG), which is a crucial effector of the humoral immune response.

We have developed and validated a reliable, sensitive, and robust analytical method for the characterization of IgG N-glycans. If the method is applicable to biotherapeutics, the main goal was to develop a sensitive method that enables the analysis of IgG N-glycans in blood samples from patients.

To achieve this goal, procainamide was used for the glycan labeling. The analytical method is based on two steps: first, an enrichment step using online solid-phase extraction (online SPE) with HILIC chemistry, followed by a separation step using the same chemistry (HILIC) coupled to fluorescence (FLD) and mass spectrometry (MS) detectors. Several parameters were optimized using an experimental design, and the method was validated using NIST mAb standard and human IgG pool samples.

Thanks to this method, we were able to investigate N-glycan patterns in two clinical applications. The first one is patients hospitalized in Intensive Care Unit (ICU) due to sepsis or severe COVID-19. The second application involved pregnant women living with Human Immunodeficiency Virus (HIV+) who initiated their antiretroviral therapy (ART) after conception (HIV+After) or before conception (HIV+Before), as well as uninfected pregnant women (HIV-) and their infants.

For the first application in ICU, hospitalized patients with sepsis were compared to COVID-19 patients who were categorized into two groups based on their clinical outcomes (survival or death). Our data indicated that a specific bisecting N-glycan, FA2B, is a potential biomarker of mortality risk in COVID-19 patients from the first day of admission to the ICU. Our results are consistent with other studies suggesting that an increase in total bisecting is associated with enhanced affinity of IgG to the FcγRIII receptor. This interaction induces antibody-dependent cell-mediated cytotoxicity (ADCC) and IgG pro-inflammatory effector functions in cases of severe illness. Although our study is the first to define a specific N-glycan as an indicator of poor prognosis.

For the second application, our results showed an increase in agalactosylation (absence of galactose residue) in HIV+After women compared to HIV- and HIV+Before women. This finding indicated that initiating ART before conception may correct alterations in IgG glycosylation profile induced by HIV infection. The diverse N-glycan profiles of maternal IgG could impact their transfer across the placenta and their effector functions in the newborn infant.

In conclusion, both investigations demonstrated that the developed method is sensitive and reliable for investigating clinical samples. Furthermore, future studies on larger cohorts of patients should be performed to investigate N-glycans as potential biomarkers in these specific infections.

OP4-A3

Monitoring by LC-MS/MS of MPO-dependent oxidation of LDL and HDL in serum

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Since myeloperoxidase (MPO) was discovered in atherosclerotic plaque, the role of this enzyme was intensively studied in cardiovascular diseases. Among those researches, the MPO-dependent oxidations of apolipoprotein B100 and A1 in the development of atherosclerosis is promising. This oxidation occurs both in the subendothelial space and in the blood circulation. By oxidizing apoA-1, MPO induces dysfunctional HDL and cholesterol efflux deficiency while modifications on apoB-100 promote atherosclerosis by foam cell formation and endothelial inflammation. Several modifications of apoA-1 and apoB-100 are well described in the literature and seem relevant in patients with

cardiovascular diseases (CVDs) [1,2]. However, no analytical method monitors at once these apolipoprotein modifications in a clinical perspective.

We developed an analytical method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to monitor relevant apolipoprotein peptide oxidations mediated by MPO. We first selected three peptides for each apo; one control peptide and two peptides oxidized by MPO in vivo. We thoroughly validated two methods on serum including: (i) serum dilution or apolipoproteins isolation from serum by using a lipid removal agent; (ii) trypsin digestion; (iii) LC-MS/MS parameters to measure peptides and their respective oxidized form. With both methods, we demonstrated that samples spiked with increasing concentration of synthetic peptides followed a non-linear regression (R2 = 0.99). Moreover, thanks to patients for which the apoA-1 and B-100 were measured by immunoassay, we tested the linearity of the method by sequential dilutions with satisfactory results (R2 between 0.96 and 0.99 for native peptides and 0.81 to 0.99 for corresponding oxidized peptides). Finally, the calculated concentrations of ApoA-1 and B-100 with our measurements of their peptides by LC-MS/MS were correlated with the measured concentrations with the immunoassay reference method.

These methods coupled to the monitoring of the ratio 3-chlorotyrosine/tyrosine, a measure of MPO activity, was then applied to several groups of patients from the CHU-Charleroi Marie-Curie Hospital (Belgium): (i) 23 patients admitted in the cardiovascular unit (STEMI, NON-STEMI & severe CAD = CVD) (ii) 30 patients admitted in intensive care unit (ICU) for COVID infection (=COVID), (iii) 11 ICU patients with sepsis as positive control (=SEPSIS); (iv) finally, 24 healthy volunteers were selected (=HV).
Our results confirmed that the oxidation of peptide containing W72 on apoA-1 was a specific marker of patients with CVDs as demonstrated by Huang et al. (2014) [1]. The results on apoB-100 demonstrate that the MPO-dependent apoB-100 oxidation is less specific. Septic patients were characterized by an oxidative burst with high MPO activity as illustrated by the higher 3-chlorotyrosine/tyrosine ratio but few apolipoprotein oxidations. More interestingly and as COVID patients are considered as septic patients, our results show that patients have an intermediary oxidative stress status between sepsis and CVD patients. This also demonstrates that beyond the oxidative stress, specific APO-peptides oxidized by MPO could be interesting and more specific markers for CVD residual risk monitoring.
These results reinforce again the oxidative role of MPO in cardiovascular diseases and prompt us to use this LC-MS/MS method in larger cohort of patients with cardiovascular risks.

OP4-B1

Charge to move forward in Volume-restricted Metabolomics

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In metabolomics, advanced analytical workflows are needed to study biochemical changes in small-volume biological samples, notably for samples originating from 3D microfluidic cell culture models, individual zebrafish larvae and neonatal clinical blood sampling. Recent work from our lab revealed that capillary zone electrophoresis-mass spectrometry (CE-MS), regardless of utilizing a sheath-liquid or sheathless interface, is a strong analytical tool for probing polar and charged metabolites in biological samples with a good reproducibility. Moreover, in a simulated metabolomics study, CE-MS was able to find the right set of differential metabolites between controls and cases. These studies clearly indicate the value of CE-MS for biomarker discovery and comparative metabolomics studies.
Given our ambition to address volume-restricted biomedical questions with metabolomics, we report in this presentation on the development of new CE-MS-based analytical workflows for the highly

efficient and sensitive analysis of polar (endogenous) metabolites in neonatal plasma and individual zebrafish larvae. As only nanoliters of samples are consumed by a single CE-MS analysis, multiple injections/assays can be performed on the same valuable volume-limited sample allowing for technical replicates and/or probing different classes of ionogenic metabolites. We show how these new CE-MS-based workflows can be employed in a reliable way for the quantitative analysis of creatinine, and many more endogenous compounds, in neonatal plasma samples using a starting amount of less than 5 microliter, whereas gold standard clinical chemistry approaches require often a minimum of 100 microliter for only creatinine determination. Hence, the proposed CE-MS-based workflow will contribute to minimizing both the amount and frequency of blood collecting required for diagnostic purposes in a neonatal setting.

We also demonstrate the utility of a new CE-MS workflow for the profiling of metabolites in extracts from individual zebrafish larvae and pools of small numbers of larvae. More than 70 endogenous metabolites could be observed in a pool of 12 zebrafish larvae, whereas 29 endogenous metabolites were detected in an extract from only 1 zebrafish larva. So far, zebrafish has proven to be a very effective model for stress research, in particular for studies on the effects of cortisol, with a clear role of the glucocorticoid receptor during stress. However, the role of the mineralocorticoid receptor (MR) on mediating the effects of cortisol is less known. By using wild-type (WT) and ubiquitous MR-knockout (MRKO) zebrafish larvae exposed to exogenous cortisol treatment, our CE-MS-based metabolomics workflow revealed the implication of metabolic pathways solely activated via MR. Taken together, CE-MS has the potential to identify novel pathways and mechanisms of action in zebrafish larvae and is a viable analytical approach for volume-restricted metabolomics.

OP4-B2

Ligand-protein interaction's monitoring by CE-MS in the context of drug development: BGE and capillary coating optimizations

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Previously, our team developed affinity capillary electrophoresis (ACE) methods using a DAD [1–3]. ACE proved to be a reliable approach for validating fragment hits and determining their dissociation constants [1,2]. The next step in this project was to develop an ACE method compatible with MS. Indeed, MS detection can extend the applicability of our approach to compounds with little or no UV-vis absorption. MS also enables the confirmation of the ligand identity. This additional output avoids misinterpretation of the results (false structural assignment of binders), which is a valuable asset during a drug discovery campaign. However, detections by MS require a volatile buffer and a permanent coating. Our previous methods used a Tris-Hepes buffer and a dynamic PEO coating, which were not compatible with MS detectors. Overall, to study ligand-protein interactions under near-physiological conditions using CE-MS, we need a volatile BGE buffering at pH 7.4 and a permanent, antifouling coating stable at physiological pH. The antifouling coating is mandatory because interactions of the protein and/or ligand with the capillary wall decrease their free concentration in solution, leading to a bias when assessing affinity. In this work, we studied protein adsorption on silica using soft and hard proteins of different molecular weights, as well as small molecules that mimic ligands with various acid/base and hydrophilic characteristics. We evaluated the properties of n-methylmorpholine (NMM) as a buffer for CE-MS applications requiring physiological conditions. We observed that NMM in itself can reduce protein adsorption to silica. We also synthesized and investigated poly(n-isopropylacrylamide)-grafted polydopamine (PDA) coatings for CE-MS interaction

studies. By monitoring the peak shapes and migration times' stability, we studied several critical parts of the protocol, such as the PDA deposition (grafting vs one-pot deposition), the end capping, and the storage conditions. Finally, the optimized conditions were validated by determining a known dissociation constant.

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OP4-B3

Simultaneous quantification of therapeutic monoclonal antibodies and neutralizing anti-drug antibodies in patient serums using capillary electrophoresis coupled to tandem mass spectrometry

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Monoclonal antibodies (mAbs) are demonstrating major successes in various therapeutic areas like oncology or immune disease treatment. Insights regarding their evolution after administration to the patient remain limited. Thus, recent clinical researches are indicating important variabilities among patients concerning clearance for the same mAbs. Also, a significant fraction of patients is demonstrating an immune response to mAbs treatment in the form of the expression of neutralizing anti-drug antibodies (NADA). Neutralization of therapeutic mAbs by NADAs can compromise their efficacy and hasten their clearance, which in most adverse cases could lead to complete inactivation. Therefore, clinical follow-up of patients treated using mAbs appears crucial, however it is currently performed solely through quantification using ELISA immunoassay.

Here, we first investigated the interaction between IFX and the corresponding NADA in vitro using mass photometry (MP) which was recently introduced to measure the molecular mass of biomolecules. MP data allowed to determine the stoichiometry between the two IgGs, in addition to provide information regarding IFX/NADA neutralization kinetics and the conditions of equilibrium. Consequently, a novel analytical strategy based on capillary electrophoresis hyphenated to mass spectrometry (CE-MS/MS) was developed to perform the absolute quantification of infliximab (IFX), used for the treatment of Crohn's disease, and the corresponding NADA concomitantly. The analytical workflow was derived from a competitive screening assay that allowed the absolute quantification of the residual amount of active IFX by CE-MS/MS analysis. Two different mAbs internal standards incorporating stable heavy-isotopes were added systematically to samples. Afterward, affinity purification was performed in order

to isolate mAbs targeting TNF-α, followed by proteolytic digestion. Peptide mixtures obtained were finally analyzed using CE-MS/MS.

CE-MS/MS results demonstrated the possibility to perform an accurate and specific quantification of active IFX and free NADA contained in serum samples for concentrations ranging from 1 to 30 µg/mL (7 - 208 nM), corresponding to levels typically reported for treated patients. The CE-MS/MS analytical workflow was applied to the quantification of 13 serum samples originating from patients treated in gastro-enterology for Crohn's disease. Results demonstrated a robust and consistent quantifications for both IFX and NADA, compared to ELISA experiments performed using commercial kits. As a consequence, CE-MS/MS data allowed to discover 2 patients expressing a significant immune response to the treatment.

In complement, CE-MS/MS analysis was performed for model serum samples spiked with a fixed concentration of IFX and increasing concentrations of purified NADA. CE-MS/MS quantification of active IFX at equilibrium for the different model serum samples enabled to determine experimentally a dissociation constant between IFX and NADA of 14.8 ± 1.2 nM, using an original data treatment and mathematical calculations procedure adapted to MS/MS experiments.

CE-MS/MS enabled to target neutralizing antibodies in a specific manner compared to commercial ELISA assay that may detect anti-IFX antibodies regardless of their neutralizing capacities. As a consequence, CE-MS/MS simultaneous quantification could be further used for the analysis of patient serums in order to identify unambiguously the occurrence of immune response in the form of NADA expression and estimate the level of immune response against the mAbs.

OP5-A1

Molecular networking approach for clinical analysis: Benefits of creating in-silico databases of all drugs marketed in France as well as centrally authorized drugs approved by the European Medicines Agency for their identification

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Introduction : In clinical toxicology, analysts face difficult problems, resulting in important clinical issues. Untargeted screening remains a challenge, due to the large number of molecules to be detected. LC-HRMS, the reference method for screening, generates a large amount of data, without tools to visualize and organize the MS data. Another drawback is the lack of a comprehensive MS/MS database. Our objectives were to i) apply molecular networking (MN) for screening interpretation, ii) build an in-silico MS library of numerous marketed drugs in France and the European Union, and iii) apply this database (DB) for drug identification in patient plasma.

Methods : Plasma was crushed with acetonitrile. After centrifugation, supernatant was injected onto an LC-Orbitrap QExactive LC-HRMS system. For MS, positive MS (m/z 50-1500) and data-dependent MS/MS scans were recorded using CID activation mode. For MN, a data preprocessing using MZmine has been performed prior to MN generation. The MN data were then analyzed and visualized using

Metgem or Cytoscape application. Finally, MetWork annotations, using an on-line tool (https://metwork.pharmacie.parisdescartes.fr) were filtered and this file was used to annotate the previously obtained MN. In parallel, an in-silico database of all commercialized drugs in Europe was built using a python script and queried for further drug annotation.

Results : The rationale behind our strategy was to build a dynamic MS/MS in-silico database of drugs in Europe. Steps involved i) extraction of smiles structures of all drugs commercialized in France from the open access ROMEDI, a dataset from data.gouv.fr website as well as the centrally authorized drugs dataset from the EMA website (www.ema.europa.eu/en/medicines), ii) generation of in-silico MS/MS spectra using CFM-ID 2.0, iii) and final compilation of all spectra in a MGF format to create this large database (around 1800 molecules), which will be interrogated to annotate patient's MN.

First, a comparison was made between an experimental DB (containing 155 drugs and metabolites) and the in-silico MS/MS spectra using CFM-ID. CFM-ID gave comparable results in 50% of cases. In a second step, for patient, additional drugs annotations were possible, increasing the number of annotated compounds, even without standards available. In a retrospective study, in addition to common drugs of abuse, benzodiazepines or neuropsychotics, drugs from antiepileptic, antifungal, immunosuppressant were found. Finally, additional metabolite annotations were possible using MetWork. This strategy unambiguously identifies phase-1 or phase-2 metabolites, isobaric and isomeric molecules (like tramadol, amitriptyline, venlafaxine and their respective metabolites).

Conclusion : For screening, our results show that MN opens perspectives in clinical toxicology. Available databases are currently a major bottleneck in drug identification. In-silico based databases generated with CFM-ID from different sources may help to expand drug annotation to assist clinicians in poisoning investigation. Area of improvement may consist in refining the generation of in-silico drugs MS/MS spectra and implementing thresholds to increase the matching specificity. To avoid false positives as much as possible, it is necessary to identify metabolites to confirm the diagnosis of poisoning by the parent compound, and the MetWork webserver can be of great help in confirming and propagating annotations.

OP5-A2

Integrating large metabolomics dataset from chemodiverse natural extracts and biological screenings into knowledge graphs to enhance bioactive natural product discovery.

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Integrating diverse information types in natural products (NPs) research, like metabolite profiling (UHPLC-HRMS2), annotations, and several biological screening results, is challenging. Typically, data are processed in an aligned manner to generate feature tables across samples, which is essential for performing Molecular Networking, and enabling precise comparisons within similar groups. However,

the integration of new samples or comparison across different batches faces obstacles due to experimental variations. To overcome these challenges, innovative sample-centric approaches have emerged to explore varied datasets of Natural Extracts (NEs) over time (Gaudry et al. 2022). In addition, there is a growing need for a comprehensive knowledge-driven framework that integrates all types of data, which has led to the adoption of Knowledge Graphs (KG) in metabolomic projects (Gaudry et al. 2023). KGs provide structured representations of complex datasets through RDF semantic web data standardisation (RDF-SemanticWebStandards 2014), this enables an easier exploration and connection of information (Caufield et al. 2023). For example, KGs allow linking a sample's taxonomy, spectral annotations, and bioactivity with existing public knowledge. As a result of a collaboration, a multidimensional KG comprising more than 250 million triples was created. It incorporates UHPLC-HRMS2 data from over 3,000 NEs, fractions, and pure compounds, alongside taxonomic information, chemo-informatics results, and bioassay outcomes for tuberculosis, obesity, anticancer, and antiviral models. We will detail the essential components for constructing a KG based on experimental data. Additionally, we will demonstrate the potential of using SPARQL queries (SPARQLQueryLanguageforRDF 2018) to explore the KG, showcasing their potential in guiding NEs selection to expedite the discovery of NPs.

Keywords: Knowledge graph, RDF, sample-centric, SPARQL query, drug discovery.
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OP5-A3

Chemometrically estimated repeatability in UHPLC equipped with a noise filter and the evaluation of their statistical reliability

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The function of mutual information (FUMI) theory using noise parametrization by the difference method can chemometrically provide a relative standard deviation (RSD) of peak area from a single measured chromatogram of an analyte through the stochastic behaviors of baseline noise in an HPLC system. While, in an HPLC analysis, a noise filter is usually applied to smooth baseline and improve a signal-to-noise ratio (S/N) of a chromatogram. The purpose of the present study is to examine the

effect of noise filter processing on the statistical reliability of repeatability assessment in a UHPLC system based on the FUMI theory.

A UHPLC with UV detection (UHPLC-UV) system for determining ergosterol was applied as an example of the repeatability assessment. In the present UHPLC-UV system, the ODS (ACQUITY UPLC BEH C18 column, 150×2.1 mm i.d., 1.7 µm, Water) and an acetonitrile-methanol-acetic acid (50:50:0.1, v/v/v) mixture were used as stationary and mobile phases, respectively. The cut-off frequency of the noise filter in the UHPLC-UV system was set at 1, 2, or 4 Hz. The series of calculations based on the FUMI theory were performed using software (TOCO19, Hayashi Pure Chemical).

By the present UHPLC-UV system, the peak of ergosterol appeared at a retention time of 8.3 min. The data of 18001 points on the chromatogram processed with the noise filter were applied to a series of calculations based on the FUMI theory. The real histogram of the baseline noise areas obtained from the chromatogram were coincided with the normal distribution. By the histogram analysis, it is found that the baseline noises on the chromatogram processed with noise filter are stochastically approximated with the mixed random process of the first order autoregressive process (AR(1)) and white noise. In the comparisons of precision profiles (plots of RSD of peak area against concentrations of an analyte), the RSD (n = 1) estimated by the FUMI theory fell within the 95% confidence intervals of RSD (n = 6) estimated by repetitive measurements, showing the repeatability assessment in the UHPLC-UV system can be performed by the FUMI theory using a single measured chromatogram of an analyte.

To examine the statistical reliability of repeatability assessment in the UHPLC-UV system, the six values of SD of baseline noise area with width k (s(k)) were determined from baseline noises on six chromatograms processed with a noise filter. And, the average of s(k) was obtained from the six values of s(k) to alternatively apply as the population SD. According to the chi-square distribution at freedom degree of 50, the 95% confidence intervals of s(k)/the average of s(k) were ranging from 0.804 to 1.195 when the population SD was provisionally defined at 1. All values of s(k)/the average of s(k) were within the 95% confidence intervals, indicating the RSDs of peak areas estimated by the FUMI theory (n = 1) and those by repetitive measurements (n = 50) have equivalent reliability in the repeatability assessment for the UHPLC-UV system with noise filter.

OP5-B1

Metabolomics insights into solvent neurotoxicity using 3D brain organoids.

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Glycol ethers, such as propylene glycol butyl ether (PGBE), are organic solvents used in many industrial processes, and their ubiquitarian presence constitutes a source of human exposure. Associations have been found between occupational solvent exposure and neurogenerative diseases, but neurotoxicity is still not systematically assessed for this family of chemicals.

To investigate neurotoxicity and its impact at the molecular level, in vitro models are used in the frame of New Approach Methodologies promoted in modern toxicology practices. In this context, human stem cells-derived 3D brain spheroids were used in this work. They were obtained by aggregation from neural progenitor cells and comprised neurons, astrocytes, and oligodendrocytes. Such a complex model enabled a wide investigation of brain metabolism. In particular, it was hypothesized from previous data that solvents could damage the myelin sheath, a lipid-rich multilayered membrane produced by oligodendrocytes, which is critical for neuronal signal transmission in axons.

For the proposed toxicological evaluation, brain organoids were exposed to incremental concentrations (0, 5, 10 and 20 mM) of PGBE and its metabolite 2-BPA for 2 days and 1 week.

Untargeted chemical profiling was achieved using a double extraction strategy. After polar metabolite extraction and protein precipitation in a methanol/water (4:1) mixture, the resulting pellet was re-extracted in isopropanol for lipidomics. Polar metabolites were measured in UHPLC with a zwitterionic Hilic approach (Waters Premier BEH zHilic) at basic pH (20 mM ammonium acetate, pH 9.2). Lipidomic analysis relied on reversed-phase LC (Waters Premier BEH C18) with 10 mM ammonium acetate and a water / acetonitrile / isopropanol gradient. Both analyses were performed with High Resolution Mass Spectrometry on an Orbitrap Exploris 120 instrument in data-dependent MS/MS.

For the most complete possible annotation, two different tools were involved in polar metabolomics data processing: MSDIAL, and the recently released MARS software. This careful manual curation and identification process resulted in a list of 120 level 1 features complemented by 86 level 2/3 features in negative mode, and in 73 level 1 features with 170 additional level 2/3 features in positive mode.
OPLS discriminant analysis showed altered levels of ribonucleotides and carbohydrate phosphates of the glycolysis pathway, suggesting increased energy metabolism after acute exposure (48 hours), followed by a strong decrease after prolonged treatment (1 week), consistently for both PGBE and its metabolite 2-BPA.

Based on the MS^2 annotation of 713 high-quality lipid features, lipidomic analysis revealed that exposure to both PGBE and 2-BPA caused significant decreases of phosphatidylethanolamines, sphingomyelins and hexosylceramides. These lipid alterations are characteristic of demyelination.
These results were complemented by other omics analyses, namely proteomics and transcriptomics. Supervised multiblock sPLS-DA showed that combining all omics results allowed samples to be separated according to a dose-dependent response to PGBE exposure, and that lipidomics displayed the highest contribution to this group separation. Moreover, the integration of multiple omics layers revealed some consistent relevant biochemical pathways, such as axonogenesis, myelination and lipid metabolism.

Taken together, these results pave the way for a better understanding of glycol ether neurotoxic modes of action.

OP5-B2

Development of a workflow targeted on phase II biomarkers by on-line SPE-LC-(ESI-)-HRMS in human urine for exposomic study purposes.

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The chemical hazard exposure of the population from environmental, industrial and food sources is of health concern in the scientific community. Indeed, working on biological fluids (blood, urine) enables us to gain insight into both the individual's metabolic levels (by measuring biomarker effect) and their internal exposure to chemical hazards via their urinary metabolites (biomarker exposure). The metabolomics science made possible to reveal as much useful information as possible, developing analytical methods without preconceived ideas to meet this challenge. Although the first answers to the question of biomarkers of effect could be demonstrated, the exploitation of the raw data obtained, and the ultimate identification of the substances revealed, often presented considerable difficulties before the problem could be solved. Over the last ten years, approaches focusing on the research question at hand have proved far more effective, as suggested by lipidomics and steroidomics, for example. In this study, we were interested in the urinary fraction of phase II metabolites, including glucuronide and sulfate conjugates, which is also highly relevant to reveal biomarkers of endocrine disruption[1], metabolic disorder of phenylalanine[2] or eicosanoids[3].At the same time, many chemical hazards of medium polarity are eliminated in this glucuronide form via the kidneys. These include bisphenols [4, 5], 2-bromophenol [6], certain polycyclic aromatic compounds [7] such as 1-nitropyrene [7] or phenanthrene [8], certain mycotoxins such as ocratoxin A [9], pesticides such as orthophenylphenol [10], phthalates or even first light PCBs such as PCB3 [11]. Instead of analysing them under their aglycone form after hydrolysis, retaining information on their conjugation seems relevant, sometimes to earn sensitivity but also to linked to the body's detoxification process simultaneously. In our study, we demonstrate firstly the relevance of urinary monitoring of phase II metabolites by on-line SPE coupled with LC-(ESI-)-HRMS, by studying biomarkers of effect and exposure such as steroids and Bisphenol A conjugates in a targeted manner. The study population could be discriminated according to gender by the former markers, or according to dietary exposure by the latter. To take the study of phase II biomarkers present in urinary extracts a step further, the aim is now to reveal non-targeted sulfate and glucuronide biomarkers by simultaneously optimizing a suitable bioinformatics reprocessing method and efficient chromatographic separation.

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OP5-B3

Metabolomic profiling of mouse hippocampus to unravel the effects of chemogenetic modulation of astrocytes in a model for temporal lobe epilepsy

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Astrocytes are a major glial cell type in the central nervous system. They provide metabolic support to neurons and can react to synaptic transmission through activation of neurotransmitter receptors and intracellular calcium signaling cascades. In response, astrocytes can also release neuroactive substances, termed gliotransmitters, to influence the activity of surrounding neurons. Current evidence shows a role for astrocytes in epilepsy, a neurodegenerative disorder characterized by spontaneous seizures. To investigate the specific role of astrocytes during seizures, chemogenetic modulation can be used. It is a cell-type specific approach based on designer receptors exclusively activated by designer drugs (DREADDs) and allows spatiotemporal modulation of a given cell type. In previous research, we found that activation of Gq-DREADDs increases calcium transients in astrocytes, and in a mouse model for temporal lobe epilepsy we observed a significant reduction in seizures 24h after Gq DREADD activation. In the current study, we investigated the corresponding metabolic changes in the hippocampus to gain further understanding of how astrocytes are involved in epilepsy and how chemogenetic modulation mitigates seizures.

We used the intrahippocampal kainic acid model for temporal lobe epilepsy. Under general anesthesia, mice were intrahippocampally injected with the chemoconvulsant kainic acid or 0.9% NaCl to obtain epileptic and control mice, respectively. Two weeks later, mice were injected with an adeno-associated viral vector containing the Gq-DREADD sequence, or its control. After three weeks, all mice received a single injection of the DREADD agonist clozapine N-oxide. Exactly 24h later, mice were decapitated, and the hippocampus was dissected and subsequently stored at -80°C. Sample preparation included the addition of internal standards, homogenization and filtration followed by evaporation to dryness and reconstitution in water. Afterwards, samples were analyzed using capillary electrophoresis (Agilent CE system) coupled to high-resolution mass spectrometry (Q Exactive Plus from Thermo Scientific). Electrospray ionization was performed in positive and negative modes. Peaks were annotated using a reference library, resulting in a total of 561 annotated peaks. Identification of altered metabolites was performed using a combination of chemometric tools (partial least squares – discriminant analysis and random forest).

The data analysis showed 11 metabolites that were altered in both epileptic animals and in epileptic animals with Gq-DREADD activation. These included adenosine monophosphate, phytic acid, glucosamine-6-phosphate, cadaverine, β-aspartylglycine, and other metabolites involved in the phospholipid metabolism and in glycosylation. Nine of these metabolites showed a trend towards restoration of the concentrations in healthy animals. Interestingly, only 3 out of the 11 metabolites were also significantly altered upon chemogenetic modulation of healthy subjects. We are currently investigating how these compounds could be involved in the pathophysiology of epilepsy and how Gq-DREADD modulation of astrocytes mitigates seizures on the metabolite level.

OP6-A1

Analytical Quality by Design: only probability matters !

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Since the adoption of the ICH Q8 document concerning the development of pharmaceutical processes following a quality by design (QbD) approach, there have been many discussions on the opportunity for analytical procedure developments to follow a similar approach. Authorities such as US Pharmacopoeia and ICH have now prepared regulations to implement Analytical QbD (ICHQ14, ICHQ2(R2), USP-1220, ...) and introducing the idea of Analytical or Assay life cycle. The life cycle steps of an analytical method, that are development, optimization, validation, transfer, comparisons and bridging are all interconnected and should ensure the results produced remains of acceptable quality. Indeed, analytical results are used to make crucial decisions about the development of the drug products to assess their efficacy and safety.

This presentation aims at showing the different steps to smartly use all information gathered throughout the life-cycle of the analytical procedure to ensure its fitness of purpose. Combining scientific knowledge with adequate statistical methodologies has a crucial role to play: such as design of experiments, statistical modeling, design space or MODR definitions and probabilistic statements. Accumulating the knowledge of the assay performances gathered at each step ensures the release of a useful analytical procedure, that are under control and that allows making the right decisions with confidence. Indeed, what only matters in the end is the probability to have reliable results.

OP6-A2

Robustness and Validation of a Size-Exclusion Chromatography Method for Monoclonal Antibody Mass Variants Analysis: An AQbD-Inspired Approach

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The quantification of aggregates and fragments in monoclonal antibodies is a critical aspect of batch release and stability studies. This presentation will focus on the robustness assessment and validation of a size-exclusion chromatography (SEC) method tailored to analyze the mass variants of a therapeutic monoclonal antibody, based on a platform approach. Despite the recognized preference for capillary gel electrophoresis in fragment quantification, this study will evaluate the SEC method's performance in this context, in addition to fragments.

Inspired by the principles of Analytical Quality by Design (AQbD), the robustness of the method was rigorously assessed using a design of experiment (DoE) methodology. This approach enabled the identification and control of critical method parameters to ensure reliable performance under varying conditions.

The validation of SEC methods presents unique challenges not fully addressed by the ICH Q2(R2) guidelines, particularly concerning accuracy assessment. In this study, a dual validation strategy was employed. The first approach followed the classical ICH Q2(R2) framework, evaluating each validation

parameter independently. The second, more integrative approach, assessed total error, simultaneously evaluating precision and bias to provide a holistic view of method performance.

This presentation outlines a comprehensive approach for SEC methods, offering insights into robustness assessment and practical guidance on method validation. The findings and proposed guidelines aim to bridge the existing gaps in the literature and regulatory guidance, enhancing the reliability and applicability of SEC in the quantification of monoclonal antibody mass variants, including aggregates and fragments.

By advancing our understanding and validation of SEC methods within an AQbD-inspired framework, this work contributes to the broader goal of ensuring high-quality analytical results in the biopharmaceutical industry.

OP6-A3

Using the Analytical Target Profile (ATP) for Efficient Procedure Lifecycle Management

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The Analytical Target Profile (ATP) as outlined in the USP <1220> and ICH guideline Q14 stands as a cornerstone in the management of analytical procedure lifecycle, linking various stages to ensure procedure fitness for purpose use. This presentation will explore the key role of the ATP in determining analytical procedure attributes and performance criteria, guiding procedure design, validation, and facilitating ongoing procedure performance and continual improvement of procedure. Strategies for defining ATP performance requisites will be discussed, and case studies presented. An example of ATP will be defined based on the drug product (DP) requirements, where the use of the Total Analytical Error (TAE), as a combined accuracy and precision criterion, will be shown to be practical in defining a replication strategy to obtain the reportable result (runs and replicates) that allows achieving the desired analytical procedure performance to ensure proper characterization of the drug product (e.g.,for release, or stability studies). This presentation will also discuss about the use of the TAE as a very powerful tool to define guard bands on the product specifications, in order to ensure the decision on the product quality, based on the analytical result, or to be able to state that the assay uncertainty is too large to make an informed decision about the DP. The presentation will also explore the risk-based approach outlined in ICH Q14 for identifying established conditions and reporting categories for post approval changes, enhancing the understanding and implementation of ATP-driven methodologies in pharmaceutical development.

OP6-A4

Understanding links between Total Analytical Error, Measurement Uncertainty, Analytical Target Profile and Quality Target Product Profile.

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For decades, the pharmaceutical industry has focused on validating analytical processes to demonstrate the assay or bioassay's effectiveness in terms of accuracy, precision, and linearity. Over time, it has become evident that the primary goal is to ensure that laboratory results meet sufficient quality standards.

Numerous publications have illustrated that achieving acceptable accuracy and precision may not always guarantee appropriate result quality. The recent introduction of ICH Q2(R2), endorsing the concept of "combined accuracy and intermediate precision" or what has long been recognized as "Total Analytical Error," underscores the need to accurately convey the relationship between measurement uncertainty and result quality during method validation.

In this presentation, we will provide an overview of Target Measurement Uncertainty (TMU) as a crucial concept encompassing bias, precision, and their respective uncertainties across various concentration levels within the working range. We'll discuss how TMU can be derived from the product or intermediate acceptance limits (Quality Target Product Profile – QTPP) to establish a well-defined Analytical Target Profile (ATP).

Lastly, we'll explore how TMU and QTPP can aid in identifying zones of uncertainty, especially when a measurement closely approaches a specification. We'll delve into how replication and a comprehensive understanding of the assay (e.g., Target Measurement Uncertainty - MMU) can minimize these zones of uncertainty, ensuring that the measurement's quality consistently aligns with its intended purpose.

OP6-B1

Overcoming matrices inter-individual differences and in-source isobaric fragments for LC-MS native lactic acid enantiomeric quantification in pediatric human feces

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In humans, L-lactic acid is the major enantiomer formed during intermediary metabolism, such as a source for gluconeogenesis in the liver or as a waste product of anaerobic glycolysis or amino acids metabolism. Conversely, D-lactic acid mainly originates from bacterial production in the intestinal tract and can be detected in stool. Compared to the L-isomer, D-lactic acid form is toxic, and excessive concentrations can have fatal consequences for human (1). The simultaneous quantification of L- and D-lactic acid represents a significant analytical challenge, as enzymatic assays lack sensitivity, while chromatographic techniques hyphenated to mass spectrometry (LC-MS) are underexplored (2). In the

context of the development of pediatric formulations, there is a need for a reliable and sensitive method capable of detecting the production of D-lactic acid, even at subclinical elevation levels.

To address this, we have developed a straightforward, fully automated, highly sensitive method for the simultaneous determination of L- and D-lactic acid using a single microextraction procedure. Stools were accurately weighed and homogenized with 0.1% orthophosphoric acid. After centrifugation, the lactic acid enantiomers were extracted from the supernatant (fecal water) using an innovative sample preparation technique based on 20µL Volumetric Absorptive Microsampling (VAMS) tips from Neoteryx (Mitra) to obtain highly clean samples and ensure significant elimination of matrix interferences. Enantiomeric separation was achieved using an Astec Chirobiotic R (ristocetin A selector) chiral column under gradient conditions composed of A) H2O+NH4HCO2 45mM and B) ACN/MeOH 98/2. Fine-tuning of the gradient and mobile phase enabled proper enantiomeric chromatographic resolution, along with the reduction of carryover that can occur in isocratic mode. Compared to ESI, the use of UniSpray technology (Waters Corp.) reduced in-source fragmentation by a factor of 4, which was associated with isobaric coeluting interference from the sample matrix. Accurate quantification of the two enantiomers was very challenging due to inter-individual variations, which led to solvent mismatches in calibration, where neat solvent and pooled stool were unable to provide accurate results for different individual samples. To overcome this bottleneck, we applied the concept of internal calibration using the internal standard/analyte ratio to calculate the concentration (3). Therefore, the internal standard concentrations were precisely investigated to obtain a linear concentration-response range of 0.5 – 250µg/mL for each enantiomer. The performance of this calibration method is currently under evaluation using real clinical samples.

The developed method will be applied to a cohort of 1300 pediatric individuals in the context of infant formulas screening.

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OP6-B2

RETENTION AND ENANTIOSELECTIVITY MODELLING OF STRUCTURALLY DIVERSE PHARMACEUTICALS ANALYZED ON POLYSACCHARIDE-BASED STATIONARY PHASES

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More than 60% of the newly commercialized drugs possesses chiral properties. Since one enantiomer in a racemic mixture might be ineffective or toxic, strict guidelines are defined for the development of

a chiral drug. The separation of chiral drug molecules, mostly by means of chromatography, is therefore an intensively studied domain. While the separation of enantiomers is important in many fields of industrial and pharmaceutical analysis, the selection of a suitable chromatographic system (chiral selector and mobile phase) is not trivial. As a consequence, chiral method development requires considerable experimentation and is often highly demanding with respect to time, material and labour. This study aims to develop models to predict the retention, enantioseparation and elution sequence of structurally diverse enantiomers. More specifically, Quantitative Structure Enantioselective Retention Relationship (QSERR) models are built that describe the relationship between molecular descriptors and retention/separation.

Retention times and elution sequences of 48 structurally diverse chiral molecules were determined on four polysaccharide chiral stationary phases, Chiralcel OD-RH (cellulose tris(3,5-dimethylphenylcarbamate) selector), Lux amylose-2 (amylose tris(5-chloro-2-methylphenylcarbamate) selector), Lux amylose-1 (amylose tris(3,5-dimethylphenylcarbamate) selector) and Lux cellulose-3 (cellulose tris(4-methylbenzoate) selector), using either an acidic or a basic mobile phase. Both achiral and in-house developed chiral descriptors were used as independent variables to build the models. Specifically, our conformation-dependent chiral descriptors were calculated for each molecule from molecular dynamics simulations in implicit and explicit solvent. In addition to 167 simple ensemble-averaged descriptors per simulation, we calculated 334 derivative descriptors that measure the asymmetry in the distributions of the original chiral descriptors, as these may correlate better to the difference in binding affinities for the chiral stationary phases.

Linear regression techniques, such as stepwise multiple linear regression (sMLR) and partial least squares (PLS) regression, were applied to model the retention or separation as a function of the descriptors. In a first step, models were built with only achiral descriptors to model the global retention of the molecules. Subsequently, models were built with only chiral descriptors to predict the enantioseparation and elution sequence.

The global retention was predicted well by the achiral sMLR models. As a result, they may predict when an unknown chiral molecule will elute approximately. The models built with only chiral descriptors satisfactorily predicted the enantioseparation and elution sequence for different chromatographic systems, which demonstrates the importance of simulations in a realistic environment to obtain physically meaningful chiral descriptors.

OP6-B3

Isotope effect in high-performance liquid chromatography: Good or bad for bioanalysis?

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Isotopically labelled compounds have attracted increasing interest in the pharmaceutical industry in the last decade [1]. In particular, more and more attention is paid to the kinetic isotope effect [2] in biological (living) systems and among them in humans, in the sense that undeuterated and deuterated biologically active compounds may have different pharmacokinetic, toxicokinetic, and most likely even pharmacodynamic properties. Such differences led to the approval of some deuterated chemical species as drugs for clinical use by regulatory bodies since 2017 [3]. Contrary to this, in bioanalysis it is believed that isotope effects do not play any significant role or don't even exist and therefore,

isotopically labelled (primarily deuterated) analogues of target analytes are considered to be optimal internal standards in bioanalysis with mass-spectrometric (MS) detection [4]. Only very few published studies have attempted the simultaneous separation of enantioisotopologues. Recently we observed baseline separation of partially deuterated isotopologues of a few amphetamine derivatives in high-performance liquid chromatography (HPLC) using achiral columns. In addition, the simultaneous separations of enantiomers and isotopologues (i.e. enantioisotopologues) were achieved on polysaccharide-based chiral columns [5]. For several compounds the isotope effect was tunable and could be switched from a "normal" to "inverse" by making changes to the mobile-phase composition. A stronger isotope effect was observed in acetonitrile-containing mobile phases compared to methanol-containing ones with both chiral and achiral columns. In a separation system where both "normal" and "inverse" isotope effects were observed the "normal" isotope effect was favored in polar organic solvents while increasing content of the aqueous component in the reversed-phase (RP) mobile phase favored an "inverse" isotope effect. This observation indicates that polar, hydrogen bonding-type noncovalent interactions are involved in the "normal" isotope effect, while apolar hydrophobic-type interactions are mostly responsible for the "inverse" isotope effect. In this presentation somehow neglected isotope effect in HPLC and its consequences for bioanalysis will be discussed based on our recent studies.

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OP6-B4

Exploring chiral supercritical fluid chromatography for preparative scale.

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Chiral drugs development is growing continuously in the pharmaceutical business and in academic laboratories alike, as it can be observed from the number of single enantiomers launched in recent years. Chromatographic preparative enantioseparation is the preferred method to obtain milligram amounts of pure enantiomers in the first step of the development of a therapeutic molecule.
Supercritical fluid chromatography has many advantages over liquid chromatography and is therefore chosen for the small scale enantioseparation in our laboratory. More widely, thanks to further upgrades in instrumentation and chiral column design this technique became the mainstream one, both in analytical « routine » and in preparative scale taking advantage of its intrinsic properties which render SFC a green method.

In this lecture, examples of chiral separation method development will be presented, with a focus of the preparative scale.

OP7-A1

Interest of pI determination in the quality control of virus-like particles of human papillomavirus

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The importance of quality control of protein reagents to improve the reproducibility of research data has been highlighted in numerous publications [1-3]. QC tests to assess identity, purity, content as well as stability of biologics have to be carried out before their use in downstream experiments, whatever their source, i.e. from commercial suppliers or produced by researchers themselves.

The binding properties of different human papillomavirus (HPV) types to laminin 332, a receptor implicated in the virus binding to the host cells, are currently under investigation in our laboratory. Because HPV in vitro production leads to low virus titers, virus-like particles (VLPs) of HPV are used as model. HPV-VLPs are formed by the self-assembly of viral structural proteins. They are produced using the baculovirus expression vector system. Several QC methods using SDS-PAGE, electron microscopy and ELISA with a conformational antibody are performed before their use in interaction studies by affinity capillary electrophoresis and bio-layer interferometry.

The aim of this study was to evaluate the interest of implementing the determination of the pI of intact HPV-VLPs using imaged capillary isoelectric focusing (iCIEF) in the QC tests. Several parameters, namely the addition of a non-ionic surfactant and urea, focusing time and voltage, were investigated to achieve reproducible analysis of the intact viral particles. An HPV-VLP sample diluted in the iCIEF matrix was analyzed by electron microscopy to demonstrate that the pI of the intact particle, and not of the viral structural protein, is determined. The performance of the developed method was studied in terms of repeatability and intermediate precision. The variability of the pI value of several HPV-VLP batches was investigated. Interestingly, one batch of HPV-VLPs successfully passed QC tests based on SDS-PAGE, electron microscopy and ELISA but not that related to the pI determination. It appeared that the binding properties to laminin 332 of this HPV-VLP batch were different compared to other tested productions. Finally, we applied the optimized method to the analysis of a VLP-based vaccine after adjuvant dissolution. This study demonstrates that the pI determination constitutes a valuable asset in the QC of viral particles.

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OP7-A2

Development of an enantio- and chemo-selective screening method for asparagine/aspartic acid and glutamine/glutamic acid residues in proteins

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The stereoinversion of amino acid residues has been reported as one of the post-translational modifications of proteins. Especially, isomerization of aspartic acid (Asp) residues has been found in the proteins derived from patients of age-related diseases, such as cataract and Alzheimer's disease. Besides, D-asparagine (Asn) residue has also been found in mouse lysozyme stored under physiological conditions. Therefore, relationships between the isomerization of various amino acid residues and deterioration of the proteins or aging/diseases are matters of concern. Regarding the comprehensive determination of D-form residues in proteins, the combination of hydrolysis pretreatment and enantioselective analysis of the resultant free amino acids is effective. However, there are still several issues; amino acid residues are partially racemized under severe reaction conditions, Asn and glutamine (Gln) residues are indistinguishable from Asp and glutamic acid (Glu) residues due to their deamidation during hydrolysis. These problems have made the screening of isomerized amino acid residues in proteins difficult, and development of a precise analytical method is expected to find new biomarkers for protein functions or therapeutic targets of diseases.

In the present study, the protein samples were heated at 60ºC for 4 h in the presence of [bis(trifluoroacetoxy)iodo]benzene to convert Asn and Gln residues to diaminopropionic acid (DAPA) and diaminobutyric acid (DABA), respectively, remaining the chiral center. The proteins were then gas-phase hydrolyzed at 90ºC for 24 h in the vapor of deuterium chloride to form free amino acids, and to isotopically label amino acids racemized during hydrolysis. Concerning the accurate and enantioselective determination of amino acids, we have developed multi-dimensional chromatographic techniques. To perform the precise determination of DAPA, DABA, Asp and Glu with chiral discrimination, the separation conditions for two-dimensional (2D) chiral LC and detection conditions using MS/MS were investigated. As a result, the target analytes derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole were purified from other compounds by a microbore ODS column (Singularity RP18, 1.0 x 250 mm) in the first dimension. The respective target fractions were introduced into the second dimension, and amino acid enantiomers were separated by a Pirkle-type column (Singularity CSP-013S, 1.5 x 250 mm). For the detection of DAPA, DABA, Asp and Glu, the precursor/product ion pairs (ESI, positive ion mode) of 431/239, 445/161, 299/149 and 313/149 were selected, respectively.

Integrating the Hofmann-rearrangement reaction followed by hydrolysis using deuterium chloride and 2D chiral LC-MS/MS determination, the simultaneous and enantioselective analytical method for Asn/Asp/Gln/Glu residues in proteins was developed. Using the developed system, L-amino acid residues including Asn (DAPA) and Gln (DABA) were successfully detected in the model peptide (H-Gly-Asp-Asn-Ala-Glu-Gln-Gly-OH) consisting of only L-form residues (by MS/MS) with minimum isomerization, whereas non-negligible D-forms were detected by the fluorescence detection. The system was applied to the analysis of amino acid residues in the lens proteins obtained from cataract patients. As a result, not only D-Asp but also D-Asn residues were detected in all the tested samples of 4 cataract patients. Further investigations about the newly found D-amino acid residues, such as isomerized positions and biological significances, are ongoing.

OP7-B1

Charge Variant Separation and Characterization of Various Antibodies Using CZE-MS, cIEF-MS, and IEX-MS on Intact and Subunit Level

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The analysis of antibodies is an ever-evolving field of research. Depending on the host cell, the purification process, or different storage conditions, antibodies can differ, for example, in their glycosylation profile or amino acid sequence. The analysis of post-translational modifications (PTMs) of monoclonal antibodies (mAbs) is therefore quite complex and time-consuming and can generally be performed on an intact, subunit, or peptide level. The analysis of intact mAbs provides information on the actual proteoform and requires minimal sample preparation, however, does not allow for detailed information on the position of the protein modification. In contrast, the subunit analysis enables fragmentation experiments providing valuable information about the position of the modification in the molecule.

In this work, we compared capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), and ion exchange chromatography (IEX) coupled with mass spectrometry (MS) to analyze charge variants on the intact level. CZE-MS was performed both on the intact and subunit level. For all intact separation approaches, monoclonal antibodies were diluted in water or background electrolyte, while for subunits, mAbs were enzymatically digested and further reduced using DTT in 4M urea. CZE and CIEF were coupled to the MS using the nanoCEasy interface.

CZE-MS enables the separation of proteoforms with reduced intramolecular disulfide bridges from the main form, on an intact and subunit level, respectively. In the case of the subunit approach, these proteoforms were based on an incomplete sample reduction, which was later addressed by using urea in the reduction approach. In addition to that, additional proteoforms such as the mAb glycosylation pattern or C-terminal lysin clipping were analyzed.CIEF-MS and IEX-MS were performed on an intact level only. Both methods were used to analyze the glycosylation pattern of the antibodies as well as the presence of C-terminal lysine residues. Small changes in the mass of the antibody compared to the main form were also evaluated.

Overall, the three presented separation methods show a separation of acidic and basic variants from the main form with subsequent online characterization of the mAb and its variants by mass spectrometry. However, both the separation and the ESI-MS data differ for all separation modes, regarding the ability to characterize both charge- and size variants. In this presentation, the performance of the three hyphenated separation methods to MS will be compared regarding selectivity, sensitivity, and ease of use.

OP7-B2

Novel approach for the isolation of β-estradiol metabolites from human serum samples employing a 3D-printed device

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β-Estradiol and its metabolites play crucial roles in human physiology, particularly in the regulation of female sexual characteristics and the menstrual cycle. Additionally, estrogens can influence the pathogenesis of various diseases. These compounds could be potentially act as biomarkers for conditions such as different types of cancer or Parkinson's disease.

Continuous efforts are made to enhance analytical methodologies, both in terms of chromatographic separations and sample preparation techniques. The sample preparation process is paramount yet simultaneously the most time-consuming, labor-intensive and error-prone stage of the analytical method. Analysts encounter challenges due to the low concentrations of endogenous compounds in the rage of nano- and pictograms per milliliter and the limited amount (around 100 – 200 µL) of the biological material obtained from the patients. This necessitates the use of highly sensitive and specific techniques following proper optimization of chromatographic conditions and mass spectrometer operation.

The aim of this study was to develop a new, efficient technique for the preparation of human serum samples for the analysis of β-estradiol and its metabolites that would be be fast, accurate, precise, and above all, characterized by very high sensitivity, sufficient for detecting low-molecular-weight endogenous compounds at physiological levels. We utilized a 3D-printed device designed for dispersive solid phase extraction (DSPE) and compared different sorbents (C18, HLB) and desorption solvents (methanol, ethanol, isopropyl alcohol and acetonitrile). This technique was also compared with a traditional DSPE approach. Samples were analyzed using a previously developed LC-MS/MS method. The chromatographic separations were performed using a Poroshell C18 column (3.0 x 100 mm; 2.7 µm), thermostated at 40°C. Mobile phase A was water with 0.1% FA, phase B was methanol with 0.1% FA. The flow rate of the mobile phase was 0.5 mL/min. The most suitable extraction conditions were proven to be a C18 sorbent and ethanol as the desorbing agent. Good linearity for the target analytes was obtained in the range of 0.1 – 50 ng/mL with R2 ranging from 0.978 to 0.9979 for 10 of the analytes (4-methoxyestradiol, 16-epiestriol, estriol, 2-methoxyestrone, 2-methoxyestradiol, β-estradiol, α-estradiol, 16-hydroxyestrone, 17-epiestriol, estrone). The analytical signal for hydroxy metabolites (2-hydroxyestardiol, 4-hydroxyestradiol. 4-hydroxyestone) was not sufficiently high for the compounds to be appropriately quantified. As compared to traditional DSPE using an Eppendorf tube, the novel extraction technique has allowed for similar or higher recoveries and higher precision. The presented method is suitable for the quantification of a group of estrogen metabolites and could potentially find purpose in clinical practise. However, the developed procedure is not sufficient for the determination of the hydroxy metabolites, which are going to be the focus of our next project.

OP8-A1

A comparative study of analytical methods for assessing mRNA integrity in lipid nanoparticles: spotlight on mRNA-lipid adducts, fragments, and oligomers.

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The development of mRNA lipid nanoparticles (LNP) brings great hope for both preventive and therapeutic vaccines. The rapid advancement of these new technologies goes hand-in-hand with the development of novel analytical methods to characterize LNPs. One critical quality attribute (CQA) of mRNA Drug Substance (DS) and Drug Product (DP) is mRNA integrity. Indeed, mRNA molecules are susceptible to degradation, as evidenced by the frequent need for ultra-low-temperatures storage of this type of vaccine. If the mRNA is degraded, it may no longer be translated correctly into the target antigen, leading to a loss of vaccine efficacy.

Different analytical methods can be used to assess mRNA integrity. Robust analytical methods are crucial for enhancing our understanding of mRNA degradation mechanisms, optimizing formulation stability, and maximizing vaccine efficacy.

In this study, three analytical methods will be compared: capillary gel electrophoresis (CGE) with fluorescent detection, CGE with UV detection, and ion-pairing reversed-phase chromatography (RP-IP-HPLC) with UV detection. The advantages and disadvantages of each method will be highlighted. Furthermore, the impact of mRNA fragmentation, mRNA oligomerization, and the formation of mRNA-lipid adducts on protein expression will be investigated and discussed.

OP8-A2

Characterization and relative quantification of oxidation in a monoclonal antibody by a multi-level approach based on mass spectrometry.

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Monoclonal antibodies (mAbs) are considered as the most rapidly growing class of therapeutics to treat several diseases. Critical quality attributes associated to mAbs such as sequence assessment, aggregates or charge variants have to be monitored for safety concerns. The hyphenation of LC methods with mass spectrometry, along with the implementation of innovative fragmentation techniques, has proven to be a valuable asset to characterize proteoform populations of mAbs. Based on these advantages, we present herein a multi-level approach to characterize and relatively quantify oxidation in a mAb, from intact native analysis to peptide mapping.

Mass spectrometry analyses were coupled to different chromatographic separations in denaturing or non-denaturing conditions, depending on the purpose. The mAb analyzed, produced by Sanofi, was submitted to oxidative stress by tert-butyl hydroperoxide (tBHP), overnight, either at room

temperature or 37°C, to increase the level of methionine (Met) oxidation. Size and charge variants were respectively characterized at the intact and middle levels by size-exclusion chromatography (SEC) and cation-exchange chromatography (CEX) hyphenated to native mass spectrometry (nMS). Middle-down mass spectrometry (MD-MS) was coupled to reversed-phase (RP) chromatography, exploiting a combination of several MS/MS fragmentation techniques. Finally classical bottom-up (BU) provided further confirmation and quantification of the oxidation sites.

The characterization study of the mAb was performed from the intact to the peptides level on the reference and stressed materials. The first step aimed at the analysis of the size and charge variants of the mAb through the combination of nMS either with SEC or CEX, respectively. Overall, the different populations separated in the chromatographic dimensions at the intact and middle levels were identified thanks to an accurate mass measurement (< 30 ppm) and resolution (3000 at m/z 5000). CEX-nMS highlighted that the Fc subunit was more prone to oxidation compared to the F(ab')2 part. However, MD-MS experiments were necessary to precisely localize these modifications. In this case, MD-MS approaches were more informative than BU since coexistence of multiple oxidation sites could be observed in a sole MS/MS fragment. Oxidation sites were confirmed through detection of diagnostics ions. Relative quantification of oxidation by MS-MS approach was performed in parallel on the RPLC chromatogram and on the MS/MS spectra, which was corroborated by BU analysis.

Several trends in Met oxidation kinetics were observed at each level of characterization: firstly, higher incubation temperature induces higher overall Met oxidation level, secondly, Met of the Fc domain are more prone to oxidation induced by tBHP, finally among the Met of the Fc domain, M253 has the highest oxidation kinetics. Altogether, this multi-level approach by mass spectrometry-based techniques allowed to tackle the precise location of oxidation sites and provided consistent quantification results of oxidation for an improved characterization of mAbs.

OP8-A3

Endotoxin Quantification by a Chemical Instrumental Analytical (U)HPLC-Assay

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Endotoxins are ubiquitous in our environment, leading to a constant risk of endotoxin contaminations in pharmaceutical and biopharmaceutical products. Endotoxins are non-covalently integrated into the outer membrane of Gram-negative bacteria and cover about 75 % of the cell surface. They are released during proliferation, growth, and bacterial membrane lysis in large quantities. Endotoxins are strong immunostimulants, even quantities as low as pgmL-1, introduced into the human bloodstream via contaminated medical devices, surgical equipment, or drugs, can trigger severe reactions such as fever, sepsis, or potentially fatal organ failure. Consequently, strict quality control of these contaminants is requested by the health authorities worldwide such as the FDA and EMA.

Endotoxin testing typically is performed with biological assays such as the rabbit pyrogen test, the monocyte activation test, or the Limulus Amoebocyte Lysate (LAL) assays. LAL is considered as the gold standard in current compendial but also development phase of endotoxin testing. While highly sensitive, all these biological assays have significant drawbacks. They work all in a very limited small dynamic concentration range and are all susceptible to strong sample matrix interferences. This may lead to false-negative results, a phenomenon known as "low endotoxin recovery" which endangers

patients' health. Moreover, e.g. the LAL assay is validated with endotoxin recovery values (accuracy) of 50 – 200 %, and a precision of 25 %. Those numbers express the large experimental error of the most common endotoxin quantification assay.

In comparison to biological assays, a chemical instrumental analytical assay presents a viable solution to reduce the experimental error, extend the dynamic concentration range and solve the specificity issues of the biological tests. Our group has developed the so called HPLC-Kdo-DMB assay [1, 2] that is based on the rare, endotoxin specific sugar acid 3-Deoxy-d-manno-oct-2-ulosonic acid (Kdo), present in each endotoxin molecule as endotoxin marker. With that the assay overcomes the huge heterogeneity of different endotoxin preparations obtained from different bacteria or different purification procedures. Kdo is quantitively released from each endotoxin molecule by mild acidic hydrolysis. Sensitive detection is obtained by Kdo derivatization with the fluorophore 1,2-Diamino-4,5-methylenedioxybenzene dihydrochloride (DMB). Matrix effects are minimized by the separation of Kdo-DMB by RP-(U)HPLC from potential interfering matrix compounds. Consequently, the chemical endotoxin quantification approach strongly reduces the likelihood of "low endotoxin recovery". The current limit of quantification of the endotoxin assay is 30 EUmL-1, with ongoing research efforts to reduce it to 0.25 EUmL-1, a health authority requirement for pharmaceutical applications.

The novel assay has been successfully used to monitor efficiently the time dependent endotoxin release in different crude bioreactor cultivations [3] and for endotoxin removal filter development. It was further used to assess the efficacy of downstream process filtrations and to analyze high protein load samples.

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OP8-B1

The Effect of Column Length and Gradient Steepness on the Separation of Oligonucleotides in Ion Pair Reversed Phase Chromatography

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The number and variety of different pharmaceutical products - such as some new small molecule drugs, monoclonal antibodies, antibody-drug conjugates, and cell and gene therapy products - is growing rapidly. Importantly, these new drug candidates are becoming increasingly complex, whether in terms of increasingly complex molecular composition or mode of action. More than ever, the industry needs new analytical methods to characterize, formulate, and perform release testing on these new drugs. Increasing the throughput and amount of data generated by an analytical method will help bring more effective drugs to market faster.

Among analytical techniques, liquid chromatography separations offer several capabilities and advantages. The presentation will focus on reversed-phase separations, but the same rules apply to any retentive elution mode for large solutes (ion exchange, HILIC, HIC or affinity chromatography).

We show that very short columns (a few millimeters to centimeters) are required to effectively retain and separate large molecule analytes. The recently introduced "ultra-short column" approach allows complex separations to be performed in 0.5 - 3 minute intervals. This can dramatically reduce method development time.

Some generic (platform) gradient approaches will also be discussed, based on the consideration of the "homologue rule". Oligonucleotides can be thought of as compounds in a homologous series. In liquid chromatography, it is an empirical observation that retention increases in logarithmic (or follows a concave power function) steps (increments) with increasing number of homologous units. This behavior means that when running linear mobile phase gradients for oligonucleotides, the retention of the larger oligonucleotides will increase not proportionally but in logarithmic increments. In other words, both retention and selectivity converge to a plateau. As a consequence, the selectivity between n-1 mers will converge to 0 for larger oligonucleotides. Therefore, the selectivity will always decrease for the larger oligonucleotides when running linear gradients. If a linear %B increment results in a logarithmic time distribution of peaks across the chromatogram, then - based on the properties of inverse functions - a logarithmic (or concave) %B program in time should result in a more homogeneous peak distribution in the time domain. Much more uniform peak distributions can be obtained by running generic concave gradients instead of traditional linear gradients.
Various examples will be shown to illustrate the potential of ultra-short columns and the new “logarithmic gradient” approach.

OP8-B2

Rapid isomer-specific identification and relative quantitation of new psychoactive substances in street samples

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New psychoactive substances (NPS) are structural derivatives of conventional illicit drugs, designed to circumvent the law. Synthetic cathinones, a class of designer drugs representing two-third of all NPS seized in Europe, frequently are positional isomers of the forbidden drugs. Reliable identification of these isomers poses a challenge in forensic casework, and current methods often require laborious and time consuming procedures.

Here we present a new methodology for the fast and highly selective analysis of cathinone isomers encountered in street samples using trapped ion mobility mass spectrometry (TIMMS). Samples were infused directly into the TIMMS instrument via positive electrospray ionization, requiring only dissolution and dilution as sample preparation. Employing appropriate TIMS tunnel pressures, voltage gradients, and mobility calibration, cathinone isomers could be unequivocally assigned based on their unique combination of accurate mass and IM profile. Addition of a crown ether during sample dilution reduced mobilogram complexity, allowing efficient resolution and reliable annotation of cathinones in mixtures, even when these are positional isomers.

With the developed workflow, the NPS composition of unknown samples could be established, also revealing the relative abundance of the individual cathinones. The new method was successfully used

for the unambiguous identification of NPS isomers in confiscated street samples in less than 5 min per sample.

OP8-B3

Microflow liquid chromatography coupled to nanoelectrospray ionization as a strategic combination for high-throughput bioanalytical methods with challenging sensitivity requirements

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In the vast majority of laboratories dealing with bioanalysis (including metabolomics), high resolution mass spectrometry (HRMS) systems with electrospray ionization are hyphenated to liquid chromatography platforms (LC), which utilize an analytical flow regime of 0.2-1 mL/min. Such configurations prioritize analytes of higher abundances or ionizability, and tend to suffer from detrimental phenomena like matrix effects and/or ion suppression. A far higher sensitivity can be obtained with nanoelectrospray thanks to a better ionization efficiency, tolerance to matrix effects and a background reduction. These advantages are crucial to reliably access low-abundant metabolites such as lipid mediators (steroids, eicosanoids, phosphorylated complex lipids) irrespectively of biological matrix.

In this work, we explored microflow (µF) chromatographic regimes in range of 1-5 µL/min with a novel microfabricated monolithic multinozzle (M3) electrospray emitter. The latter allows for flow splitting to obtain multiple stable nanosprays. Current setup (5 nozzles, 600 nL/min per nozzle) was considered for high-throughput non-targeted lipidomic profiling of 3D cell cultures and compared to an established LC/HRMS protocol based on analytical flow (AF) employed routinely in our laboratory. With an identical runtime of 20 minutes and using the same MS, processing of acquired data with MS-DIAL v4.92 returned 10254 (µF-M3, 0.2 µL sample) vs 10422 (AF, 2.0 µL sample) features. Matching of fragmentation spectra produced 2165 (µF-M3) vs 1190 (AF) hits. Both setups demonstrated comparable robustness with 128% (µF-M3) vs 123% (AF) CVavg on all signals and 1.4% (µF-M3) vs 0.7% (AF) CVavg on retention times for all features in the batch. Subsequent manual curation revealed 1371 (µF-M3) and 854 (AF) distinctive lipid species: a remarkable 60% improvement produced from 10x lesser amount of the injected material. Interestingly, the µF-M3 configuration featured significantly lower degree of adduction and dramatically improved ionization for cholesterol esters, mono- and diacylglycerols, otherwise hardly detectable with a conventional setup.

SO-1

Endogenous steroid analysis by supercritical fluid chromatography-mass spectrometry: super possibilities and critical aspects

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The first step to detect doping with endogenous steroids like testosterone is the analysis of the urinary steroid profile for the athlete biological passport (ABP). It consists of six endogenous steroids and their ratios, which are monitored individually and longitudinally for an athlete. If a parameter is observed to exceed the athlete's individual thresholds, the origin of steroid must be determined by Isotope Ratio Mass Spectrometry (IRMS). To harmonise steroid profiling performed by different anti-doping laboratories, the World Anti-Doping Agency (WADA) has issued a technical document for the analysis of endogenous anabolic steroids. This document establishes the combination of gas chromatography and mass spectrometry (GC-MS(/MS)) as the standard and mandatory method for quantification of urinary steroids. Though the negligible matrix effects in electron ionisation (EI) make GC-MS an excellent choice for quantification, there are less separation mechanisms available for the discrimination between isomers. Considering the downsides of GC-MS and developments in alternative methods, approaches on e.g. LC-MS have been proposed to perform urinary steroid profiling.

Supercritical fluid chromatography (SFC) is an alternative separation technique, but so far it has not received much attention in antidoping analyses. Due to the nature of supercritical fluids, SFC combines the advantages of LC and GC. The gradients employed for separation are faster than common GC methods and many different stationary phase chemistries are available for SFC to address the separation of frequently occurring isomeric pairs. While the utility of SFC coupled to tandem mass spectrometry (SFC-MS/MS) in antidoping analyses was demonstrated for stimulants and exogenous steroids, methods for the analysis of endogenous steroids are limited to clinical applications.

In our work, we developed a novel ultra high-performance supercritical fluid chromatography coupled to tandem mass spectrometry (UHPSFC-MS/MS) method for the quantification endogenous steroids for the urinary steroid profile in the ABP. The achieved sensitivity is comparable to modern LC MS/MS instruments, so less than 1 mL of urine is needed for accurate quantification of the urinary steroid profile and the extraction could be performed in 96-well plates, thus increasing the analytical throughput in comparison to GC-MS-analysis. With a chromatographic run-time of 10 minutes, it is possible to analyse 80 samples per day. The method was validated according to the corresponding technical document for limits of quantification (LOQ) and measurement uncertainties. Authentic urine samples from athletes (n=132) were analysed, and the results were compared with routine GC-MS urinary steroid profiling. Regression analysis showed agreement in results between the two platforms. Furthermore, the use of preparative UHPSFC for sample purification and fraction collection prior to IRMS analysis was explored, demonstrating the wide potential of this technique for the analysis of endogenous steroids in the antidoping context.

SO-2

Ultra-miniaturized weak affinity chromatography: towards a screening strategy of native membrane proteins in fragment Based Drug Discovery: Adenosine Receptor as a case-study.

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In the field of fragment-based drug discovery (FBDD), weak affinity chromatography (WAC) has found its place in the arsenal of biophysical methods for detecting weak interactions (µM to mM) between a target protein and small ligands called fragments. In weak affinity chromatography, the protein target is immobilized on the chromatographic column and a library of fragments is screened by injecting pools of fragments. The popularity of WAC for fragment library screening is linked to its multiplexing capacity and the significant reduction in reagent consumption (protein target and fragment). We have recently demonstrated that its miniaturization (75 µm i.d capillary column) opens fragment screening to membrane proteins, especially GPCR superfamily which are pharmaceutical targets of great interest as they have crucial roles in physiological and diseases-related processes. Our methodology relies on the immobilization of the target membrane protein, embedded in a biotinylated membrane-mimicking environment (nanodisc), on in-situ synthesized monolithic support modified with streptavidin. While the ability to detect weak interactions is primarily dependent on the amount of active protein immobilized per unit volume of stationary phase, non-specific interactions are a common bottleneck in fragment screening and a source of potential false positives/negatives in the identification of hits.

We will present the current limits of weak affinity chromatography in terms of detectable affinities (Kd range) due to high non-specific retention and to the limited quantity of active recognition sites for the poly(GMA-co-EDMA) monolith. To reduce non-specific interaction, we envisioned the development of a more hydrophilic monolith (poly(DHPMA-co-MBA), synthesized with a more hydrophilic crosslinker (Methylene bis acrylamide, MBA) and a diol acrylate monomer (2,3-Dihydroxypropyl methacrylate, DHMPA). We demonstrate that this new monolith fulfils the key performance criteria with reduced non-specific interactions and a twofold increase in the number of active binding sites. To further increase the ND density a three-dimensional molecular assembly of NDs was then considered (multilayer grafting alternating layers of streptavidin and biotinylated NDs). The number of active binding sites is about 60% higher than in the first stage.

The increase in the number of membrane protein binding sites (a gain of up to a factor of 3 over the poly(GMA-co-EDMA) monolith) and the reduction of non-specific interactions makes it possible to extend the range of detectable affinity, as demonstrated with the identification and characterization of affinities of very low affinity ligands (Kd values of several hundred micromolar) for the adenosine receptor AA2AR used as a model protein, which was not possible before. The affinity was confirmed by competition experiments.

SO-3

Advancements in neoglycoprotein synthesis and monitoring strategies

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Glycoproteins have proven their unignorable values in therapeutic and vaccine development. Glycosylation plays important roles in modulating the biological functions of glycoproteins, such as protein folding, stability, solubility, and immunogenicity. Natural glycoproteins, produced by cultured cells, often carry heterogeneous N-glycans, due to the complexity of N-glycosylation processing in the biosynthesis. For pharmaceutical applications, preparation of well-defined and ideally homogenous glycoproteins is of paramount importance. Therefore, close attention has been drawn to the development of glycoengineering strategies to better control the glycan structures and the glycosylation profile. Such achievements are realized through methods like the genetic engineering of host cells, but also via chemical or enzymatic remodeling. Alongside these advancements, there is a concurrent need for rapid analytical methods that can efficiently characterize glycoengineered products thereby accelerating the development phase and ensuring quality in the finished product.
In this context, we have developed an analytical platform for the full characterization of glycoproteins, based on LC-ESI-HRMS at peptide and intact level. Additionally, we have introduced a new advanced MALDI in-source decay (ISD) Fourier-transform ion cyclotron resonance (FT-ICR) MS method for the direct glycosylation analysis in intact glycoproteins. The use of a sodium-doped MALDI matrix enabled the generation of glycans as ISD fragment ions during ionization, that can be further fragmented through collision induced dissociation (CID). The obtained results demonstrated high repeatability and accuracy in glycan profiling, consistent with conventional methods, and facilitate the identifications of glycosylation differences between standard and glycoengineered proteins.

The second part of this work is focused on the development of glycoengineered vaccines starting from recombinant non glycosylated protein antigens. The novelty of our approach lies in combining chemoselective glycosylation of the protein with minimal synthetic glycans (functionalized N-acetyl glucosamine) as substrate for a class of enzymes known as endoglycosidases in a transglycosylation reaction. These enzymes can catalyze both the hydrolysis of the chitobiose core of N-glycans between two N-acetyl glucosamine (GlcNAc) residues and transglycosylation reactions transferring an activated N-glycan at the anomeric position to a protein bearing a single GlcNAc residue. A protein model was glycosylated with GlcNAcs chemically activated with different functional groups. These newly GlcNAc-modified proteins have been tested using endoglycosidases to perform a transglycosylation reaction with a synthetic oxazoline-activated oligosaccharide. Glycosylation yields were determined by HILIC-MS at intact glycoprotein level. The most effective GlcNAc functionalization strategy, identified based on its ability to provide a suitable combination of chemical and chemoenzymatic glycosylation, will be applied to glycosylate Ag85B, a known tuberculosis antigen, to find a new effect way to produce a neo-glycoconjugate vaccine.

SO-4

Navigating Experimental Metabolomics Knowledge Graphs to Highlight Anti-Infective Natural Products Within a Large Collection of Plant Extracts

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The escalating threat posed by Multidrug-Resistant Tuberculosis (MDR-TB) caused by Mycobacterium tuberculosis (Mtb) has spurred the necessity to find new antibiotics to treat tuberculosis. In our study, we employed an innovative 3R infection model using the amoeba and professional phagocyte Dictyostelium discoideum infected with Mycobacterium marinum as stand-ins for macrophages and Mtb, respectively. We used this model in a high-throughput phenotypic assay which enabled simultaneous assessment of a compound's impact on both the host and the pathogen[1].

To find novel anti-infective compounds, a registered diverse collection of 1600 plant extract samples (Pierre Fabre Library, PFL) was screened using this assay. In parallel, this collection was analyzed by untargeted UHPLC-high-resolution tandem mass spectrometry (UHPLC-HRMS/MS)[2]. The resulting data allowed us to turn this physical extract library into a virtual chemical space by generic systematic metabolites profiling of all samples. The metabolome was then used to try and correlate different bioactivity results at the extract level with specific chemicals in them.

One plant extract that showed high anti-infective activity in the screening was of Stauntonia brunoniana (roots). We then applied High-resolution HPLC micro-fractionation bioactivity profiling[3] to highlight Natural Products (NPs) responsible for the bioactivity of this extract. In the present case, all the entities detected in bioactive fractions were annotated as stilbenes.

The massive metabolomic data organized in the form of a knowledge graph (KG)[4] helped us to explore the virtual chemical space of all over 780'000 features in the collection of extracts[5]. Querying this KG to search for MS2 spectra (or features) similar to those from the bioactive stilbenes annotated in S. brunoniana facilitated the search for potentially stilbene-rich plant extracts. This query generated a list of 14 candidate extracts, that were investigated for the isolation of new and/or bioactive compounds. Targeted isolation yielded a total of 11 stilbene oligomers of different types (5 of which are newly reported NP structure). They were fully characterized by NMR and electronic circular dichroism (ECD) to establish their absolute stereochemistry. The anti-infective potential of these pure stilbenes was assessed in M. marinum infected amoeba. The dose-response of these compounds was also investigated in the aforementioned biological assay to determine their IC50 and revealed some anti-infective scaffolds with promising potential.

Based on this panel of confirmed structures, an estimation of stilbene amounts was attempted in the 14 shortlisted extracts using the semi-quantitative Charged Aerosol Detector (CAD) coupled with UHPLC-PDA-HRMS/MS. The objective was to establish if there was a correlation between the stilbene-content of an extract and its biological activity. This chosen example serves as a proof of concept for the use of a KG to integrate bioactivity and metabolomics, to efficiently identify bioactive NPs in collections of plant extracts.

SO-5

Characterization of Human Papillomavirus virus-like particles using iCIEF

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Human Papillomavirus (HPV) virus-like particles (VLPs) have become an important research tool due to their structural resemblance to the native virus and inherent lack of infectivity. Ensuring consistency between VLP batches is essential for reliable research data [1]. While traditional quality control (QC) methods such as ELISA, SDS-PAGE and electron microscopy can assess protein identity, purity and morphology, they may not reveal variations that could impact functionality [2].

This study aims to establish an imaged capillary isoelectric focusing (iCIEF) method as a complementary QC tool for HPV-VLPs to identify potential functional differences among HPV-VLP batches. A robust and reproducible iCIEF method was developed, specifically designed for intact VLP analysis. The method development involved optimizing parameters like the addition of non-ionic surfactant and urea, focusing time and voltage to ensure the integrity of the VLPs during analysis. Electron microscopy confirmed that the iCIEF method measured the isoelectric point (pI) of the intact VLP particles. The performance of the developed method was evaluated for repeatability and intermediate precision.

The pI value of several HPV-VLP batches was determined. Interestingly, one batch passed standard QC tests (SDS-PAGE, electron microscopy, and ELISA) but exhibited a distinct pI compared to other batches. Further investigation revealed that this batch showed altered binding properties to laminin 332, a receptor implicated in HPV infection. This finding suggests that iCIEF can detect variations that potentially impact the functionality of HPV-VLPs. Finally, the optimized iCIEF method was successfully applied to the analysis of a VLP-based vaccine after adjuvant removal.

This study establishes iCIEF as a valuable tool for in-depth quality control of HPV-VLPs. The ability of iCIEF to detect functional variations highlights its potential as a complementary technique. Integrating iCIEF into routine quality control procedures can help improve the consistency and functionality of HPV-VLPs.

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SO-6

Detailed characterization of monoclonal antibodies charge variants using capillary electrophoresis and tandem mass spectrometry

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Monoclonal antibodies (mAbs) used as therapeutic agents have become an indispensable tool in modern medicine and are knowing a huge growth in the pharmaceutical industry. The structural complexity of mAbs involves that their production and conservation must be strictly regulated. They can undergo post-translational modifications (PTMs) that may have a negative impact on their pharmacological properties. Thus, it is important to be able to characterize those modifications. Capillary zone electrophoresis (CZE) is a separation method particularly adapted for the analysis of mAbs charge variants, as they are visible and separated during the analysis. However, the selectivity provided by CZE in the case of intact mAbs is not completely understood. Mass spectrometry (MS) could be used in order to obtain structural informations about the PTMs linked to the separation selectivity provided by CZE.

In this work, we present the development of a novel analytical strategy based on CZE-UV separation of mAbs charge variants followed by systematic offline characterization of the variants CE-MS/MS. The analytical workflow consisted of collecting fractions of each separated charge variants of intact mAbs in CZE-UV. The separation and collection were repeated multiple times in order to enrich the fractions. Subsequently, each sample containing a type of charge variant was digested into peptides and characterized using CE-MS/MS analysis. To improve the identification, the mAb had also been submitted to proteolytic treatment (carboxypeptidase b and endoglycosidase) before a CZE-UV separation.

CZE-UV separation was realized to different types of mAbs demonstrating specific electrophoretic separation. Therefore, CZE-UV data enabled the confident identification of the nature of mAbs depending on their mobilities and natural charge variants distribution. In the case of infliximab, results demonstrated the possibility to distinguish unambiguously the innovator product Remicade and its biosimilars Remsima and Flixabi. Enzymatic treatment using carboxypeptidase b allowed to demonstrate the differences observed between biosimilars, corresponding to infliximab, were due to dissimilarities regarding C-terminal lysine residues. CZE-UV analysis of pembrolizumab (PBZ) exhibited peculiar charge variants distribution, presenting a total of 5 charge variants (2 basic, one main and 2 acidic variants) which could not be identified to be originating from C-terminal lysine residues and/or N-glycans. Based on mobilities observed for PBZ charge variants, fluidic calculations were applied in order to enable collection of the fraction corresponding to the separated variants. By separating the different forms of pembrolizumab with CE-UV, we could collect independent fractions from the 5 charge variants. Then, we characterized in a detailed manner the primary structure of the separated charge variants after proteolytic digestion into peptides followed by CZE-MS/MS. This method allowed the precise characterization of an important extend of PTMs to identify the position of the amino acid that could have undergo the modification. The attribution of the modification enabled to determine further the separation selectivity achieved using the CZE-UV method. Thus, it demonstrated the relevance of the electrophoretic separation to distinguish faint changes occurring on such complex macromolecules.

SO-7

Towards automated decision support in the analytical reliability assessment of biomarker candidates: a multicriteria optimization framework.

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Abstract

Introduction: Biomarkers are objectively measured and evaluated characteristics that serve as indicators of normal biological functions, pathogenic processes or pharmacological responses to therapeutic intervention (B. D. W. GROUP et al.,2001). Consequently, several validity criteria have to be carefully assessed upon identification (Morrow et al, 2007) in particular their specificity, sensitivity and reproducibility (González-Domínguez et al, 2024). During the discovery step, various indicators and approaches are commonly used to assess the quality of biomarker candidates in untargeted metabolomics experiments, most of which rely on variability estimators, such as coefficient of variation, and potentially some other analytical parameters, such as linearity (Theodoridis et al., 2012). However, in order to prepare the translation from discovery to validation, it is important to assess specific aspects of measurement quality, taking the overall complexity of metabolomic data. Given the various facets of candidate biomarker consistency, highlighting those that fully maximize measurement quality becomes a multi-criteria optimization problem. The aim of this work was to implement an optimisation framework for the multi-criteria classification of biomarker subsets based on their analytical quality.

Methods: The PROMETHEE model (Brans et al, 2016) was used as a basis for the optimisation framework. First, a survey was conducted among metabolomics experts to select relevant quality criteria and allocate initial weights estimate in the optimization process. A list of indicators was identified, which was then complemented by a literature review of the various methods currently used in metabolomics to define the quality of candidate biomarkers. On this basis, a subset of 10 criteria was selected to describe different dimensions of the analytical quality, namely linearity of the signal, mass/charge ratio variability, retention time variability, signal intensity, spectral purity, repeatability and reproducibility (i.e. coefficient of variation and newly developed indicators : intra batch dispersion, intra batch dispersion range, inter batch dispersion, dispersion ratio (SALANON et al., 2024)). Then, flexible optimization was carried out to adjust weights using a data-driven strategy. For that purpose, original data were transformed using weighted Principal Component Analysis model to avoid computational problems due to correlations, when the number of candidate biomarkers is higher than 10. If not, the initial weights were retained.

Results: The final classification results were found to be well-balanced between the different types of analytical criteria. The algorithm's performance was tested using several use cases in metabolomics, including the management of the analytical redundancy either from a single dataset or from a multiplatform approach, statistical redundancy (from biological correlation) and classification of biomarker candidates. While some results were fully in accordance with the metabolomics expert choices, other situations suggested a complementary viewpoint offered by the multicriteria optimization strategy.

Conclusion: Taken together, the results highlighted the benefits offered by including automated decision support to assess the analytical reliability of biomarker candidates. It opens the door to a more reliable and faster assessment of biomarker candidates optimizing their translation into current practices.
Key words: automation, multicriteria optimization, decision making, candidate biomarkers

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SO-8

Phage therapy: quality control challenges and advances

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Bacteriophages (phages) are viruses that specifically target and eliminate bacteria. They have been used as antimicrobial agents for over a century, notably in the former Soviet Union. Unfortunately, the differences between Soviet medical practice and reporting of clinical data and the current regulatory standards upheld by Western countries make it impossible for said countries to draw any conclusions about the safety and efficacy of phage therapy, without additional high-quality studies. However, due to the global increase in antimicrobial resistance, the potential of phages as a viable alternative treatment strategy has gained more interest in the last two decades, encouraging research in the field. In Belgium, more than a hundred patients have already benefited from phage therapy. Although the initial treatments were administered under the provisions of Article 37 of the Declaration of Helsinki (Unproven Interventions in Clinical Practice), the majority of patients received phage treatment thanks to a novel, dedicated regulatory framework established in 2018, known as "The Magistral Phage". This pragmatic personalized therapy approach allows the use of phage products as active pharmaceutical ingredients (API) in a magistral preparation, specifically designed for an individual patient. The process necessitates the certification of the product, which includes a genomic passport of the phage and a quality control (QC) of the product batch, by a laboratory recognized by the national health authority. Our presentation will delve into five years of experience with the QC of therapeutic phage products intended as API. We will illustrate the current flow chart of the certification process employed in Belgium, based on the findings from more than 60 batches of phage productions. The choice of approaches and methodologies for genetic analyses and pyrogenicity assessment will be discussed, and potential future developments, supported by data generated through ongoing research projects, will be addressed. Advances in technology and methodology, guided by regulations such as the upcoming general chapter 5.31 in the European Pharmacopoeia, will play a key role in refining QC processes. Ultimately, this will enhance the reliability of phage therapy products, paving the way for their wider acceptance in the medical community.

PS01-01

A simple LC-MS/MS assay of E6011, a novel anti-fractalkine human monoclonal antibody, in monkey serum - comparison with ligand binding assay -

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As a growing number of therapeutic antibodies are on the drug development pipelines and are on the market recently, it is required to establish a robust assay for the determination of therapeutic antibodies. Although ligand binding assays (LBA) have been typically used for evaluating pharmacokinetics (PK) of therapeutic antibodies, we have developed a simple assay for quantifying a therapeutic antibody in cynomolgus monkey serum by liquid chromatography with tandem mass spectrometry (LC-MS/MS) using E6011, an anti-fractalkine human monoclonal antibody, as a model drug.

To find signature peptides of E6011, in silico search by the Skyline software indicated that TLADGVPSR from light chain of IgG2 is a promising candidate. Procedures in denature, reduction, alkylation, and tryptic digestion were optimized. Monkey serum samples were processed with ammonium sulfate and were fortified by a stable isotope labeled signature peptide as the internal standard (IS). The peaks of the signature peptide and the IS in processed pellets were separated on a C18 reverse phase column under a gradient elution. E6011 in monkey serum was quantifiable from 3 µg/mL without any selectivity issues in individual sera. Relative error and relative standard deviation of quality control samples at four concentrations in the reproducibility study were within ±15% and 15%, respectively, which ensured assay reproducibility. The LC-MS/MS assay of E6011 was then applied to a monkey PK study. Serum E6011 concentrations in monkeys after a single intravenous administration of E6011 at 1 mg/kg were determined. E6011 concentrations were determined by the LC-MS/MS and an LBA with electrochemiluminescence detection. Comparison of PK results by the two assay platforms (LC-MS/MS vs LBA) will be also shared.

PS01-02

Analytical developments towards new metrological references for the quantification of testosterone, aldosterone and 17β-estradiol in human serum.

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Steroid hormones are blood biomarkers used for the diagnosis and therapeutic monitoring of patients. A slight variation of these hormones, in the range of ng/mL or even pg/mL, can either indicate the development of diseases (e.g. tumors, reproductive disorders, Down syndrome during pregnancy) or exposure to endocrine disruptors. Therefore, it is crucial to have robust, specific and accurate methods at these concentration levels. However, external quality assessment programs (e.g. College of American Pathologist Accuracy-based Programs) have revealed bias and variability in the

measurement results at low concentration levels for some hormones (e.g. concentration <15 pg/mL for 17β-estradiol). Thus, there is a need to improve the quality and standardization of steroid hormone assays, as highlighted by the Center for Disease Control and Prevention program aiming to standardize measurement of 17β-estradiol and testosterone. One way to guarantee the reliability of diagnostic test results is to establish metrological traceability of results to the International System of Units (SI), through the development of reference methods and reference materials. Reference materials exist for testosterone and 17β-estradiol, but the concentrations are too high (e.g., 31 pg/mL for 17β-estradiol). Dedicated reference methods for testosterone and 17β-estradiol also exist, respectively, but they are not multiplex.

In the present work, we first performed a literature review to select a list of hormones with clinical relevance and for which there is a need for measurement quality. The number of annual biological tests was also assessed to determine which steroid hormones are the most commonly monitored. Three steroid hormones have been selected: aldosterone, testosterone and 17β-estradiol, and the targeted LOQs in the serum matrix have been set at 10 pg/mL for testosterone and 5 pg/mL for aldosterone and 17β-estradiol, with uncertainties below 10%.

Then, a sample preparation and analysis method using the techniques of isotopic dilution LC-MS/MS was optimized with the aim of achieving the targeted LOQs. For this purpose, extraction and re-concentration methods were developed specifically for the three hormones. Also, a LC-MS method previously developed in the laboratory was upgraded by transferring the analysis to a new instrument (XEVO TQ-S micro, Waters), changing the column (biphenyl vs C18 phase), the additive of the mobile phase (acetic acid vs ammonium fluoride) or adapting the gradient. The LOQs are currently estimated at 35 pg/mL for testosterone, 6 pg/mL for aldosterone, and 60 pg/mL for 17β-estradiol. To reach the target LOQs, modifications of the sample preparation method is considered on-going, such as the use of molecular imprinted polymers (MIP) for the extraction, or increasing the volume of initial human serum sample intake.

At the same time, interferences were investigated to assess the method’s specificity. Following the literature review that provided a list of potentially interfering molecules, the gradient was further optimized to separate the testosterone from its isomers, the epitestosterone and the DHEA, and the 17β-estradiol from the 17α-estradiol. Subsequently, analyses on a high-resolution Orbitrap mass spectrometer (QExactive Focus, ThermoScientific) have been performed on several matrix samples to demonstrate that no additional interference is observed for the three molecules of interest.

PS01-03

Affinity selection- mass spectrometry for Identifying Ligands of Acetylcholinesterase from Topsentia ophiraphidites and Docking Studies for the Dereplicated Ligands

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Acetylcholinesterase (AChE) inhibition has been successful used as a cholinergic strategy to treat Alzheimer’s related cognitive decline and still stands as an important target [1]. In this context, affinity selection-mass spectrometry (AS-MS) has been acknowledged as a high-throughput screening (HTS)

technique for prospecting ligands from natural product libraries [2,3]. In this work, an AS-MS assay with AChE immobilized onto magnetic beads (AChE-MB) has been used to search for AChE ligands in samples of the sponge Topsentia ophiraphidites collected in the archipelago of Fernando de Noronha, Brazil. Ligand dereplication disclosed 6-desmethyl-6-ethyl-9,10-dihydrospongosoritin A and 3,5-dibromo-O-methyltyrosine, while 3-bromo-5-iodo-O-methyltyrosine and 3,5-di-iodo-O-methyltyrosine were directly annotated from the GNPS network. Affinity Ratio (AR) towards AChE showed little difference among the halogenated ligands. In addition, molecular docking analysis with human AChE enhanced important π-alkyl and π-π stacking intermolecular interactions of the (R, R) stereoisomer of 6-desmethyl-6-ethyl-9,10-dihydrospongosoritin A and the Peripheral Anionic Site (PAS) of AChE. Among the halogenated substances, there were similar interactions with AChE, in accordance with AR results. Altogether, this study stimulates further investigations of these ligands in other assays to elucidate their mechanism of action.

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PS01-04

mRNA/LNP Multiattribute Quantitation of Payload(s), Size and Heterogeneity With Size Exclusion Chromatography Coupled to Multiangle Light Scatteringps

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Cell and gene therapies offer an opportunity for curing previously intractable diseases. However, bringing increasingly complex therapeutics to patients comes with a formidable challenge of ensuring their safety and efficacy. These drugs are multicomponent ensembles (carrier + payloads), which means there are multitude critical quality attributes (CQAs) that must be monitored at each stage of their development – creating bottlenecks and delaying the process [1]. High-throughput analytical methods that permit multiattribute measurements constitute a potential solution to this issue. Among the most efficient are chromatographic separations that can be coupled to advanced detectors, as with size exclusion chromatography paired with multiangle light scattering (SEC-MALS), which is of growing importance to emerging new modalities (AAVs, mRNA/LNPs etc.) [2, 3].

Significant attention is being placed on creating fit-for-purpose SEC methods for lipid nanoparticles (LNPs) and their payload drug substances. Optimized widepore, low adsorption SEC columns (>450Å) have been developed such that new techniques can be envisaged. Past investigators have applied a positive surface potential gel permeation resin to perform LNP size exclusion chromatography, which may bias the analysis. Here, we have alternatively investigated non-ionic, hydrophilic packing materials for SEC of LNPs and their impact on analyte recovery. We have compared the recovery of several small to medium sized LNP materials under different conditions and performed MALS mediated size, mass and heterogeneity measurements.

Moreover, we developed and optimized conditions for a complete online disruption of the LNP carrier which enables a direct measurement of the nucleic acid payload(s) with a 2 ng as the limit of detection (LOD). We show that efficient size based separation is crucial for highly accurate and linear dual payload quantitation of gene editing therapy oriented LNPs containing both mRNA and gRNA. Various denaturing agents and LNP formulations were investigated to ensure universality of the disrupting conditions and the creation of a robust platform method.

In summary, we demonstrate the versatile use of SEC-MALS to extract multiple CQAs [4] (purity related e.g. RNA integrity or presence of process related impurities or strength related such as mRNA content) measurements for LNP RNA drug products and drug substances. With its deployability, SEC-MALS is coming to establish a cost and time-effective means to comprehensively characterizing a range of new nucleic acid therapies.

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PS01-05

Vizualisation of the bivariate dispersion structure for the robust assessment of the repeatability and reproducibility of analytical measurements.

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Abstract: Introduction: Assessing repeatability and reproducibility in analytical chemistry is commonly based on dispersion indicators, calculated for each detected variable using repeated measurements of Quality Control samples (QC) collected throughout the data acquisition sequence. Knowing that analytical variability is conditional to many sources, different problem affecting the results can occur during the measurement process. However, these events are often poorly described by classical dispersion indicators, such as standard deviation, and the aim of this study was to develop a visualization method to better capture and understand their dispersion structure, both within and between analytical batches.

Methods: We consider a Euclidean space defined by a plane made of the “measurement order” (X-axis) and the “intensity” (Y-axis) of the analyte signal. Given a finite set of QC repeated measurements S = {S_1, …..,Sn} where the coordinates of S_1 are (x_1,y_1), there is a unique convex hull CH(S) of S in the plane, defined as the smallest convex set that contains all data points S. The proposed visualization takes advantage of this axis system to display the convex hull of the intra and inter-batch(es) dispersion structures for a given feature allowing then to describe its variability more efficiently. The relevance of these indicators and the associated visualization methods were highlighted based on simulated data and a metabolomics case study involving liquid chromatography coupled to mass spectrometry

measurements of the NIST SRM1950 reference material analyzed on the same instrument over more than one year within different projects.

Results: The visualization method allowed the potential drift within each batch to be reliably described along the sequence. The distribution of the dispersion computed from simulated data, with different types of bias (additive, multiplicative) showed that the structure of the bivariate dispersion remained unchanged as far as the bias was constant. Moreover, , the visualization of real experimental data involving non-constant bias revealed different types of structures with various shapes. In practice, observing this difference could lead to detect problems occurring during the data acquisition process. Moreover, this approach allowed the dispersion to be investigated at different time points, which is of particular interest in the assessment of the system suitability. This visualization tool can also be used to automatically highlight common behaviors between analytes.

Conclusion: The proposed visualization method, allowed an efficient evaluation of the structure of variability, both within and between analytical batches. Combining this visualization method with different indicators will contribute to reveal potential issues in the sequence and make diagnostic for further choice of an adapted correction method.

Key words: Reproducibility, Precision, LC/MS, Dispersion, Drift

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PS01-06

Enhancement of LC-MS analysis of intact glycoprotein

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Glycosylation is one of the common protein modifications that regulates their biological properties. For certain diseases, its characterization enables diagnosis and prognosis. The conventional approach for the glycosylation characterization is based on the analysis of the glycopeptides obtained after the enzymatic digestion of the glycoforms of a given glycoprotein. It is also possible to analyze the intact glycoforms using liquid chromatography coupled with mass spectrometry[1]. This approach preserves crucial information, namely the link between the structure and the activity or the number of glycoforms.

For several years, our focus has been on human chorionic gonadotropin (hCG), better known as the pregnancy hormone. hCG consists of two non-covalently linked subunits, hCGα and hCGβ. It has eight glycosylation sites, resulting in numerous glycoforms, some of which could serve as biomarkers of pregnancy-related pathologies. We demonstrated that the analysis of hCG glycoforms is possible keeping the protein intact using nano liquid chromatography (nanoLC) coupled with high-resolution mass spectrometry (HRMS) featuring an Orbitrap analyzer[2]. NanoLC enhanced sensitivity and its coupling with HRMS led to the detection of more than seventy different glycoforms of hCGα and hCGβ in concentrated hCG samples (fertility drugs containing recombinant hCG or purified from women's urine). The impact of two different acidic additives, namely formic acid (FA) and trifluoroacetic acid (TFA), into the LC mobile phase was investigated. It was found that only TFA enabled the separation of hCGα and hCGβ glycoforms in reversed-phase liquid chromatography (RPLC). However, even at low concentrations, TFA induced a strong signal suppression and led to the formation of numerous adducts, which considerably complicates the interpretation of MS data[3].

It thus, became necessary to investigate the effect of other additives on the separation (resolution, efficiency) and detection (intensity, adducts formation) of hCG glycoforms in nanoRPLC-HRMS. Chemically closed to TFA, difluoroacetic acid (DFA) has been evidenced to combine the same separation properties while increasing MS sensitivity[4]. Nevertheless, for hCG glycoforms, DFA adducts were still detected. Using the "all ion fragmentation" (AIF) mode of the Orbitrap we succeeded to drastically limit these adducts. The "On/Off" elution properties of proteins were highlighted in the study of monoclonal antibodies[5]. The implementation of a gradient comprising multiple isocratic elution steps showed an improvement in separation. We aimed to evaluate the performance of such a step gradient with the goal of improving the separation of the hCG glycoforms. It was found that separation of the hCGα glycoforms as a function of their composition is possible through successive isocratic elution steps, each having a precise elution strength, i.e. a precise percentage of acetonitrile content in the LC mobile phase. This improved separation further reduces the ion suppression effect, potentially revealing new low-intensity glycoforms not previously detected.

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PS01-07

Development and validation of a High-Performance Liquid Chromatography coupled Mass Spectrometry (HPLC-MS/MS) method for ABT-263 quantification in two different biological matrices

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Context: Cancer treatment remains a critical challenge in modern medicine, particularly in prostate cancer. Conventional methods such as chemotherapy and radiotherapy can induce cancer cells into a dormant state known as cellular senescence, contributing to treatment resistance and recurrence. To address this, senolytic drugs like ABT-263 (Navitoclax®) are investigated to target senescent cells as a second-line treatment. ABT-263 is a powerful BCL-2 inhibitor, which bind to and inactivate anti-apoptotic proteins that are overexpressed in prostate senescent cells.

Objective: This project aims to develop and validate a bioanalytical method for quantifying ABT-263 in various matrices, facilitating subsequent in vitro and in vivo studies.

Methods: A liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed to quantify ABT-263, adhering to ICH M10 guidelines on bioanalytical methods validation. Parameters such as chromatographic column, mobile phase composition, and M/Z transition were optimized. Validation encompassed sensitivity, selectivity, matrix effects, recovery, accuracy, precision, linearity, carry-over, dilution integrity, and stability. These assays were replicated in culture media and mouse plasma to mimic the conditions of upcoming in vitro and in vivo studies.

Results: Method development revealed unforeseen, non-specific binding to plastic and glass surfaces, participating in significant carry-over effects in the method. To mitigate this, the concentration range was narrowed to 5-500 ng/mL, despite high sensitivity (<< 1 ng/mL) and wide linearity range (1-5000 ng/mL). The adjusted method met ICH criteria for carry-over, sensitivity, and selectivity. Accuracy (93.1% - 109%), precision (CV ≤ 13.3%) and linearity (R2 ≥ 0.9992) were demonstrated in both matrices. Quality control samples preparation was precise (89.7% - 98.2%) and accurate (CV ≤ 10.6%). Matrix effects and recovery had relative standard deviations below 15%. Dilution integrity was confirmed up to 10-fold in culture media (91.6% - 96.9%) and plasma (101.1% - 111.1%).

Discussion: Four LC-MS/MS methods for ABT-263 were published in the literature, all using human plasma or urine as matrices. Therefore, our method was validated using two matrices that has never been used for this drug. Furthermore, despite employing a less sensitive mass spectrometer than other research teams, we successfully validated a method that demonstrates equal or superior sensitivity compared to those previously published. Also, our approach demonstrated improved robustness by incorporating isopropanol into the organic mobile phase.

Perspectives: Successful validation of this bioanalytical method for ABT-263 in culture media and mouse plasma is crucial for initiating subsequent in vitro and in vivo studies. These studies aim to explore combining frontline treatments with senolytic drugs to potentially reduce cancer recurrence associated with cellular senescence.

PS01-08

Electrochemical Biosensors: Practical Solutions for Microorganism Identification

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Various detection technologies are available for identifying pathogens, broadly categorized into methods targeting either the whole microorganism or its metabolites. These encompass general approaches like microscopy and culture-based techniques, as well as specific assays such as biochemical tests, Polymerase Chain Reaction (PCR), DNA sequencing, Fluorescence in situ Hybridization (FISH), Enzyme-Linked Immunosorbent Assay (ELISA), Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), and Next-Generation Sequencing (NGS). Electrochemical biosensors stand out among these techniques due to their speed, sensitivity, and cost-effectiveness. Biosensor methodologies hold promise for detecting pathogens in diverse media like blood, serum, urine, food, and water, with minimal sample pre-treatment, thereby simplifying detection processes. Detection of pathogens using electrochemical biosensor systems offers a powerful tool for rapid and sensitive detection (1). These systems utilize electrochemical signals to determine the presence of specific pathogens, typically comprising electrodes coated with bioreceptors to detect a particular target pathogen. The advantages of using electrochemical biosensors for pathogen detection include high sensitivity, rapidity, specificity, and portability, offering them suitable for various applications such as food safety, medical diagnostics, environmental monitoring, and bioterrorism prevention. Additionally, the efficient design of electrochemical biosensors facilitates straightforward integration into portable devices, enhancing their accessibility and usability in field settings or remote locations. Molecularly Imprinted Polymers (MIPs) within electrochemical biosensors play a crucial role, serving as a vital link between selective molecular recognition and efficient detection. These synthetic polymeric structures are intricately designed with

binding sites precisely tailored to mimic the shape, size, and chemical characteristics of target molecules, such as bacteria or other pathogens. Functioning as recognition elements, MIPs exhibit remarkable selectivity within electrochemical biosensors, effectively capturing and binding the target molecules (2). In this presentation, various electrochemical-based detection methods for microorganisms such as bacteria and fungi will be addressed. Methods will also be discussed for distinguishing bacteria with highly similar genetic structures from each other through electrochemical techniques mediated by MIPs. Although electrochemical methods offer significant potential and numerous advantages, there have been relatively few publications documenting their application in fungi detection thus far. In this discussion, we will also explore the detection of fungi with the assistance of biosensors.

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PS01-09

Transacylation and hydrolysis of the acyl glucuronides of ibuprofen and its α-methyl-substituted analogues investigated by 1H NMR spectroscopy and computational chemistry: Implications for drug design.

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Drugs and drug metabolites containing a carboxylic-acid moiety can undergo in vivo conjugation to form 1- β-O-acyl glucuronides (1-β-O-AGs). In addition to hydrolysis, these conjugates can undergo spontaneous acyl migration, and anomerisation reactions, resulting in a range of positional isomers. Facile transacylation has been suggested as a mechanism contributing to the toxicity of acyl glucuronides, with the kinetics of these processes thought to be a factor. Previous ^{1}H NMR spectroscopic and HPLC-MS studies have been conducted to monitor the degradation rates of the 1-β-O-AGs of three nonsteroidal anti-inflammatory drugs (ibufenac, R-ibuprofen, S-ibuprofen) and a dimethyl-analogue (termed here “bibuprofen”). These studies have also determined the relative contributions of hydrolysis and acyl migration to their reactivity in both buffered aqueous solutions and human plasma. Here, a detailed kinetic analysis is reported, providing the individual rate constants for the acyl migration and hydrolysis reactions observed for each of the 4 AGs, together with the overall degradation rate constants of the parent 1-β-O-AGs. Computational modelling of the reactants and transition states of the transacylation reaction using density functional theory indicated differences in the activation energies that reflected the influence of both substitution and stereochemistry on the rate of transacylation/hydrolysis

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PS01-10

Absolute quantification and structural characterization of therapeutic monoclonal antibodies after administration to patients using capillary electrophoresis-tandem mass spectrometry

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Monoclonal antibodies (mAbs) are demonstrating major successes in various therapeutic areas like oncology or immune disease treatment. However, insights regarding their evolution after administration to the patient remain limited. For instance, Infliximab (IFX) is a chimeric monoclonal antibody (mAbs) approved mainly for the treatment of Crohn's disease. Because IFX is administered repeatedly over extended period of times for the treatment of a chronic pathology, it is important to monitor its concentration in patient serums in order to adjust the treatment if necessary. Thus, the clinical practice indicated significant differences of IFX clearance among patients and in some cases unexpected responses like the persistence of clinical symptoms or the expression of anti-drug antibodies, without providing any tangible interpretation. Patients follow-up is currently performed solely through quantification using ELISA immunoassay. Moreover, post-translational modifications (PTMs), which may impact IFX activity, cannot be characterized using this technique.

We developed a novel analytical strategy based on capillary electrophoresis hyphenated to tandem mass spectrometry (CE-MS/MS) for the absolute quantification and concomitant structural characterization of IFX in human serum. A dedicated serum purification process was designed to provide optimal sensitivity and compatibility with the CE-MS/MS analysis. Purified IFX peptides obtained from tryptic digestion were separated and characterized by CE-MS/MS. Regarding PTMs characterization, an original normalization was developed to estimate modification levels occurring strictly during IFX residence time inside the system of the patient.

CE-MS/MS method demonstrated the successful quantification of IFX in spiked serum for concentration ranging from 0.4 to 25 µg/mL. CE-MS/MS absolute quantification was compared to ELISA assay showing a good correlation between the two techniques, whereas ELISA exhibited systematic biases highlighting the occurrence of uncontrolled matrix effects.

Structural characterization of IFX was performed simultaneously using the same dataset. CE-MS/MS data allowed to successfully characterize the structures of six major N-glycosylation and establish a detailed glycoprofiling of IFX in serum samples. Also, six PTMs of interest, including asparagine deamidation and aspartic acid isomerization, were precisely characterize regarding localization and modification levels.

CE-MS/MS analytical strategy was applied to serum samples originating from 24 patients treated for Crohn's disease using IFX. CE-MS/MS results demonstrated the robust quantification and structural characterization of IFX provided by the method. Results exhibited an important disparity regarding the evolution of IFX concentration after administration between the different patients. Results showed the possibility to identify faint differences of IFX glycoprofilling for patient samples that highlighted the possibility to identify IFX glycoforms presenting faster serum clearance. Finally, CE-MS/MS data

obtained from analysis of patient samples demonstrated a gradual Asp57 deamidation during IFX residence time in the patient's system that was not described in the literature. This residue is located in the region of IFX directly interacting with TNF-α, and therefore the modification may alter the activity of IFX. The study therefore illustrated the possibility provided by CE-MS/MS to achieve an additional dimension of characterization regarding the outcome of mAbs after administration, which appears to be crucial to understand the impact on the clinical response or the apparition of adverse effects.

PS01-11

Qualitative and quantitative evaluation of the metabolism of various drugs for five types of CYP2D6 SNPs

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The behavior of drugs within the human body remains a crucial research indicator for predicting the efficacy and side effects in individual patients. The drug-metabolizing enzyme that has the most significant impact on drug metabolism is cytochrome P450 (CYP). Genetic polymorphism is most frequently observed in CYP2D6, with over 200 genetic variations identified. It is known that about 25% of drugs used in clinical practice are metabolized by CYP2D6. In this study, we established a cell line expressing CYP2D6 SNPs (i.e., CYP2D6. *10, *14, *21, *49, and *56) using HEK293FT cells to assess alterations in metabolism and toxicity associated with CYP SNPs. We conducted qualitative and quantitative analysis of metabolites using mass spectrometry by developing cell lines that express CYP2D6 SNPs and isolating and purifying CYP SNP microsomes. Among the SNPs, fivetypes (i.e., CYP2D6. *10, *14, *21, *49, *56) exhibited a significant decrease in intrinsic clearance compared to CYP2D6 1. Our research results provide insights into inter-individual variability in new drug candidate groups and precision medicine applications in predicting hepatotoxicity and drug metabolism changes caused by SNPs, in drug development stages.

PS01-12

The investigation of structural characteristics of biologically active natural polymers using solid-state NMR experiments

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The naturally occurring polymers discovered in widespread plants, such as Symphytum grandiflorum DC (creeping comfrey) and Symphytum asperum (prickly or rough comfrey), show biological activity towards human hyaluronidase Hyal-1, which is one of the most important enzymes in the metabolism of hyaluronic acid (HA). The detailed study of these poorly water-soluble polymers requires characterization of their structural properties and improvement of their solubilities. The latter question remains problematic so far. However, the structures of the polymers can be accessed using NMR spectroscopy. We conducted 1H liquid-state and the 13C solid-state NMR experiments. The recorded 1H NMR spectra are confirmed by the 13C solid-state NMR results. Two different experiments were chosen to assign the 13C signals to the respective atoms of the monomer structures: The 13C{1H}

cross-polarization (CP)/MAS NMR experiment and a dipolar-dephasing solid-state NMR pulse sequence. The 13C{1H} (CP)/MAS NMR experiment is a routine experiment for spectral assignment of carbon signals. The advantage of the CP/MAS experiment over the directly excited 13C MAS NMR experiment is an intensity improvement (of up to a factor of four) in addition to a faster recycling delay. A dipolar dephasing solid-state NMR pulse sequence allows the distinction between the proton-bound carbon atoms and the carbons that are not directly bound to protons. The resulting 13C{1H} CP/MAS NMR spectra are in accordance with the expected structures of both polymers. The determination of the molecular weight of Symphytum Asperum was performed by size exclusion chromatography (SEC).

PS01-13

Exploring Chiral Recognition: Docking Simulations and Experimental Validation on chiral Polysaccharide-based Stationary Phases

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Chirality plays a fundamental role in pharmaceuticals and bioactive compounds, often influencing their pharmacological properties. In this study, we employed docking simulations to investigate the retention behavior and elution order of various chiral molecules, including the pharmaceutical Thalidomide, on five polysaccharide separative chiral columns. These molecules were specifically designed as potential RAGE (Receptor for Advanced Glycation Endproducts) ligands, offering a unique platform for chiral separation. To validate our docking studies, two additional molecules and the enantiomers of Thalidomide were screened, further elucidating their elution patterns. The findings within this work underline the complex interactions governing chiral recognition and could offer insights into the development of efficient chiral separation methods for pharmaceutical applications. Importantly, our findings suggest that this method could save time and resources by avoiding complex experiments and reducing the need for certain chemicals, thus aligning with green and sustainable practices

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PS01-14

Electrochemical biosensor designing for investigation of the interaction between DNA and Ciprofloxacin

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Ciprofloxacin (CF) is a broad-spectrum fluoroquinolone first introduced in the 1980s. The chemical name of SF is 1-cyclopropyl-6-fluoro-4-oxo-7- (piperazine-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (Figure 1) and it is a second-generation fluoroquinolone derivative that is frequently used in medicine and veterinary medicine. CF inhibits bacterial DNA gyrase IV and stabilizes the DNA-topoisomerase-drug complex, resulting in bactericidal and bacteriostatic effects. Moreover, treatment with SF increases the release of ROS, which contributes to its overall bactericidal effect. CF shows activity against gram-negative bacteria, some gram-positive bacteria (including Staphylococcus aureus but not

Streptococcus pneumoniae), and some atypical pathogens. SF; It has been approved by the FDA to be used in the treatment of urinary tract infections, sexually transmitted infections (gonorrhea and chancroid), skin, bone, and joint infections, prostatitis, typhoid fever, gastrointestinal infections, lower respiratory tract infections, anthrax, plague and salmonellosis. It is also used against SARS-CoV-2 in the pandemic that affects the world. Regarding its use in human or animal treatment processes, it is likely to be detected in natural sources in environmental analysis.

In this study; an electrochemical biosensor was designed for interaction between fluoroquinolone antibiotic CF and DNA (dsDNA and ssDNA) sequences by using pencil graphite electrode (PGE). Consequently, the developed biosensor provides a suitable stage for the analysis of CF-DNA interaction sensitively. In the present study, the interaction of the antiviral drug was investigated with the DNA-modified electrodes. The binding mechanism of the antiviral drug CF with dsDNA and ssDNA was determined by using electrochemical voltammetry methods. For this purpose, DNA-modified electrodes were prepared and interacted with CF then measured by electrochemical analysis. In summary, it was shown that the label-free detection of CF–DNA interaction could be done with sensitive, faster, and less laborious techniques by using disposable electrochemical biosensors.

PS01-15

Enhancing microflow LC-MS/MS analysis of neuromedin U through reduced aspecific adsorption

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Neuromedin U (NmU) is a 2.7 kDa neuropeptide belonging to the neuromedin family and is highly conserved throughout mammalian species, making it a great candidate for investigating its preferential release in (neuro)pathological conditions. Major challenges are faced when trying to quantify neuropeptides in mouse brain extracellular fluid, since these peptides are usually present at concentrations in the low picomolar to femtomolar ranges. In vivo sampling techniques, such as microdialysis, usually provide very low volumes, necessitating highly sensitive methods. Miniaturized ultra-high-pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) is potentially suited for this purpose. However, sensitivity can still be an issue due to aspecific adsorption, a phenomenon where the molecules of interest bind non-specifically to various surfaces during analysis, impeding accurate quantification. These surfaces include tubes, vials, pipette tips and various parts of the LC system. Therefore, it is necessary to minimize aspecific adsorption during every step of the method, by using appropriate solvents, materials and LC conditions.

In this study, a UHPLC-MS/MS method to quantify NmU was designed using a Waters AQUITY UPLC M-class system equipped with a CSH C18 (130Å, 1.7µm, 150µm x 50mm) iKey separation device coupled to a Xevo TQ-XS mass spectrometer in multiple reaction monitoring (MRM) mode. During method development, at the level of sample preparation and analysis, different approaches to combat aspecific adsorption were investigated.

Before injecting samples, the system was passivated with a 40 µg/mL bovine serum albumin (BSA) solution. BSA acts as an adsorption competitor to NmU, saturating the non-specific sites in the system.

This step was crucial for obtaining sufficient sensitivity. Varying compositions of the solvents, used to dissolve lyophilized NmU standard and perform further dilutions, were tested. The highest responses were obtained using water/acetonitrile (ACN)/formic acid (FA) (70/30/0.1 v:v:v) as dissolution and dilution solvent. By adding 10% (v:v) of both ACN and FA to the UHPLC vial before injection, signal intensity was further increased.

Next, the UHPLC conditions (mobile phase gradient, column temperature, trapping flow rate, trapping temperature and trapping duration) were investigated to further improve sensitivity and to minimize carryover. A gradient with an initial organic modifier fraction of 5% in the mobile phase while keeping column temperature at 45°C resulted in the best signal. Trapping, using a Symmetry C18 trap column, at a flow rate of 10 µL/min and a trapping volume of 30 µL, resulted in the highest signal without significant carryover.

The limit of detection of this optimized method was estimated in the 100 pM range with standard solutions prepared in water. Lower peak areas were consistently found for spiked microdialysis samples, indicating that further method optimisation is necessary before NmU can be accurately quantified in in vivo samples.

PS01-16

Study on pesticides contamination in Chinese Proprietary Medicine (CPM) products retailed in Singapore

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Chinese proprietary medicines (CPM), being natural in nature, have been perceived by the public as relatively low risk. However, they are not completely free from the possibility of toxicity or other adverse effects. Potential harm can occur due to inherent toxic properties of the herbs, as well as contaminants or adulterants in the CPM. Pesticides, used in herb cultivation and storage, can persist as residues in these products, leading to contamination in herbal complementary health products (CHPs). Notably, organochlorine pesticides, banned in the United States and Europe since the 1970s due to their carcinogenic and genotoxic effects, have raised significant health concerns, including seizures, dementia, and endocrine disruption potential.

A study on pesticide contamination for a range of products sampled from Singapore’s market was initiated by the Health Products Regulation Group (HPRG) in Health Sciences Authority in 2023. In this study, 50 different CHPs were sampled from the local market, comprising 10 Chinese Proprietary Medicines (CPM), 20 Traditional Medicines (TM), and 20 Health Supplements (HS). A total of 22 common pesticides (7 organochlorine, 12 organophosphorus, and 3 pyrethroid) were screened from these samples. The results of this study have been presented in this poster; these findings would be useful in facilitating the review of compliance of these products with respect to pharmacopeia standards.

PS01-17

Clinical Calcium Ion Analysis with Isomeric Calcium Ionophores in Ion-Selective Electrodes

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Calcium ions are crucial in numerous physiological processes and their precise measurement is significant for various clinical diagnostics and therapeutic interventions. Ionophore-based ion-selective electrodes (ISEs) provide a convenient, accurate, and specific method for Ca2+ determination in clinical diagnostics. As one of the most popular and widely used calcium ionophores, Calcium Ionophore I (Ca I), also called ETH 1001, was introduced some 50 years ago, addressing the limitations of earlier ionophores by a significantly enhanced selectivity over alkali metal cations.

In this work, two calcium ionophores, Calcium Ionophore I, (R, R)-Calcium ionophore I (diethyl (17R,18R)-13,17,18,22-tetramethyl-14,21-dioxo-16,19-dioxa-13,22-diazatetratriacontanedioate), and its isomeric form, (R, S)-Calcium ionophore I (diethyl (17R,18S)-13,17,18,22-tetramethyl-14,21-dioxo-16,19-dioxa-13,22-diazatetratriacontanedioate), were compared. The seemingly subtle structural change was found to give an important influence on the resulting stability of the calcium-ionophore complex and the selectivity of the resulting membrane electrode. This was evidenced by thin layer transfer voltammetry, a technique that allows one to assess binding constants, complex stoichiometries and selectivities. This is achieved by reducing the membrane film thickness to eliminate mass transport limitations and the use of lipophilic TEMPO as novel lipophilic redox probe. The complex stoichiometry was confirmed to be 1:2 by changing the ionophore to ion-exchanger ratio in the film.

The comparative performance of the two isomeric ionophores were further assessed by incorporating them in the Eaglenos Blood Gas Analyzer (model: EG-i30) containing the EG10+ test cartridge containing appropriately formulated screen-printed ISEs (https://www.eaglenos.com/en/). The cartridges were evaluated in whole blood samples and directly compared to the commercialized instrument data, achieving an average bias of -2.2% for the original ionophore and -7.4% for its isomer. The isomeric form was found to give unsuitable bias data, suggesting the presence of undesired matrix effects.

PS01-18

Unveiling the potential of ion-pairing RPLC for mRNA characterization

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The SARS-CoV-2 pandemic shed lights on the use of mRNA as effective vaccine modality, accelerating new developments for prophylactic and therapeutic applications. With this rapidly emerging class of therapeutic drugs, comes the need to develop robust methods to properly characterize the in vitro-produced mRNA samples.

Ion-pairing reversed phase liquid chromatography (IP-RPLC) presents significant promise in this regard. This technique takes profit of the mRNA negatively charged backbone that complexes with the positively charged ion-pairing agent added to the mobile phase. The mRNA-ion pair complex displays an increased affinity to the stationary phase compared to the mRNA alone, resulting in enhanced retention on a C18 type column. This allows separating mRNAs based on both their size and composition.

IP-RPLC has been extensively studied for small oligonucleotides analysis but has been poorly evaluated for analyzing larger RNA. In this context, we explored in a systematic way the effect of a wide range of ion-pairing agents of different hydrophobicity on the retention and selectivity of RNAs between 200 and 6000 nucleotides in lenght. The ion-pairing agents were evaluated alone or in combination, at various temperatures, pH, and concentrations. Eventually, the optimal conditions will be applied on real mRNA-based drug substances. By highlighting key parameters for a proper use of ion-pairing agent in RPLC, our work lays the foundation for improved nucleotide analysis and is bound to facilitate the comprehensive characterization of newly produced mRNA.

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Camille Malburet and Marc Francois-Heude are Sanofi employees and may hold shares and/or stock options in the company. Jonathan Maurer and Davy Guillarme declare no competing interests.

PS01-19

LC-MS/MS determination and pharmacokinetic study of selected breast cancer drugs in clinical practice

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Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors palbociclib (PAL), ribociclib (RIB), and abemaciclib (ABE) have been introduced for the treatment of hormon-dependent breast cancer, in combination with endocrine therapy anastrozole (ANA), letrozole (LET) or fulvestrant (FUL). Their pharmacokinetic (PK) data is actively collected and evaluated in clinical practice, to elucidate possible causes of inter-individual differences in efficacy and toxicity, track adherence, and evaluate strategies for therapeutic drug monitoring (TDM) [1].

In this work, plasma samples from 13 patients treated with different combinations of the drugs of interest were collected in multiple timepoints after dose administration. The analytes were quantitated using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and non-compartmental PK analysis was conducted to estimate the maximal concentration (Cmax), time to reach Cmax (Tmax), elimination half-life (T1/2), 1st order elimination constant (ke), trough concentration (Cmin), and area under the 24-hour concentration-time curve (AUC0-24). The obtained results were compared to the published literature values and target ranges [1]. The PK parameters obtained for RIB (N = 5), ABE (N = 1), and FUL (N = 2) were all within the expected ranges. The patients treated with PAL (N = 7) showed some inter-individual variability and non-adherence – the concentrations/AUC of the adherent patients were near to the suggested target ranges, but additional long-term monitoring would be advisable to closely assess the impact on the progression-free survival.

High inter-patient variability was discovered for LET (N = 2), with one patient having 2.76× higher AUC than the other. This is likely a consequence of hepatic impairment due to metastasis.

The main encountered challenge with PAL and RIB was their specific dosing schedule (once daily for 21 days and 7 days off-treatment). It is during the off-treatment period that the patients are usually scheduled for blood tests to determine the feasibility of the next treatment cycle according to absolute neutrophil count, which is not a suitable time for PK measurements. Moreover, due to the off-period, the steady state is effectively achieved for only two weeks each month. Accurate collection of data on dose administration and sampling times is therefore of critical importance. When adequately collected and coupled to clinical data, this information is valuable for clinical interpretation, giving insight into the underlying effects affecting drug concentrations, efficacy and toxicity. Future perspectives include further clinical interpretation and long-term follow-up of patients with "inadequate" concentrations, to evaluate the benefits of TDM. Assessing patient adherence with PK data is another endpoint with strong public health significance. Finally, developing analytical methods for alternative samples such as dried blood spots would enable a more patient-friendly sampling regimen and facilitate further development and implementation of the proposed methodology.

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PS01-20

Potential for drug-drug interactions of breast cancer drug abemaciclib – an in vitro metabolism study

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Abemaciclib (ABE) is a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor registered for the treatment of hormone receptor-positive, human epidermal growth factor 2 receptor-negative breast cancer in combination with anastrozole or fulvestrant. It is currently investigated in further clinical trials for the application in endometrial and ovarian cancer with concomitant letrozole (LET) [1,2]. ABE has been shown to undergo extensive hepatic metabolism to active metabolites - ABE-M20, ABE-M2, and ABE-M18 being the most prominent, with plasma exposures of 26%, 25% and 13%, respectively [3]. The aim of this work was to evaluate the potential for drug-drug interactions mediated by ABE and its metabolites in vitro, using CYP3A4 as the most susceptible liver enzyme.

The hydroxylation of testosterone (Te) to 6β-hydroxytestosterone (OH-Te) was used as the marker reaction. Direct, time-dependent and metabolism-dependent inhibition/induction assays were performed, respectively, by a) incubating both ABE and Te with the enzyme, b) incubating ABE with the inactive enzyme, delayed addition of Te and start of the reaction, c) incubating ABE with the active

enzyme and a delayed addition of Te. A second set of experiments was performed with the combination of ABE and LET, with and without Te. Control samples were prepared by incubating each drug separately under all of the tested conditions. All incubations were performed with 50 mM potassium-phosphate buffer, pH 7.4, in the total volume of 100 µL, on a shaking bath at 37 °C for the duration of at least 15 min. Generative system, containing glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP, was injected to start the reactions. Drug concentrations were 100-150 µM when used as substrates, or 1 µM when used as enzyme inhibitors/inducers. The reactions were stopped by the addition of 900 µL of cold acetonitrile. After vortex-mixing, centrifugation, and evaporation of the supernatant, the dry residue was dissolved in 65% methanol and analysed by LC-MS. The analytes were separated on a Phenomenex Gemini C18 column and detected using the parent ions: m/z 507.6 (ABE), 523.0 (ABE-M20), 479.0 (ABE-M2), 532.0 (ABE-M18), 305.4 (Te), 289.2 (Te-OH), and 286.0 (LET).

ABE showed time-dependent induction and metabolism-dependent inhibition of CYP3A4, while LET showed direct inhibition of CYP3A4. These findings point to a risk of drug-drug interactions between ABE, LET and potential other drugs metabolised via CYP3A4.

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PS01-21

Chiral targeted brain metabolomics in volume-limited biological samples

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The quantification of metabolites with neurotransmitter, gliotransmitter or neuromodulator roles in the healthy and diseased brain is important to further elucidate the mechanisms behind neurological diseases and, thus, to unravel possible novel drug targets and find new therapeutics. Indeed, the D-enantiomers of different hydroxy acids and amino acids are studied as potential biomarkers in metabolic disorders, but also in cognitive disorders. Therefore, their enantioselective analysis is crucial in further understanding brain functioning under physiological and pathological conditions. In this project, a miniaturized UHPLC-ESI-MS/MS method will be developed allowing the sensitive (picomolar to micromolar range) and enantioselective analysis of 28 amino acids and hydroxy acids in a single analytical run. The final aim is to analyze microdialysis samples with a limited volume of maximal 20 µL. Since these are chiral molecules, as first step chemical derivatization will be performed with

diacetyl-tartaric anhydride (DATAN) to obtain a different retention for the enantiomers on an achiral stationary phase, by turning enantiomers into diastereomers, and to increase sensitivity and stability of the target metabolites. Furthermore, isotope-coded DATAN will be used to form an isotopically labeled internal standard (IS) for all analytes of interest to achieve correction for matrix effects for each metabolite. For the development of the method, the derivatization reaction was performed with (+)-DATAN on dried amino acid standard solutions, as described by Pandey et al. [1]. The reaction took place at 75°C for 2h. The derivatized solution was analyzed in either positive or negative electrospray ionization mode on a UHPLC-ionKey system coupled to a Xevo TQ-XS triple quadrupole mass spectrometer, and on a UHPLC - Xevo TQ-MS (both from Waters, Milford, MA, USA). Different elution gradients with water and acetonitrile, both with 0.1% of formic acid, as mobile phases A and B, respectively, were tested. For the miniaturized iKey system separations were performed on a BEH C18 150 µm x 50 mm (1.7 µm, 130 Å) with a flow rate of 3 µL/min, while for the other UHPLC-MS system separations occurred on an Acquity BEH C18 column 2.1 x 100 mm (1.7 µm, 130 Å) with a mobile phase flow rate of 0.3 mL/min. Both columns were kept at 60°C. At the moment, capillary and cone voltages and collisional energy are further optimized as well as precursor and product ions for multiple reaction monitoring (MRM) mode.

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PS01-22

An automated CZE-MS and Genedata-RefinerMS based analytical workflow for plate-based milligram scale siRNA synthesis.

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In recent years, synthetic oligonucleotides have been increasingly used as therapeutic agents [1]. They can target specific genes or proteins to modulate their activity, offering potential treatments for various diseases such as cancer, genetic disorders, and viral infections. In the early phase of pharmaceutical research, a small-scale synthesis of designed oligonucleotides is often applied. The most widely applied method for the synthesis of therapeutic oligonucleotides is solid-phase synthesis [2]. The production of oligonucleotides undergoes rigorous characterization and quality control (QC) to ensure safety and efficacy. For milligram-scale synthesis of oligonucleotides, a high-throughput synthesizer in a 96-well plate format is used. This kind of automated synthesis system enables a production of up to 10,000 compounds/year custom-designed single-stranded oligonucleotides. To ensure timely and reliable QC of the synthesis, an automated high-throughput analytical platform is highly desired, which requires a separation technique with a short run time and a fully automated data processing workflow.

The most widely applied and well-established analytical approach for the characterization of oligonucleotides is ion-pairing reverse-phase liquid chromatography (IP-RPLC) with either UV or MS detection. Here, we present an analytical workflow for the plate-based QC of milligram-scale synthesized single stranded siRNA. The crude reaction mixture of ss siRNA (sense or antisense) was first analyzed using a chip-based capillary zone electrophoresis (CZE, ZipChipTM) coupled to high-resolution mass spectrometry (HRMS) via nano-electrospray in the positive ion mode (nESI+) [3]. The

data is processed using a Genedata Expressionist RefinerMSTM-based workflow for MS spectra processing, deconvolution, library search, and final reporting. CZE-MS provides advantages over IP-RP LCMS, such as mild separation conditions, no usage of ion-pairing reagents to avoid a source of contamination and carryover, and short analysis time (4-6 min vs. 20 min), etc. Additionally, the ultra-high sensitivity (amount injected is ~3 fmol) on the chip allows the detection of oligonucleotides by direct injection of a highly diluted crude reaction mixture.

Key words:
Oligonucleotide, mass spectrometry, automated oligo synthesis platform, adduct clustering, charge clustering, isotope clustering, impurities, identification, Gendata Expressionist, deconvolution, capillary zone electrophoresis, ZipChipTM, quality control.

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PS01-23

Fluorescent compounds as pH tracing standards for capillary isoelectric focusing analysis of labeled proteins and their glycoforms using laser-induced fluorescence detection

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Capillary isoelectric focusing (cIEF) is a powerful analytical method used to separate proteins and their post-translational modifications. However, it suffers from lower limits of detection of the analyzed compounds when the absorbance is measured at the standard UV 280 nm wavelength. Laser-induced fluorescence detection (LIF) is able to lower the detection limit by several orders of magnitude by using suitable fluorescent labels or fluorescent analytes.

The tracing of a pH gradient by fluorescent compounds in cIEF-LIF is essential for method optimization and isoelectric point (pI) calibration of the compounds analyzed. We present a set of low-molecular-mass fluorescein-based compounds covering the pH range from 3.10 to 10.21. Thanks to the fluorescein core, the pI standards (markers) have an excitation range suitable for the most common 488 nm laser, with fluorescence emission usually collected above 500 nm.

The novel fluorescent compounds were analyzed in regard to their purity after synthesis, their fluorescence properties, and focusing ability. Criteria were established and candidate compounds below the limits were removed from the group. After thorough analysis, a set of 19 fluorescent markers was selected as suitable for marking the pH gradient. In addition, due to the significant lack of fluorescent markers at basic pI two more candidates were added (first surpassing a lowered purity limit, the second being purified by cIEF fractionation). Altogether, utilizing such set of compounds

enabled precise calibration of the pIs of very low amount of injected proteins. This approach was demonstrated using fluorescently labeled immunoglobulin glycoforms and naturally fluorescent phycocyanin.

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PS01-24

Measurement of exogenous and endogenous steroids in dried blood spots by LC-MS/MS for application to pharmacokinetic studies

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Introduction. Exogenous glucocorticoids as methylprednisolone, prednisone, prednisolone, budesonide, and beclomethasone are widely used for anti-inflammatory and immunosuppressive purposes. Middle- and long-term treatment often lead to side effects due to excessive dosing, to suppression of the hypothalamus-pituitary-adrenal axis and variable recovery after therapy discontinuation. Dexamethasone is largely used for the functional screening of hypercortisolism conditions, but inter-individual variability complicates test interpretation. Indeed, the pharmacokinetics of glucocorticoid drugs is not fully delineated, with factors such as body composition, renal and hepatic function influencing their absorption, distribution, metabolism, and clearance. Pharmacokinetics studies are complicated by the need of frequent blood sampling. Dried blood spots (DBS) from finger-prick are a convenient alternative to venipuncture as they are easy to collect, store and transport, ensuring analyte stability and low biological risk. The DBS major drawback consisting in small blood volume may nowadays be overcome by sensitive and specific liquid chromatography-mass spectrometry (LC-MS).

Aim. We aimed at developing large LC-MS/MS panels including endogenous and exogenous steroids for studying drug pharmacokinetics and the perturbation of the endogenous steroid system in endocrine and non-endocrine diseases requiring glucocorticoid testing or treatment.

Methods. MS/MS detection by APCI and ESI, both in positive and negative modes, was optimized on the API4000 Q-Trap mass spectrometer (Sciex). Chromatography was optimized on the Serie 200 HPLC (PerkinElmer) by testing Kinetex® Biphenyl, 100x3 mm, 2.6 µm; Kinetex C8, 100x3 mm, 2.6 µm; Luna C8(2), 100x3 mm, 3µm; Luna PFP (2) 100x4.6 mm, 5 µm (all Phenomenex®); and Eclipse XD8-C18, 100x3 mm, 3.5µm (Agilent). Water, methanol, and acetonitrile as solvents, and acetic acid, ammonium fluoride, and formic acid as additives, were evaluated as mobile phases. Test DBS samples were generated by pipetting 30 µL of drug-free whole blood and dried for 24 hours. Extraction procedures involving water, methanol, acetonitrile, ethyl acetate, hexane, methyl-tert-butyl ether, and their combinations, as solvents, and vortex and ultrasound bath as devices, were tested on 6 mm diameter DBS disks.

Results. Two multiple reaction monitoring panels were defined according to analyte ionization performance. Panel 1, operating with ESI-, included estrone-3-sulphate, DHEA-S, 20-α-dihydrocortisone, cortisol, prednisolone, 20-β-dihydrocortisone, cortisone, prednisone,

dexamethasone, methylprednisolone, estradiol, aldosterone and estrone. Panel 2, operating with ESI+, included 11-ketoandrostenedione, 11-ketotestosterone, 11-hydroxyandrostenedione, 21-deoxycortisol, 11-hydroxytestosterone, corticosterone, 11-deoxycortisol, 16-hydroxyprogesterone, 11-α-hydroxyprogesterone, androstenedione, 11-deoxycorticosterone, 11-β-hydroxyprogesterone, testosterone, 17-hydroxyprogesterone, 17-hydroxypregnenolone, DHEA, progesterone, pregnenolone, beclomethasone and budesonide. Effective separation of isobars was achieved with Kinetex® Biphenyl, 100x3 mm, 2.6 µm for Panel 1, and with Kinetex C8, 100x3 mm, 2.66 µm for Panel 2. For both panels, mobile phases A and B were 20% methanol with 50 µM NH4F and 100% methanol, respectively, and gradient run duration was 18 minutes. Best extraction was obtained with 500 µl acetonitrile/methanol (80/20, v/v) with 20 minutes ultrasound bath at room temperature. Endogenous steroids detectable in test DBS were DHEA-S, cortisol, cortisone, corticosterone, androstenedione, testosterone, progesterone, 17-hydroxyprogesterone, and 16-hydroxyprogesterone.

Conclusion. These preliminary results show that DBS coupled with LC-MS/MS is a promising approach for comprehensive analysis of glucocorticoid pharmacokinetics and endogenous steroid pathways. Further developments are required to facilitate future applications. (Funding:GC-KINE-PRO;PRIN2020ERLB52_001)

PS01-25

Circadian rhythm profiling of amino acids and biogenic amines in chronic hypercortisolism states: utility of dried blood spots

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Introduction. Chronic hypercortisolism is featured by deranged circadian rhythm and energy metabolism. Sustained poor glucose utilization and enhanced protein catabolism lead to diabetes, sarcopenia, and cardiovascular diseases. Protein synthesis and catabolism display circadian regulation. Nonetheless, little is known about circadian fluctuation of amino acids (AA) and biogenic amines (BA) in health, while no information was reported in hypercortisolism states. Dried blood spot (DBS) self-samplings from finger-prick represent ideal devices for circadian studies. However, DBS comparability with traditional venipuncture and informative potential were not explored yet.

Aim. This study aimed at: 1) comparing measurements of AA and BA in DBS and serum; 2) evaluating the impact of different hypercortisolism states on AA and BA daily fluctuation.

Methods. Six volunteers participated in the correlation study. Nine healthy subjects (HS), six patients with autonomous cortisol secretion (ACS) and five patients with Cushing syndrome (CS) participated in the hypercortisolism study. All followed a standardized diet for seven days. On day seven, seven paired DBS and serum samples were collected from awakening to bedtime. Twenty-one AA and twenty-one BA were measured in study samples by LC-MS/MS.

Results. Correlation study. All AA were measurable in both specimens. Correlation between DBS and serum levels were registered for fourteen AA ($P<0.001$-0.022), with highest trends for proline, valine, isoleucine, and leucine ($r=0.739$-0.794). Isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and valine showed daily fluctuations parallel in serum and DBS ($P=0.001$-0.068),

with positive quadratic trend (i.e., higher levels at awakening and bedtime, and lower levels at midday). Among BA, six were undetectable, one was only detected in serum, and fourteen were measurable in both specimens. Levels of eleven BA directly correlated between serum and DBS (P<0.001-0.047), with highest trend for putrescine, creatinine, and acetylornithine (r=0.590-0.941). Daily fluctuation was observed in DBS, but not in serum, for methionine-sulfoxide (positive quadratic trend, P=0.001), and for creatinine (P<0.001) and kynurenine (P=0.007) (both linear decreasing trend). Hypercortisolism study. Compared to HS, ACS exhibited lower histidine (P=0.026), while CS had lower asparagine (P=0.030) and higher spermidine (P=0.003). CS also had elevated spermine (P=0.028) and t4-OH-proline (P=0.032) compared to ACS. In HS, eleven compounds displayed positive quadratic rhythms (methionine, isoleucine, arginine, leucine, methionine-sulfoxide, tryptophan, histidine, citrulline, proline, valine, glutamate; P:0.001-0.037). Leucine trend was maintained in ACS and CS (P=0.001-006), whereas histidine, isoleucine, valine, methionine-sulfoxide, and tryptophan were maintained in ACS (P=0.001-0.043). Further specific fluctuations were revealed in ACS for glycine, serine, ornithine, threonine, lysine, t4-OH-proline, and phenylalanine (P<0.001-0.010), and in CS for creatinine, asymmetric-dimethylarginine and spermine (P=0.003-0.018).
Conclusions. Most of AA and BA correlated in DBS and serum. Several AA displayed circadian fluctuation consistent in the two specimens. However, fluctuations were only observed for a few BA in DBS. In health condition, defined AA fluctuated in accordance with physiologic day/night protein metabolic processes. Such panel was partially and severely altered in ACS and CS, respectively. Overall, ACS displayed disrupted histidine metabolism, whereas CS manifested increased spermidine and spermine production. Our data support the utility of DBS for characterizing amine pathways alterations in hypercortisolism states (Funding:PRIN:2017HRTZYA_002;CHRONO-IMAGE).

PS01-26

Beyond conventional: evaluating the potential of HILIC for collagen peptide mapping analysis

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Hydrophilic interaction chromatography (HILIC), with its unique retention mechanism based on multiple interactions between hydrophilic stationary phases and polar analytes, has emerged in the last decade as a promising alternative for the separation and analysis of biomolecules, both at the intact and at the peptide level. Nevertheless, reversed-phase liquid chromatography (RPLC) is still the most common choice for peptide mapping analysis, with HILIC often considered only as a complementary technique and focusing on glycosylation.

Collagen, due to its rich composition in proline residues and their specific hydroxylation to form hydroxyproline as a distinctive post-translational modification, represents an interesting subject for reassessing HILIC potential over traditional RPLC peptide mapping approaches. The presence of hydroxylated proline residues along the collagen sequence, specifically 4-hydroxyproline and the less common 3-hydroxyproline, introduce polar functionalities that can interact favorably with the hydrophilic stationary phases used in HILIC. These hydrophilic interactions may enhance the selectivity of collagen peptides, making HILIC a suitable platform for their analysis. Moreover, collagen peptides often feature multiple potential hydroxylation sites, further complicating their chromatographic behavior. By exploiting the inherent hydrophilicity of collagen peptides and the distinctive retention mechanisms of HILIC, this analytical approach holds substantial promise in revealing the structural

characteristics and modifications of collagen. This is crucial considering that even subtle alterations to the hydroxylation pattern can lead to improper protein folding and severe biological consequences, underscoring the importance of accurate analytical characterization.

This study presents a comprehensive and systematic approach aimed at developing a robust HILIC method to specifically address collagen peptide mapping analysis. A set of sixteen model peptides derived from in-silico predicted tryptic peptides (zero missed cleavages), were selected for detailed fundamental studies in HILIC. Their sequences are representative of different physicochemical properties and structural motifs typical of collagen.

The methodology explores both conventional and state-of-the-art HILIC stationary phases with different mobile phase conditions. Additionally, attention is given to the sample diluent and injection mode, as it is known to be particularly critical in HILIC. In this context, classic injection and Performance Optimizing Injection Sequence (POISe) were compared in a systematic way. By elucidating the factors influencing peptide retention and selectivity in HILIC, the aim was to identify the most suitable conditions for the optimal separation of collagen model peptides, while also clarifying the still underexplored chromatographic behavior of peptides in HILIC. This will contribute to expanding the knowledge and applicability of HILIC to peptide analysis.

Finally, following full optimization of the chromatographic separation, hyphenation with mass spectrometry leads to a thorough understanding of the method effectiveness in analyzing collagen digested samples. This involves comprehensive evaluation of peptide sequence coverage and the ability to discern hydroxylation patterns compared to RPLC analysis.

PS01-27

Biomonitoring of phthalates in the Swiss population

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Phthalates are found in many everyday objects, mainly as plasticizers, but some have been classified as endocrine disruptors, and their use has been restricted or banned in recent years [1]. The Federal Food Safety and Veterinary Office has organized two large-scale human biomonitoring campaigns in Switzerland as part of health studies, and various substances, including phthalates, are measured in the biofluids of adults and children.

Phthalate exposure is measured by analyzing phthalate metabolites in human urine, thus avoiding the need to deal with ubiquitous environmental phthalate contamination. Phthalate metabolism in humans consists of hydroxylation and glucuronidation. Enzymatic hydrolysis is performed to cleave the glucuronides in urine, followed by a protein precipitation [2].

Thirty metabolites, representing 16 phthalates, were selected and analyzed with liquid chromatography–mass spectrometry. The mass spectrometer was operated in negative ionization mode, using a scheduled multiple reaction method by selecting two transitions per substance (one quantifying and one qualifying transition) to ensure selectivity. Corresponding isotopically-labelled standards were added in equal amounts to all samples to ensure robustness.

The analytical challenge of this study is to handle more than 2500 samples. Method development efforts focus on optimizing sample preparation and analysis time based on a published method [2]. First, a transfer from a column of high-pressure to ultra-high-pressure was evaluated to shorten the analysis time. Secondly, sample preparation was evaluated by comparing three clean-up methods (online solid-phase extraction [2], offline solid-phase extraction in 96-well plates, and without solid-phase extraction). This comparison demonstrated that a sample preparation with no solid phase extraction was sufficient for this study and will be presented in detail.

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Int J Hyg Environ Health, 2017, 220, 130-141.

PS01-28

Determination of organic acids and phenolic compounds by capillary electrophoresis

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The composition of organic acids and phenolic compounds present in fermented foods such as wine or vinegar are key parameters for organoleptic properties. We have developed a capillary electrophoresis method to simultaneously quantify the main organic acids and the main phenolic compounds present in fruit juices, fruit wines and vinegars. This inexpensive method allows wine and vinegar producers to control fermentation processes and improve the sensory properties and preservation of foods.

PS01-29

Therapeutic drug monitoring of asparaginase in acute lymphoblastic leukemia (ALL) patients Swiss-wide implementation and international cross-validation.

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Asparaginase (ASNase) is a key agent against ALL and has allowed to substantially improve survival rates, especially in children.

However, considerable interpatient variability in ASNase activity has been observed, due notably to -silent- immunologically mediated enzyme inactivation in vivo, which may lead to treatment failure and discontinuation of therapy.

Plasma ASNase activity is considered the best marker of clinical effectiveness and occurs at ASNase activity in plasma higher than 100 IU/L. Monitoring ASNase activity in patient's plasma can therefore help hematologists to better appreciate individual patient's response to asparaginase therapy and can be used to optimize treatment for patients.

However, there is at present no laboratory in Switzerland that currently proposes a Therapeutic Drug Monitoring (TDM) service for asparaginase. Our project aimed thus to set up at the Lausanne University Hospital, an assay for the TDM of the two commercially available PEG-asparaginase and Erwinia asparaginase, in patients' plasma.

The validation of the ASNase assay was based on ICH M10 guidelines (European Medicine Agency – EMA) for bioanalytical method validation. Method's parameters have been optimized for the instrument Hidex Sense Microplate Reader (Hidex, Turku, Finland). The ASNase activity in patients' samples was measured by a colorimetric assay, using the analogous substrate aspartic acid ß-hydroxamate, which after enzymatic conversion, yields hydroxylamine that reacts with 8-hydroxyquinoline to give in turn indooxine that is measured spectrophotometrically at 690 nm. The method has been validated for both enzymes within the activity ranges of 3 to 100 U/L (low levels) and 75 to 1000 U/L (high levels) with a trueness between 87.4-114.0% and an intermediate precision of 0.3-9.8% for all analytes and levels. Stability studies of PEG-asparaginase and Erwinia asparaginase in plasma at room temperature and +4°C indicate suitable stability for up to 72 hours. Besides, plasma samples subjected to three freeze-thaw cycles showed comparable enzyme activity to the initial reference values. Finally, a cross-comparison with two European reference centers, the Mario Negri Institute (Milan, Italy) and the Zentrale Einrichtung UKM Labor, Universitätsklinikum (Münster, Germany), showed that our results fell between those of the two centers, with an average difference of +5.3%, comprised within the ±15% acceptance limits. External Quality Controls organized by the Referenzinstitut für Bionanalytik (RfB, Germany) were as well successfully performed. The assay will be deployed as a routine service at the Lausanne University Hospital and throughout Switzerland by fall 2024.

PS01-30

A multiplex LC-MS/MS assay for the Therapeutic Drug Monitoring of ivacaftor, lumacaftor, tezacaftor, and elexacaftor and their active metabolites, in Cystic Fibrosis.

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Drugs modulating the cystic fibrosis transmembrane conductance regulator (CFTR) protein, nicknamed 'caftors' (i.e., ivacaftor, lumacaftor, tezacaftor, and elexacaftor) are revolutionizing the management

of individuals with Cystic Fibrosis (CF) patients care. Currently, approximately 90% of individuals with CF carry CFTR variants eligible for caftors. Caftors are mainly metabolised by cytochromes P450 3A whose enzymatic activity is influenced by environmental factors and is sensitive to inhibition and induction by concurrent drugs. Caftors are characterized by an important interindividual pharmacokinetic variability. The caftors are therefore good candidates for a Therapeutic Drug Monitoring (TDM) program [1].

To that end, a multiplex method by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, LC Vanquish with a TSQ Quantis MS, Thermo Fisher Scientific, USA) has been developed and validated in the laboratory of Clinical Pharmacology at the University Hospital in Lausanne. The assay allows the simultaneous quantification of all caftors currently registered in Switzerland along with their major pharmacologically active and clinically relevant metabolites ivacafor-M1, tezacaftor-M1 and, for the first time, elexacaftor-M23. The analytical method implies the reverse phase separation of the seven caftors and their metabolites on a C18 Xselect® HSS T3 (2.1 x 75 mm, 3.5 µm) (Waters, Milford MA, USA) using a stepwise gradient of 0.2% formic acid and acetonitrile over 3.6 min, followed by a rinsing step and re-equilibration to initial solvent composition within 3.4 min. A 50 µl-aliquot of plasma is vortex-mixed with 150 µl acetonitrile containing each internal standard (I.S.). After centrifugation of the mixture at +4°C for 10 min at 12’700g, a 150 µl-aliquot of supernatant is diluted 1:1 with ultrapure MilliQ water. Stable isotopically labeled I.S. were used for the quantification of respective parent drugs, and for some metabolites.

In accordance with ICH10 guidelines, the comprehensive analytical validation comprised the assessment of trueness, precision, accuracy profile, lower and upper limits of quantification, linearity, crosstalk, carryover, matrix effects, integrity to dilution, stability studies in plasma in various conditions and after multiple freeze-thaw cycles. The validated calibration ranges cover patients' observed plasma concentration range with an accuracy between 89.8-107.9% and a 1.3-10.9% precision for all analytes and levels. Inter-laboratory cross-comparison tests were also successfully performed with two laboratories already proposing a dosage of caftors in France and Germany with an average bias between 4.3-13.7% (n=25) for the four parent drugs.

This assay is being implemented for clinical research application at the Lausanne University Hospital and will be deployed throughout Switzerland by fall 2024. The TDM of caftors should be notably considered in case of suboptimal clinical response, suspected clinical toxicity, drug- or food-interactions, special populations (i.e., pediatrics), and for monitoring short-term adherence, particularly considering the extremely high cost of these innovative treatments.

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PS01-31

Quantification of recombinant human serum albumin (rHSA) in serum-free preservation medium for human donor corneal tissues, by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

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Safe and efficient preservation of corneal tissues in eye banks constitutes one of the major challenges for the quality of tissues for transplantation. Cornea Syn® (Eurobio Scientific, France), is a serum-free, synthetic medium, used for organoculture of human corneal tissues. Recombinant human serum albumin (rHSA) is used for the preparation of Cornea Syn® preservation medium, and its long-term stability has therefore to be ascertained. For such application, we have developed and validated an assay to quantify rHSA concentrations in Cornea Syn® batches after enzymatic digestion by trypsin, followed by selected quantification of a 'signature' peptide by LC-MS/MS.

Given its high molecular weight of 66'438 Da, the rHSA protein cannot be easily detected by tandem MS/MS, and a trypsin digestion approach was considered. In silico peptide digestion of the rHSA sequence was first realized using online software Expasy (SIB Swiss Institute of Bioinformatics, Switzerland) and a series of putatively promising 'signature' peptides were identified. Peptides were fragmented in silico using online MS/MS Fragment ion Calculator software (Institute for Systems Biology, Seattle, WA, USA).

Tryptic digestion was realized using the Rapid Digestion Trypsin Kit from Promega® (Madison, WI, USA) that is active at high temperatures (up to 70°C) allowing an enhanced denaturation of rHSA and providing a better availability to trypsin cleavage sites and higher digestion yield overall. In these conditions, the trypsin digestion can be carried out in only 1h at 70°C. The reaction was stopped using formic acid, to bring the pH of the solution outside trypsin activation range.

The LC-MS/MS instrument comprised a Vanquish liquid chromatograph coupled to a TSQ Altis triple quadrupole detector (ThermoFisher Scientific, Waltham, MA, USA). The MS/MS transitions of peptides proposed in silico have been tested by carefully tuning collision energy. The chromatographic separation of peptides was performed using a bioZen Peptide XB-C18 column (50 x 2.1 mm, 2.6 µm particle size) (Phenomenex, Torrance, CA, USA). LC and MS parameters were optimized for achieving the best chromatographic separation and mass spectrometry sensitivity for the investigated peptides. The analysis time is only 6 minutes, ideal for routine analysis. Matrix-matched calibration curve was established using a blank, rHSA-free, otherwise identical to Cornea Syn® medium, spiked with rHSA standard substance at concentrations ranging from 1.5 µg/mL to 1500 µg/mL.

Among 8 peptides that have been evaluated, the 'signature' peptide QTALVELVK was selected for the quantification while three other peptides were kept in the method for confirmation (qualifiers) purposes. The peptide ASGITFSNSGMHWVR (retention time of 3.02 min), was used as internal standard for QTALVELVK (retention time of 2.99 min). The method achieved good performances in terms of trueness (97.8% to 103.9%), repeatability (2.8% to 8.0%) and intermediate precision (3.6% to 9.7%).
In conclusion, a UHPLC-MS/MS assay has been developed and validated for the quantification of rHSA in Cornea Syn® conservation medium batches. This rapid method is applied to compare batch-to-batch reproducibility and to ascertain Cornea Syn® media stability.

PS01-32

Quantification of NNAL and anabasine with consecutive analysis of nicotine, cotinine and 3'-hydroxycotinine in urine using LC-MS/MS to determine tobacco smoking status and nicotine use from nicotine-containing alternatives for smoking cessation studies

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Tobacco cigarette use remains a major public health problem and smoking cessation strategies using Electronic Nicotine Delivery Systems (ENDS) with e-liquids are proving to be effective. Tobacco smoking status can be determined in urine by quantifying 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) or anabasine. NNAL is an endogenous metabolite of nicotine-derived nitrosamine ketone (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNK), a known carcinogenic tobacco-specific nitrosamine. NNAL is present in urine for a long period after cigarette smoking due to its long half-life, i.e. 10-18 days, although the urinary concentrations are very low. Anabasine can be found at slightly higher concentrations, but this tobacco alkaloid has a shorter half-life, i.e. 10-16 hours. Both are useful biomarkers in smoking cessation studies, because nicotine-containing alternatives, e.g. e-liquids, typically do not contain NNK or anabasine. To investigate nicotine use and the nicotine metabolite ratio in these studies, simultaneous quantification of nicotine and its endogenous metabolites cotinine and 3'-hydroxycotinine is needed.

We aimed to develop an LC-MS/MS method with a relatively simple sample preparation to measure NNAL and anabasine in urine at their cigarette-use cut-offs (i.e. 47pg/ml and 3ng/ml respectively) to determine tobacco cigarette abstinence, and to quantify nicotine, cotinine and 3'-hydroxycotinine (>100ng/ml).

Sample preparation involved benchtop methanol extraction and online solid phase extraction (SPE) of 15µL deglucuronidated urine for NNAL and anabasine quantification and 15µL pre-diluted urine (50x) for nicotine, cotinine and 3'-hydroxycotinine analysis. The samples were extracted using 600µL methanol containing the isotopic internal standard mix. After centrifugation, the supernatant was dried and reconstituted in 150µL 0.01% NH_4OH/10% methanol, after which 100µL was injected. The sample was loaded on the online SPE column (Oasis HLB Column 15µm 2.1x20mm, Waters) using 100% mobile phase A (0.01% NH_4OH/100% water) at a flowrate of 1ml/min. After 1 minute, mobile phase B (100% acetonitrile) was increased to 15% and at 2 minutes the sample was backflushed onto the analytical column (XBridge C18 3.5µm 4.6x100mm, Waters). Next, the analytical gradient was increased to 20%B, stabilized until 2.2min, then increased to 35%B (3min), stabilized again until 3.5 minutes and finally increased to 50%B (4.5min). Both columns were washed at 100%B and re-equilibrated over a total run-time of 9 minutes. Instrument contamination was avoided by diverting the nicotine, cotinine and 3'-hydroxycotinine peaks of the undiluted samples to waste. One calibration curve was used covering 0.025-250ng/ml for all 5 compounds, which achieved R>0.99 for each set of 6 consecutive points for quantification. The lower limits of quantification (LLOQ) for this method were 50pg/ml for NNAL, 0.75ng/ml for anabasine and 5, 1 and 2.5 ng/ml for nicotine, cotinine and 3'-hydroxycotinine, respectively. The overall accuracy was 90.8-100.3% with a precision of 4.1-12.1%.

In summary, an LC-MS/MS method was developed to consecutively measure low NNAL and anabasine concentrations in urine to determine tobacco cigarette abstinence, whilst quantifying high nicotine, cotinine and 3'-hydroxycotinine concentrations to determine nicotine use and metabolite ratios. Using

a relatively simple sample preparation, this method can be used in smoking cessation trials investigating use of nicotine-containing alternatives and in which high sample numbers are expected.

PS01-33

Caffeine metabolic ratios from diet to estimate CYP1A2 activity: association with olanzapine plasma concentrations

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Introduction: Olanzapine is an antipsychotic drug with high pharmacokinetic variability due to clinical, environmental and genetic factors [1]. It is metabolized by cytochrome P450 (CYP) 1A2, the activity of which can be estimated by plasma caffeine metabolic ratios (CMR), with monitored caffeine administration (standard phenotyping test) [2].

Objective: We aimed to investigate the association between dose-normalized olanzapine plasma concentrations (C/D ratios) and CMR, with dietary caffeine instead of monitored administration, to explore the clinical relevance for olanzapine dose personalization.

Method: The study used therapeutic drug monitoring data from the PsyMetab cohort, including 222 steady-state olanzapine C/D ratios from 146 psychiatric patients. Plasma CMR (paraxanthine/caffeine concentrations) were measured randomly with respect to dose and time of last caffeine intake, in the same sample as olanzapine, with caffeine originated from the diet (i.e. without specific intake). Olanzapine, caffeine and paraxanthine plasma concentrations were quantified by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. Linear mixed-effect regression models were applied to examine the association between CMR and olanzapine C/D adjusted by clinical covariates (sex, age, body mass index and smoking status). Receiver operating characteristic (ROC) curves were performed to identify a CMR threshold predicting at best a twofold increase in (slower metabolizers), and a halving of (faster metabolizers), C/D ratios.

Results: Olanzapine C/D ratios were significantly associated with CMR ($\beta=-0.16$, $p=0.02$), female sex ($\beta=0.37$, $p=0.02$) and smoking ($\beta=-0.91$, $p<0.001$), but CMR explained only 1.9% of the C/D variability. Patients with CMR lower than percentile (p)10 (CMR<0.4, slower metabolizers), or higher than p90 (CMR>2.9, faster metabolizers), had C/D ratios significantly higher (+27%, p=0.043), and lower (-27%, p=0.046), respectively, than patients with CMR between p10-90 (normal metabolizers). Using ROC curves, CMR <0.6 discriminate a C/D ratio twice above the median value (slower metabolizers; area under the curve (AUC), specificity, and sensitivity: 0.71, 0.81 and 0.57). No CMR threshold to discriminate a C/D ratio lower than half of the median value could be considered, as the obtained AUC was 0.56.

Conclusion: This study showed that dietary CMR, used to estimate CYP1A2 activity, were significantly associated with olanzapine C/D ratios. However, the explained variability was low, probably because olanzapine is also metabolized by other pathways such as uridine diphosphate glucuronosyltransferase 1A4 (UGT1A4), mitigating the impact of CYP1A2. Future studies must determine how the inclusion of other factors (e.g. UGT1A4) could be used, in addition to dietary CMR, to identify slow and/or fast metabolizers to personalize olanzapine doses.

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PS01-34

Methodological Support for Clinical Analysis of Patients in Critical Condition

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Critically ill patients admitted to intensive care units after planned surgery or during emergency hospitalization, are patients at high risk of developing infectious complications and poor outcome. Along with the use of generally accepted scales for assessing the severity of the condition of patients in intensive care and the use of non-specific biomarkers, such as lactate, procalcitonin, C-reactive protein and interleukin-6, a scientific search is being conducted for new markers with high sensitivity and specificity and a noticeable response to treatment.

In clinical studies conducted in patients with acute surgical diseases of the abdominal organs (n=58); in patients after cardiac (n=79) or neurosurgical (n=82) surgeries; as well as in patients in critical condition upon admission to the intensive care unit (n=79), the diagnostic and prognostic significance of a number of aromatic metabolites of tyrosine, phenylalanine, and tryptophan was revealed. The comparison group consisted of healthy donors (n=48). The objects of the study were samples of blood serum (n=452) and cerebrospinal fluid (n=82), in which the level of aromatic metabolites was determined by gas chromatography-mass spectrometry (GC-MS) and ultra-high-pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

In patients with acute surgical diseases of the abdominal organs with infectious complications (n=35), the median serum levels of phenyllactic acid (PhLA) and 4-hydroxyphenyllactic acid (p-HPhLA) were higher by 2.5 ($p<0.001$) and 1.5 ($p=0.048$) times, respectively, compared with patients without an infectious process (n=23). In patients with an aortic dissection who subsequently developed both infectious complications (n=26) or any types of complications (n=43), the median serum levels of p-HPhLA and the concentration of sepsis-associated metabolites (PhLA, p-HPhLA, and 4-hydroxyphenylacetic acid (p-HPhAA)) 6 hours after the end of surgery were 1.4 ($p = 0.010$) and 1.6 ($p = 0.002$) times higher, respectively, compared with patients who did not develop complications (n = 36). In patients with signs of post-neurosurgical meningitis (n=30), the median levels of p-HPhLA ($p<0.001$), indole-3-lactic ($p=0.006$), and indole-3-carboxylic ($p=0.027$) acids in the cerebrospinal fluid were 2.7, 4.6, and 1.5 times higher, respectively, compared with patients without signs of secondary meningitis (n=52). In critically ill patients upon admission to the intensive care unit, regardless of the main diagnosis, the median levels of sepsis-associated metabolites PhLA, p-HPhLA, and p-HPhAA, were 3.6, 3.2 and 5.1 times higher, respectively, in non-survivors compared to survivors. Various prognostic

models were built to demonstrate the ability of metabolites to predict the development of infectious complications and poor outcome.

The results obtained indicate the high diagnostic and prognostic significance of monitoring aromatic metabolites in patients in intensive care units. To date, various analytical protocols for determining aromatic metabolites in blood serum and cerebrospinal fluid were developed using liquid-liquid extraction or microextraction by packed sorbent (MEPS), followed by GC-MS; and a simple protocol using protein precipitation and HPLC-MS/MS analysis. Thus, it is of interest to introduce the developed methods into clinical practice.

PS01-35

Association of type 2 diabetes mellitus to arsenic metabolism?

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Context: Type 2 diabetes mellitus (T2DM) is a disease with a rising prevalence worldwide, with established origins (e.g. obesity, defined by a body mass index (BMI) superior to 30). Nevertheless, an environmental aetiology should not be discard. Human population is exposed to various chemicals compounds around the world and some of them are known to affect health. Several research studies focused on the impact of trace element on health, and in particular arsenic exposure. They investigated the possible association between this trace element and diabetes with controversial results, partly due to missing speciation analysis to differentiate organic and inorganic arsenic as well as the different arsenic metabolites.

Methodology: Based on the CoLaus|PsyCoLaus cohort, we analysed urine samples by ionic chromatography hyphenated to inductively coupled plasma mass spectrometry (IC-ICP-MS). This technique allows for arsenic speciation and therefore arsenic metabolites quantification. Participants were free of T2DM at baseline and were followed for incident T2DM until first follow-up. Incident case-samples were matched (age, sex, glucose) with 1 or 2 controls. Preliminary linear and logistic regressions were performed on data adjusted for urinary creatinine, age, sex, smoking status, alcohol consumption and education level.

Results: 469 urines samples were analysed from 183 participants who developed T2DM between baseline and first follow-up interview and their corresponding controls. As expected, diabetes was associated predominantly with glucose and BMI but also with monomethyl arsenic (MMA). Glucose was associated with BMI, gender and age while BMI appeared to be associated with education level and alcohol consumption as well as MMA and dimethyl arsenic (DMA). These latter associations of BMI with arsenic metabolites would be a key to understand the influence of arsenic exposure on T2DM. No association with total arsenic concentration was observed.

Conclusion: T2DM is known to be linked to obesity and therefore BMI, nevertheless the association between arsenic metabolites and BMI highlighted by this study shall be investigated further, to understand the causal role and the underlying mechanisms.

PS01-36

Determination of sildenafil by capillary electrophoresis

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According to the WHO, counterfeit medicines account for 10% of the pharmaceutical market in low- and middle-income countries. Currently, it is easy to find cheaper medicines on the internet, but with questionable safety and standards. Based on this problem, the current work is being conducted on Viagra, which is often couterfeited and easily found online. This project aims to develop a capillary electrophoresis (CE) analytical method that allows for the quantification of the active ingredient, sildenafil, present in Viagra medication. The CE was developed by the Pharmelp association, whose goal is to make it accessible to a wider audience through prices significantly lower than those on the global CE market. The method was developed using theophylline as an internal standard allowing for the quantification of both authentic and counterfeit medications. The long term goal is to make this analytical method widely available and enable others with a capillary electrophoresis device to perform these analyses.

PS01-37

DEVELOPMENT AND VALIDATION OF A HPLC-MS/MS METHOD FOR DETERMINATION OF BUDESONIDE IN NEWBORNS AFTER INHALATION OF THE DRUG

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Introduction
Budesonide is a glucocorticoid used for the prevention and treatment of bronchopulmonary dysplasia, a chronic lung disease which is a significant cause of morbidity and mortality among preterm neonates [1]. The low pulmonary availability of inhaled budesonide requires the development of optimized methods of aerosol delivery. On the other hand, systemic penetration through the lung may cause adverse drug effects [2]. To evaluate budesonide systemic exposure, a sensitive analytical method is required to analyze drug concentrations in a small volume of blood.

Our aim was to develop and validate a HPLC-MS/MS method for the determination of budesonide concentrations in plasma and dried blood spots.

Methods
Chromatographic separation was achieved on a Kinetex C18 column (100 x 2,1 mm, 5 µm) with a mobile phase composed of 5mM ammonium bicarbonate and methanol (20:80, v/v/) at a flow rate of 0.5 mL/min. The analytes were detected using a positive ionization mode by multiple reaction monitoring. Selected m/z transitions for budesonide and triamcinolone (internal standard) were from 431 to 413 and from 395 to 375, respectively. Multiple extraction methods were tested to determine the highest analyte recovery from plasma and dried blood spots, with acceptable precision and accuracy of results.

Results
For the isolation of budesonide from plasma samples, SPE was found to be the most suitable technique with extraction efficiency > 92%. Liberation of the drug from dried blood spots was performed using ultrasound-assisted LLE with ethyl acetate, and the recovery was 73%. The method validation was performed based on the ICH M10 guideline. The lower limit of quantification for budesonide was 0.1 ng/mL in plasma and 0.5 ng/mL in dried blood spots. The calibration curves covered the concentration ranges of 0.1 – 50 ng/mL and 0.5 – 50 ng/mL in plasma and dried blood spots, respectively. Precision of the method was in the range of 3.1-10.9% and the accuracy amounted 90.8-103.5%. Budesonide was considered stable under the variety of storage conditions tested.

Conclusions
The developed HPLC-MS/MS method was adequately accurate and precise and fulfilled the recommendations on the validation of analytical assays for drugs in biological matrices. The usefulness of the method was confirmed for quantification of budesonide in clinical samples collected from a child treated with nebulized budesonide.

Acknowledgments
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PS01-38

A prick is enough! Therapeutic drug monitoring of antiseizure medication through capillary microsampling devices

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Aims: Capillary fingerprick sampling requires a lower blood volume compared to venipuncture, used as a reference-standard method, resulting in a less painful and invasive procedure. This makes it an attractive tool for therapeutic drug monitoring (TDM)(1). We aim to assess the performance of different fingerprick devices, VAMS-Mitra® and qDBS-Capitainer®, used for the antiseizure medications (ASMs) quantification in persons with epilepsy (PWE) for self-sampling(2). Additionally, we evaluated the reliability and real-life feasibility of VAMS-Mitra® devices performed at-home and shippedvia regular mail to the laboratory.

Methods: We focused on the most commonly used ASMs: carbamazepine (CBZ), lacosamide (LCM), lamotrigine (LTG) and levetiracetam (LEV). The quantification was conducted using a validated Ultra High-Pressure Liquid Chromatography-Mass Spectrometry method. The reliability of VAMS-Mitra® and qDBS-Capitainer®devices was assessed by comparing the ASMs concentrations obtained through self-sampling via fingerprick in ambulatory settings with those from venipuncture(a).The same evaluation was also conducted for VAMS-Mitra® samples collected in ambulatory settings versus those collected

at-home(b). Reliability between the different results (a; b) was performed through Bland-Altman analysis and Passing-Bablok regression. A bias close to 0 for Bland-Altman analysis and an intercept around 0 with a slope around 1 for Passing-Bablok regression demonstrate a good agreement. Moreover, to assess the at-home feasibility, we considered the percentage of VAMS-Mitra® devices that successfully reach the laboratory for analysis, along with an ad hoc survey submitted to PWE to assess qualitative data.

Results: 76 PWE (69,7% females, mean±SD age 41,5±14,7 years), were enrolled. 13,2% were on CBZ, 18,4% LCM, 43,4% LTG, and 36,8% LEV (13,2% on polytherapy). Preliminary results by Bland-Altman analysis and Passing-Bablok regression, between both thedevices sampled in ambulatory versus venous blood samples (a) and VAMS-Mitra® at-home versus VAMS-Mitra® ambulatory (b), revealed a good agreement. The at-home study showed that more than 88% of patients successfully sent the device to the laboratory, while the remaining encountered difficulties with the shipment process. The ad hoc survey assesses the challenge of the fingerprick procedure: 95% reported it as "easy" and painless during the blood sampling procedure.

Conclusion: Our results show a good correlation between capillary fingerprick sampling with both qDBS-Capitainer® and VAMS-Mitra® devices self-sampling,holding promise in improving the accessibility of TDM in PWE. Moreover, the at-home VAMS-Mitra® study, despite the logistical challenges, demonstrates the feasibility and reliability of measuring ASMs levels, enhancingthe use of these devices towards telemedicine(3).

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PS01-39

Potential Identification of An Undeclared Compound in Herbal Supplements; Beta-Methylethephenylamine

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Background:
Beta-methylphenethylamine (BMPEA) is a positional isomer of amphetamine (AMP). Previously, it was detected in some dietary supplements labeled as Acacia rigidula. Pharmacological studies demonstrated that BMPEA has pressor effects, including an increase in blood pressure and heart rate in animals. Scientists hypothesized that it would involve the etiology and/or pathophysiology of a number of neuropsychiatric diseases. Importantly, organizations like WADA, FDA, and TGA forbid the use of BMPEA because it is a serious doping agent.

Methods:
10 registered samples were analyzed by an in-house analytical method using LC-MS/MS with ESI ionization source and the MRM library. AMP and methylamphetamine were used as reference standards; the mobile phase was methanol, acetonitrile, and 0.1% formic acid. LC-MS/MS parameters (curtin gas, 30; gas 1, 45 psi; gas 2, 35 psi; ion-spray voltage, 5.5 kv; positive mode; flow rate, 0.4 ml; column, C18).

The objective is to search for potential detection, if any, of the presence of BMPEA in Saudi FDA-registered dietary supplements.

Result:
An indication of the product and precursor ions of amphetamine-like peaks was found in one of weight loss supplements at retention time (RT: 1.44min; m/z 136/91) and (RT: 1.37min; m/z 136/119), that was different from that for AMP and methylamphetamine mix (RT: 1.91min; m/z 136/91) and (RT: 1.9min; m/z 136/119).

Conclusion:
The results raised suspicion of the presence of biogenic amines or AMP analogues since there was a resemblance between the product and precursor ions. This is preliminary data, and further investigation is needed, which would allow the Saudi Food and Drug Authority to take regulatory action.

PS01-40

Comparative Analysis of Antibodies and Molecularly Imprinted Polymers for Electrochemical Detection of Dopamine

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Parkinson's disease patients commonly receive levodopa medication, which undergoes conversion to dopamine not only in the brain but also in the gastrointestinal tract through aromatic L-amino acid decarboxylase (AAAD). This peripheral conversion can lead to variations in drug absorption and efficacy. To mimic this gut-mediated dopamine production and monitor it in vitro, we developed a gut-on-a-chip platform integrated with a three-electrode electrochemical detection system. In this study, the performance of two detection approaches within the gut-on-a-chip platform were evaluated: dopamine antibodies and dopamine imprinted polymeric nanoparticles (DIPs). Imprinted polymers offer distinct advantages over antibodies, including enhanced stability, reusability, and tolerance to extreme physical and chemical conditions. These properties make them particularly suitable for continuous monitoring applications in complex biological environments such as the gastrointestinal tract. Our comparative analysis focused on several key parameters: detection limits, sensitivity, and selectivity. Characterization and comparison of the performance of dopamine antibodies and DIPs within the electrochemical detection system was performed. The results demonstrated that DIPs exhibited superior performance metrics compared to antibodies, showcasing lower detection limits and higher sensitivity for dopamine detection in simulated gut environments. Furthermore, the electrochemical signals generated by DIPs showed excellent selectivity towards dopamine even in the presence of potentially interfering compounds commonly found in biological samples. This selectivity is critical for accurate and reliable monitoring of dopamine levels specifically attributable to levodopa metabolism in the gut. Overall, our study establishes the feasibility and efficacy of utilizing imprinted polymer based electrochemical detection within a gut-on-a-chip platform for real-time monitoring of dopamine production. This approach not only enhances our understanding of peripheral dopamine metabolism but also holds promise for applications in personalized medicine and drug development, particularly in optimizing treatment strategies for patients receiving levodopa therapy.

PS01-41

Developing HPLC-UV Method for Multi-Analyte Detection of Caffeine, Nicotine, Clozapine, and Their Metabolites in Plasma Samples

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One of the psychiatric diseases that researchers have focused on is schizophrenia (SCZ). It has been reported that about one percent of the world population suffers from this disease. The disturbance of brain development due to genetic, environmental, and neurobiological factors influences the emergence of SCZ. Pharmacotherapy is the cornerstone of the treatment of SCZ. The efficacy of clozapine as a treatment for SCZ has been substantiated by evidence, establishing it as the most effective antipsychotic medication for this condition. Its selection as a leading pharmaceutical option can be attributed to its enzymatic activity. Clozapine (CLZ) is known to be metabolised by cytochrome P450 (CYP) enzymes to form N-desmethylclozapine (DCLZ). CLZ and caffeine are both primarily metabolised by the CYP1A2 enzyme. Studies have indicated that caffeine has an inhibitory effect on clozapine metabolism. Therefore, the consumption of caffeine may increase clozapine levels in the plasma by inhibiting the CYP1A2 enzyme. In the case of smoking, polycyclic aromatic hydrocarbons in cigarettes are thought to be the inducers of the CYP1A2 enzyme. As a result of the induction, smokers require higher clozapine doses than non-smokers to reach the recommended, or therapeutic, level of clozapine. This study aimed to expand previous findings on schizophrenia by examining the amount of CLZ and its metabolite DCLZ in the plasma of individuals with schizophrenia. The research is designed to examine the relationship between exogenous substances, including drugs, and their metabolites in patients who consume caffeine and nicotine. Analytes selected for analysis include CLZ, caffeine, nicotine and its metabolites (paraxanthine, theobromine, theophylline, and cotinine). The method development was conducted using a high-performance liquid chromatography (HPLC) system with an EVO C18 analytical column (250mm x 4.6mm, 5µm). The injection volume, column temperature, and flow rate were optimized. The pH of the buffer was also optimised. Finally, the gradient method was applied with 0.1% H3PO4 buffer (pH 4) (A), methanol (B), and acetonitrile (C). The flow rate was set to 1 mL/min, and the column temperature was set to 20 °C. The detector wavelength was adjusted to 260 nm. The validated chromatographic method was in accordance with the ICH guidelines. Instrumental calibration curves were constructed using standard solutions for all the analytes. The developed method is appropriate for the detection of each analyte at a concentration of 0.1 µg/mL, with the exception of nicotine, which can be detected at a minimum concentration of 0.25 µg/mL. The R2 values were greater than 0.99, indicating that the developed HPLC method can be used for the determination of the analytes and their metabolites from biological samples after the development of an efficient extraction methodology

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PS01-42

Electrochemical and Spectroscopic Methods Provide Insight Into The Interaction between Nabumetone and double-stranded DNA.

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Nabumetone, an Non Steroidal Anti-Inflammatory Drug (NSAID); it is a potent inhibitor of cyclo-oxygenase (Cox-1 and -2) that blocks the formation of prostaglandins, which are important mediators in pain and inflammatory pathways. Nabumeton is a prodrug and exerts its anti-cyclo-oxygenase activity only after absorption and activation in the liver. Like other NSAIDs, it has analgesic, antipyretic and anti-inflammatory activities. The development of precise, cost-effective, and quick techniques for detecting calf thymus double-stranded deoxyribonucleic acid (ct-dsDNA), and understanding the structural alterations that develop in ct-dsDNA as a result of exposure to active pharmaceutical components, is of critical importance. Within this framework, the objective of this research is to create an electrochemical ct-dsDNA biosensor with the purpose of quantifying Nabumetone and elucidating the mechanism by which it interacts with DNA. Through the use of Differential Pulse Voltammetry, Fluorescence, and UV Spectroscopy, this research endeavored to understand the mechanism of interaction that exists between Nabumetone and DNA. Glassy carbon electrode (GCE), platinum wire and Ag/AgCl reference electrode were used for electrochemical investigation of the interaction. The Shimadzu 1601PC double beam UV-Vis absorption spectrophotometer and Agilent Cary Eclipse fluorescence spectrophotometer linked to a Peltier heat-regulated cell holder were used for spectroscopic and fluorometric studies. The results of the electrochemical experiment showed that Nabumetone significantly interacts with dsDNA, as seen by the decreased oxidation signals of dGuo and dAdo when Nabumetone presents. The binding constant (Ksv) between nabumeton and ct-dsDNA was calculated as 4.98 $x10^3$, 4.27 $x10^4$,1.12 $x10^5$ at 3 different temperatures (293,303,308 Kº), respectively, using spectrofluorometric measurements. Furthermore, quantitative evaluation of thermodynamic data (ΔS = +36.58 cal mol-1 K-1 and ΔH = +9.46 kcal mol-1) for the Nabumeton - ct-dsDNA complex predicted the contribution of hydrophobic bonds in Nabumeton - ct-dsDNA.

PS01-44

Comparative Study of Molecularly Imprinted Polymer Immobilization Techniques on Microfluidic Electrochemical Chips for Dopamine Biosensing

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Molecularly Imprinted Polymers (MIPs) are synthetic receptors designed to selectively bind target molecules through molecular recognition sites. Their application in biosensors offers distinct advantages such as robustness, stability, and cost-effectiveness compared to natural receptors. In this study, we explore different methods for immobilizing MIPs onto microfluidic three-electrode electrochemical chips and evaluate their efficiency. Three distinct immobilization techniques were employed: covalent attachment using alkanethiols, electropolymerization, and on-chip UV polymerization. Each method offers unique advantages in terms of simplicity, speed, and control over MIP deposition on the microfluidic platforms. Dopamine, a neurotransmitter and model analyte, was chosen as the template molecule for imprinting within the MIPs. The performance of the MIPs was systematically evaluated under continuous flow conditions within the microfluidic channels. Key parameters such as sensitivity, selectivity towards dopamine over structurally similar compounds, and reusability of the MIP-modified electrodes were thoroughly investigated. Electrochemical techniques provided real-time monitoring and quantification of dopamine concentrations, highlighting the suitability of MIPs for rapid and sensitive detection in complex biological samples. Comparative analysis of the immobilization methods revealed that covalent attachment using alkanethiols offered stable and uniform MIP layers with enhanced sensitivity and reproducibility. Electropolymerization demonstrated rapid fabrication of MIP coatings directly onto the electrode surfaces, facilitating ease

of integration into microfluidic systems. On-chip UV polymerization provided a versatile approach for spatially controlled MIP immobilization, enabling selective placement of MIPs within designated regions of the microfluidic channels. Overall, this study underscores the importance of selecting appropriate immobilization strategies tailored to the specific requirements of microfluidic biosensing platforms. The findings not only advance our understanding of MIP-based biosensors but also pave the way for the development of highly sensitive and selective analytical devices for neurotransmitter detection and other biomedical applications. Future research directions may focus on optimizing these immobilization techniques further and expanding the repertoire of target analytes for MIP-based microfluidic biosensors.

PS01-45

Subunit glycoprofiling by HILIC and FcγRIIIA affinity chromatography to address the glycoengineering of Rituximab from Nicotiana benthamiana

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Monoclonal antibodies (mAbs) represent a major class of biotherapeutics, which are mostly produced as recombinant proteins in Chinese hamster ovary (CHO) cells.

Their efficacy relies partly on the ability to trigger cellular effector functions by binding, through their Fc region, to the Fcγ receptors (FcγRs) present on the surface of the immune cells. In particular, the antibody-dependent cellular cytotoxicity (ADCC) is mediated by the interaction with the FcγRIIIa receptor, mainly expressed on the surface of natural killer (NK) cells.

Glycosylation plays a key role in the ADCC of mAbs. The N-glycosylation of asparagine 297 is necessary for Fc binding to FcγRIIIa and the nature of the glycan chain affects the affinity for the receptor. For example, it is well documented that the absence of fucosylation drastically improves the affinity for the FcγRIIIa receptor and results in an increased capacity to elicit the ADCC response.

Precise characterization of mAbs glycosylation profile is thus of paramount importance to evaluate product consistency and assess biosimilarity. In this context hydrophilic interaction liquid chromatography (HILIC) can exert its unique selectivity for glycans allowing a detailed definition of mAbs glycosylation profile at subunit level, after protein digestion with IdeS and disulfide bridge reduction.

Parallel to the progression in mAb characterization, the recent advancements in genetic engineering have enabled the production of recombinant glycoproteins in alternative systems (e.g. plants) and the glycoengineering of glycosylation pathways to achieve specific glycosylation and enhanced ADCC.
In this study, we produced glyco-variants of the mouse/human chimeric anti-CD20 antibody Rituximab (RTX), the first antibody-based drug approved for the treatment of patients with recurrent B-cell lymphomas, in Nicotiana benthamiana plants.

RTX mAbs were produced in plants by transient expression using the vacuum-agroinfiltration technology, and their glyco-variants were obtained by exploiting different glycoengineering approaches.

HILIC at subunit levels was used to define the glycosylation degree and characterize the glycosylation profile of the obtained products. All RTX samples were also screened for their affinity for a commercial FcγRIIIa receptor-based chromatographic column.

The RTX protein expressed in non-engineered plant showed only partial glycosylation and a heterogeneous glycosylation profile. Among the different glycoforms detected in HILIC-MS, many of which bearing typical plant sugars, the G0 glycoform was the only one to show the desired interaction with FcγRIIIa receptor.

The plant glycosylation system was thus engineered to obtain a highly homogenous RTX glyco-variant with only the G0 glycoform present at a very high glycosylation degree. This product showed slightly higher affinity for FcγRIIIa receptor compared to G0F/G1F glycovariants of the mammalian cell-derived originator Mabthera®.

The production process of glycoengineered plant-derived RTX was then optimized to assess reproducibility and stability, and the resulting batches were tested for consistency and biosimilarity using different chromatographic approaches coupled to MS.

To the best of our knowledge, this product can be considered the first promising RTX biosimilar candidate produced in plant, and this work represents a unique example of rational design of a glycoengineered mAb based on FcγRIIIa receptor affinity.

PS01-46

Oligonucleotide Characterization: Combining Anion Exchange Chromatography with In-line Multi-Angle Light-Scattering Detection

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Oligonucleotide therapeutics are promising modalities in drug development that require advanced methods for precise characterization. The determination of the molecular weight (MW) of oligonucleotides is of central importance in this field to ensure the therapeutic efficacy and safety of the compounds developed.

While ion-pair reversed-phase in combination with mass spectrometry (MS) is the gold standard for characterization of oligonucleotides, anion exchange chromatography (AEX) is mainly used for the purification of oligonucleotides and can separate short- and long-mers. However, the high salt conditions used in AEX are not compatible with MS. This complicates process-related and rapid oligonucleotide characterization without a lengthy desalting step.

We have developed a characterization method for oligonucleotides that combines anion exchange (U)HPLC and direct in-line MW determination with the LenS3™ multi-angle light scattering detector (MALS) to accelerate oligonucleotide characterization.

Take-home message:
- The method seamlessly combines anion exchange (U)HPLC with multi-angle light-scattering for simple and rapid molecular weight determination

- Multi-angle light-scattering detection with LenS3 rapidly and accurately determines molecular weight even of low-scattering substances such as antisense oligonucleotides (~6 kDa).

PS01-47

Effect of Analytical Conditions on Oligonucleotide Adsorption in Ion-Pairing Reversed Phase and Ion Exchange Chromatographyps

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In the development of nucleic acid therapeutics, liquid chromatography is used for qualitative/quantitative analysis, impurity analysis and bioanalysis of oligonucleotides. In such analyses, the adsorption of oligonucleotides on the column hardware is one of the challenges to ensure the accuracy of the analysis. Adsorption occurs through a combination of column hardware, mobile phase and target oligonucleotide. This study aims to evaluate the influence of column hardware on oligonucleotide analysis. The column hardware used in this study was stainless steel (SUS), PEEK/PEEK-lined stainless-steel (PEEK) and bioinert coated stainless steel (YMC Accura).

In ion-pairing reversed phase (IP-RP) chromatography, the stainless-steel column showed adsorption of oligonucleotide independent of mobile phase conditions. The PEEK column also showed adsorption when the concentration of IP reagent in the mobile phase was low, while little adsorption was observed at a high concentration of IP reagent. The YMC Accura column with its bioinert coating prevented adsorption under all IP reagent conditions.

In ion exchange (IEX) chromatography, the tendency is the same as for IP-RP. The stainless-steel column is not ideal for oligonucleotide analysis in IEX. The PEEK column showed no adsorption when the mobile phase was a sodium hydroxide system. However, the PEEK column showed adsorption when using Tris-HCl buffer system, which is widely used for oligonucleotide IEX analysis. Furthermore, the adsorption became more severe as the oligonucleotide length increased. The bioinert YMC Accura hardware showed no adsorption of oligonucleotides regardless of mobile phase conditions and oligonucleotide length.

The bioinert coated YMC Accura column hardware was found to be a universal option for oligonucleotide analysis in different separation modes.

PS01-48

Native anion exchange chromatography coupled to mass spectrometry for the analysis of charge variants of IgG4-based monoclonal antibodies

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Monoclonal antibodies (mAbs) are immunologically active proteins, which have a defined specificity for certain antigens e.g. on cancer cells. Thereby, an immune response against the target cell is induced. This makes mAbs not only an efficient tool in cancer therapy but also for the treatment of

different haematological, immunological and infectious diseases. Advances in biotechnology and protein biochemistry have resulted in the formation of various novel recombinant variants. A large number of therapeutic antibodies are already available on the market and several hundred more are currently under investigation. Efficacy and/or safety of mAbs are ensured by tightly controlled critical quality attributes (CQA). One CQA is the charge heterogeneity, which is caused by post-translational modifications.

Besides capillary electrophoresis (CE), ion exchange chromatography (IEX) is a common method to determine the overall charge heterogeneity. Since most commercially available mAbs are IgG1-based and possess a high isoelectric point (pI) of usually ≥8, cation exchange chromatography (CEX) is the most suitable method for analysis. Also coupling to mass spectrometry (MS) has been successfully described [1]. However, the importance of mAbs based on IgG4 as human therapeutics is currently increasing. In contrast to IgG1 mAbs, they possess a pI <8 so that CEX is less suitable for their analysis. Therefore, anion exchange chromatography (AEX) may be an alternative approach. In this study, the successful application of an AEX method for charge heterogeneity analysis of IgG4-based mAbs coupled to MS was achieved [2]. Five different IgG4-based mAbs with different pIs (between 6.1 and 7.3) as well as the NISTmAb (pI=9.2) were analysed using BioPro IEX QF, a strong anion exchange (SAX) column with non-porous particles. To enable MS analysis under native conditions, which require high salt concentrations, a special setup combined with nanoelectrospray ionisation mass spectrometry (NSI-MS) is needed.

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PS01-49

Microwave-assisted extraction and characterization of fatty acids: a sustainable approach to fatty acid production from wheat bran

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Aiming to protect natural resources and promote a circular economy, the present project focuses on the valorization of by-products from specific agricultural crops through sustainable practices. The primary goal is to develop eco-friendly surfactants by chemically conjugating natural hydrophilic substances extracted from discarded chicory roots with hydrophobic substances derived from by-products of maize starch and flour production. Given the significant role of fatty acids in human health and industrial applications, their sustainable production is crucial. Traditional methods of producing monoglycerides from vegetable oils, rich in triglycerides, involve high-temperature chemical glycerolysis, which is energy-intensive and yields lower-quality products. Thus, exploring alternative methods for extracting and synthesizing fatty acids and monoglycerides from vegetable oils is necessary. Preliminary extractions from wheat bran corn kernels using microwave irradiation offer a clean, efficient method with higher yields in shorter times compared to conventional heating. Fatty acids were extracted and isolated from wheat bran using an alkaline hydrolysis method assisted by microwave heating irradiation. The initial method involved heating wheat bran suspended in NaOH

solution, followed by filtration and liquid-liquid extraction with n-hexane. The fatty acids were then isolated by acidification and further extraction with n-hexane. To improve the process, an alternative method was developed where wheat bran was treated with ethanol and KOH. The resulting solution underwent liquid-liquid extraction with n-hexane and the crude extract was purified via flash chromatography affording the pure fatty acids mixture. For optimization within a circular economy framework, the use of organic solvents was then minimized. The samples were then esterified and analysed through GC-MS. The proposed procedure yielded reliable and reproducible results, as the composition of the fatty acid mixtures remained consistent for all the collected samples. The optimized procedure afforded high yields minimizing the use of organic solvents. The sustainable extraction and isolation of fatty acids from wheat bran represent a promising advancement in green chemistry and circular economy. The study demonstrates that microwave-assisted extraction, combined with alkaline hydrolysis and optimized solvent use, enhances the efficiency and eco-friendliness of fatty acid production. By minimizing the consumption of organic solvents and maximizing the yield, the developed methods align with the principles of sustainability and resource valorization. The successful extraction of valuable fatty acids from waste materials not only adds economic value to low-cost by-products but also contributes to reducing environmental impact.

PS02-01

Application of inert biphenyl stationary phase in the analysis of biologically relevant metabolites

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Stainless steel has been the preferred material to manufacture HPLC columns due to its ease of fabrication and mechanical resistance to pressure. Nevertheless, metal-analyte interactions are prevalent in the chromatographic device and may lead to poor peak shapes, low recoveries, and overall poor performance. This type of Non-Specific Adsorption (NSA) has been specifically documented in the literature for compounds bearing polar heads (such as sulfates, phosphates, etc.) and several solutions have been promoted in recent years to mitigate such interactions. Inert coatings via Chemical Vapor Deposition (CVD) offer one of the best solutions while improving the analyses of several acidic and chelating-prone species. In this study, a biphenyl stationary phase was leveraged along an inert column hardware towards the analysis of steroids and their phase II metabolites in biological samples. The biphenyl stationary phase demonstrated greater selectivity, especially towards isobaric species, when compared to C18 phases; while the inert hardware offered higher peak height and better peak shape when compared to the untreated counterpart. Overall, the application of inert coatings in HPLC columns aims to mitigate NSA while offering a robust and improved chromatographic performance, without the need to make repetitive injections or implementation of mobile phase additives that could compromise the sensitivity of the detection. Details on the chromatographic improvement of other health-adverse species will also be discussed.

PS02-02

Improvements in body odor sampling and analysis for medical diagnosis applications using canine olfaction and GC-MS

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The human body odor is widely studied for the detection and identification of specific patterns of Volatile Organic Compounds (VOCs), induced by pathologies such as cancers or infectious diseases. The use of canine olfaction is emerging as a promising solution for the development of non-invasive diseases diagnosis methods. However, limitations in the employment of the dog as an analytical tool have been reported [1]. One of these limits is the lack of standardized training procedures and materials. Indeed, efficient odors sampling materials are essential to properly train detection dogs.

This experimental study aims to compare five VOCs sampling devices for sweat collection: medical gauzes, Twisters (Gerstel), polydimethylsiloxane (PDMS) pads, and patented materials for canine olfaction such as Sorbstars® (Action Europe) and Getxent® tubes (Biodesiv). An aqueous solution containing a mixture of VOCs from different chemical family found in human sweat is synthetized. Using an offline autosampler, VOCs are vaporized, and then injected in closed containers containing the devices. The protocol mimics a sweat passive sampling for one hour. Devices are then either introduced into a thermal desorption unit or extracted by Solid Phase MicroExtraction (SPME) or Dynamic Headspace (DHS). The final analysis is performed by GC-ToF-MS (Pegasus BT-ToF-MS, Leco). Extraction conditions (temperature, trapping time and nitrogen flow in the case of DHS) are optimized using design of experiments, for Getxent® tubes and medical gauzes, providing methodological recommendations for furthers studies involving GC-MS.

Reproducible results are obtained, allowing each of the five devices studied to be assigned a selectivity score (number of VOCs detected). Then, limits of quantification (ng) are determined to evaluate the sensibility of each device. None of the devices allows for the detection of 3-methyl-3-sulfanylhexan-1-ol, a volatile thiol typically found in human axilla sweat. Therefore, the development of more specific devices to extract sulfur compounds has been considered. Sampling materials made of functionalized PDMS with gold nanoparticles (that have the ability to form strong bonds with sulfur compounds [2]), have been developed for this purpose. Adsorption and desorption processes with these innovative materials are still in development and could lead to the achievement of better detection of volatile thiols emitted by the human body.

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PS02-03

Blockchain and machine learning based framework for managing EMRs: Security, Privacy, Interoperability, and Clinical Decision Support

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Managing electronic medical records (EMRs) poses major challenges in terms of security, privacy, and interoperability. EMRs are often stored centrally, exposing patient data to the risks of hacking and unauthorized disclosure. Moreover, traditional systems struggle to effectively share data among different healthcare institutions. Given these considerations, the primary objective of this work is to enhance EMR management by ensuring data security, privacy, and interoperability while improving care quality by facilitating access to relevant medical information and aiding in early diagnosis. To address these challenges, we propose an EMR storage and management framework based on a private blockchain, smart contracts, and machine learning.

Blockchain technology offers significant potential to improve clinical medicine by ensuring the security, confidentiality and traceability of medical data, while facilitating the management of medical records and promoting medical research. The main contributions are:

- Security and confidentiality of medical data using advanced encryption protocols. Data is stored in a decentralized and secure manner, reducing the risk of hacking or data loss.
- Control access to medical data, making it accessible only to authorized people. Patients thus have better control over their medical information.
- Traceability of medical data, from their creation to their access and use. This helps ensure data integrity and detects any unauthorized changes.
- Simplification and security of the management of medical records by making them accessible in a secure and transparent manner for health professionals and patients.
- Facilitates the secure sharing of medical data between research institutions, which can accelerate medical research and innovation.
- Monitoring of the journey of medicines from their manufacture to their distribution, which can help reduce counterfeiting and guarantee the quality of medicines.

The use of smart contracts in a private blockchain for the storage and management of electronic medical records offers a secure, transparent and efficient solution, guaranteeing data confidentiality, protecting patient information and providing significant benefits for clinical medical practice.

Machine learning associated with a private blockchain offers several advantages in clinical medicine: integration of heterogeneous data, treatment personalization, early disease detection, and optimization of clinical processes.

This integrated approach combining private blockchain, smart contracts, and machine learning would provide better security, more efficient management of medical data, and more personalized patient care, thus contributing to improving clinical outcomes, care quality, and the efficiency of EMR-related processes. The proposed approach ensures data security, privacy, and interoperability, providing an effective solution for automating processes and improving healthcare service efficiency. By adopting this framework for EMR management, healthcare institutions can not only improve the quality of care but also strengthen patient trust in the healthcare system.

PS02-05

Capillary electrophoresis as a green alternative to liquid chromatography for determination of alpelisib in pharmaceutical dosage forms

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Breast cancer treatment has made significant progress in recent years and new medicines are emerging continuously. Alpelisib (ALP) is a novel phosphoinositide-3-kinase (PI3K) inhibitor recently approved for HR-positive, HER2-negative, PIK3CA-mutated metastatic breast cancer in combination with fulvestrant. Alpelisib has been the subject of only a limited number of preclinical in vitro and in vivo studies using different chromatographic techniques. However, to date, no research has been published on analyzing alpelisib using capillary electrophoresis (CE). The absence of pharmacopoeial monographs for alpelisib in both the European and United States Pharmacopoeias highlights the urgent need to develop a reliable analytical method for its quality control. In this work, we have developed a CE method for the determination of ALP in bulk and pharmaceutical dosage forms in just 1.4 minutes. This was achieved with a 25 mM borate buffer at pH 9.3, with 30 kV separation voltage and 30 °C capillary temperature. The proposed method was validated according to the ICH guidelines regarding selectivity, linearity ($r = 0.9988$), precision (RSD < 5.9%), accuracy (bias < 3.0%) and robustness (RSD < 3.5%). It was applied to the pharmaceutical dosage form of ALP and was shown to be suitable for the reliable determination of ALP. Furthermore, to demonstrate the applicability of the CE as an alternative technique to more commonly used HPLC in the analysis of drugs, cross-validation of CE and HPLC methods was performed. The average difference in determined concentrations between CE and HPLC over a range of 10-100 µg/mL was 0.1 µg/mL ($p = 0.8169$, $N = 19$) meaning that there is no difference in the performance of CE and HPLC in determination of ALP in pharmaceutical dosage forms. Finally, the environmental impact of both methods was assessed using AGREE software [2]. AGREE scores of CE and HPLC were calculated to be 0.74 and 0.51, respectively which can be explained by the minimal waste produced and energy spent by CE while retaining faster analysis and a smaller amount of sample required. Because of equally reliable analytical performance and greener analysis, CE should be considered as an alternative technique to HPLC in the analysis of pharmaceutical dosage forms.

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PS02-06

On going procedure performance verification of chromatographic assay methods

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Thanks to the emergence of the concept of Analytical Quality by Design, now described in the United States Pharmacopeia's chapter <1220> "Analytical Procedure Life Cycle"[1] and in the two ICH guidelines Q14[2] and Q2(R2)[3], related to the development and validation of analytical procedures, it now appears to industry analysts that a chromatographic assay method can no longer be conceived of as a fixed, unchangeable object, but on the contrary, as possessing its own life cycle. From its design to its implementation and routine use, its continuous fit for purpose must be the subject of particular attention and the tools for monitoring its performance must be put in place.

Industry control laboratories use daily system suitability tests based on performance parameters of their chromatographic analytical methods, or regularly analyze quality control samples to verify the performance level of their methods. However, these indicators are often only observed extemporaneously, whereas they can be easily monitored using control charts to facilitate decision-making and the implementation of preventive or corrective actions before any analytical problem occurs.

Control charts of measurements and moving ranges (Levey-Jennings charts) or averages/medians and ranges/standard deviations (Shewhart charts) are widely used in industrial process monitoring and we show how these tools can be advantageously used when monitoring the performance of chromatographic assay methods.

Based on well-known interpretation rules (Western-Electric rules[4] or Westgard rules[5]), we propose a method for analyzing the results of control charts, the objective of which being the rapid identification of drifts or shifts in the measurements or their dispersion.

We illustrate this approach with the help of a graphical example, obtained from monitoring data for a system suitability test of a HPLC assay method, for which a control chart processing tool makes it possible, through the use of sets of colors, to easily identify the rules that are not respected and gives the laboratory the opportunity to implement preventive or corrective actions on the cause before its consequences affect the quality of the analytical results.

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PS02-08

Full analytical characterization of an innovative nanomedicine targeting human islet amyloid polypeptide for type 2 diabetes mellitus treatment

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Type 2 diabetes mellitus is a chronic endocrine disorder resulting in high glucose levels in the blood for prolonged periods, mainly due to insulin resistance. According to the WHO, the worldwide prevalence of diabetes in adults will reach 5.4% by 2025 and amongst diabetics, 98% are affected by type 2 diabetes mellitus. The misfolding and aggregation of human islet amyloid polypeptide (hIAPP or amylin) have been identified as an etiology of type 2 diabetes mellitus1. To prevent this pathological process, innovative two-feature long-circulating nanoparticles, called Janus nanorods (JNR), obtained by co-assembling two polymers, have been designed2. One side is dedicated to capturing hIAPP and avoiding its oligomerization or fibrillation, whereas the other side is expected to bind human serum albumin (HSA) to increase their circulation time in the bloodstream.

A full physicochemical characterization of this new JNR generation has been done. Individual nanoparticle tracking analysis (NTA) and multi-angle dynamic light scattering (MADLS) assessed their size and shape, confirming the cylindrical shape with a length of 210 nm and width of 50 nm. Then, the surface charge was determined with a zeta potential around -12.5 mV. To predict their in vivo behavior after parental administration, we recently developed a frontal analysis continuous capillary electrophoresis (FACCE). The affinity constant and stoichiometry of interaction between nanoparticles and HSA could be estimated with high accuracy3. We adapted this method also to study hIAPP/JNR affinity. This was challenging as hIAPP is prone to self-oligomerization in a pH-dependent manner4. Extensive investigations have been made to optimize the background electrolyte composition, ionic strength, as well as silica modification. A reliable method was obtained to estimate their affinity under physiological conditions, with a good linearity of calibration (R2 > 0.98). In parallel, the behavior of hIAPP oligomerization over time was monitored by a combination of capillary zone electrophoresis (CZE) to reveal small oligomers formation as well as a Thioflavin T (ThT) assay to follow hIAPP fibrillation at physiological pH4. Thanks to these two approaches, the effect of JNR on hIAPP was assessed. In particular, the ThT assay highlighted a delay in hIAPP fibrils formation.

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PS02-09

Analysis of enantiomeric purity of some fluoroquinolones antibacterials pharmaceutical formulation on different chiral stationary phases

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Today in our pharmacies are a lot of developed drugs possess chiral centers, chirality has become increasingly important topic issue in drug research and has attracted increasing consideration in the pharmaceutical industry. The main objective of this work is to applied a simple direct isocratic high-performance liquid-chromatographic methods for chiral separation and determining the enantiomeric purity of some antibacterials active pharmaceutical ingredient (API) using commercialized polysaccharides stationary phases (Chiralcel® OZ-3, Chiralcel® OD-H, Chiralcel® OD, Chiralcel® OJ, Chiralpak® AD, Chiralpak® IA and Chiralpak® IB) and online coupled with electronic circular dichroism (ECD) detector also applying different mobile phases in isocratic mode is described. The role of addition of organic additives, were also investigated . The correlations of experimental ECD traces with quantum chemical ECD calculations with TD-DFT made it possible to elucidate the absolute configuration for each enantiomer, and establish the elution order. Furthermore, molecular docking was performed to confirm of absolute configuration, elution order and analyse the binding modes of R- and S-enantiomers. Moreover, the stereoselective and the chiral recognition mechanism of racemic fluoroquinolons antibacterials drugs on Chiralcel® OD-H chiral stationary phase (CSP) has also been researched via modeling studies. It was observed that hydrogen bondings and π-π interactions are the major forces for chiral separation. The modeling studies indicated strong interactions of R-enantiomers with Chiralcel® OD-H chiral selector than S-enantiomers. This process was found to be suitable for rapid enantiomeric purity analysis and a quality control of quinolones in any matrices also racemic compounds.

PS02-10

Untargeted Metabolomics of PAC-containing Dietary Supplements by LC-MS: Data Fusion Approach For authentication and Detection of Adulteration in Botanical Materials

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Many dietary ingredients are source of PACs, which present a wide range of pharmacological properties of interest for dietary supplements (DS) applications. PAC dietary sources vary according to their composition of monomeric units, type of interflavan linkage (A- and B-type), degree of polymerization (DP) and present a very high structural complexity. Industry has been commonly using unspecific methods to analyze PACs from different sources and up to date no single analytical technique has been able to provide a full characterization of PACs of low and high DP distribution. Due

to this limitation, PAC-containing DS are commonly adulterated with cheaper sources of PACs or non-legitimate ingredients such as peanut skin extract, which in latter case can create potential public health concerns.

Chemical fingerprint of 58 botanical materials (Grape Seed-GS, Pine Bark-PB, Cranberry-CR, Cinnamon and Peanut Skin-PS) and in-house adulterated samples (5, 10, 25, 50% spiked levels with PS, GS and PB) was acquired by HILIC-MS and RPLC-MS (Orbitrap Fusion Lumos MS) and fused into a single dataset, which was processed and analyzed by Orthogonal Partial Least Square (OPLS-DA) in order to generate a prediction model to classify authentic and potentially adulterated samples.

The resulting OPLS-DA prediction model presented high predictability (Q2 = 0.760), and all authentic samples were correctly classified into their respective groups. Suspected and in-house adulterated samples were not classified as authentic, with exception of GS and CR in-house adulterated samples with low levels (5% GS and 5,10% CR) of PS. A comprehensive assessment of the complete metabolite profile of different PAC-containing botanical materials was obtained by evaluating the loading plots and heatmaps, which further allowed the identification of target metabolites to detect adulteration and confirm DS authentication. Untargeted metabolomics based on this innovative multiplatform analytical methodology was successfully applied to develop potential compendial methods for the analysis of botanical materials, allowing a comprehensive assessment of oligomeric and polymeric fractions of PAC-derived ingredients and detection of adulteration with non-legitimate and unsafe ingredients.

Metabolomics in combination with chemometric tools showed to be a powerful analytical strategy to develop more sensitive, selective and suitable methodologies for proper quality control of complex botanical materials and establishment of their specifications. The metabolomics/chemometrics approach presented herein can be applied in the pharmacopeial framework as an innovative strategy to develop suitable compendial standards, create emerging and potential digital standards that can support the advancement of quality of medicines, dietary supplements & herbal medicines.

PS02-11

Chemical derivatization for targeted brain metabolomics of volume-limited samples using LC-MS/MS

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The analysis of the brain interstitial fluid is interesting as it gives information about the release and re-uptake of neuroactive molecules. These molecules, called brain metabolites, can be released by various cells, for example neurons and astrocytes. Neurons can release neurotransmitters at the synapse to enable neurotransmission, while astrocytes can also directly influence neurotransmission through the release of gliotransmitters near the synapse. The direct analysis of the brain extracellular fluid is challenging as the metabolites under investigation have a wide variety of physicochemical properties, they are low in concentrations, and in vivo sampling is difficult. Microdialysis is an elegant sampling technique, but often results in low sample volumes, depending on its temporal resolution.

The aim of this study is to develop a liquid chromatography (LC) tandem mass spectrometry (MS/MS) method for the analysis of a wide range of brain metabolites in these volume-limited samples. To enable retention of polar metabolites in reversed phase LC, and to allow analysis of all these compounds in electrospray positive mode, chemical derivatization is performed with dimethylaminophenacyl bromide (DmPABr). This derivatization reagent reacts with primary and secondary amines, carboxylic acids and thiols. As such, it allows the analysis of many metabolites of interest in one run.

Miniaturized LC-MS/MS was performed on an Acquity UHPLC system coupled to a TQ-XS triple quadrupole MS (both from Waters), which was operated in electrospray positive mode. An IonKey source was used, integrating the analytical column (BEH C18 ™ separation device (130 Å, 1.7 µm, 150 µm x 50 mm), Waters) for the separation at 60°C, and the electrospray ionization probe with a capillary voltage of 3 kV. Using a flow rate of 3 µL/min, gradient elution was performed, with 0.1% formic acid in water and in acetonitrile as mobile phases A and B, respectively. One µL of sample was injected.

Optimization of the LC and MS parameters and of the derivatization reaction were performed using a mixture of 8 compounds, which included glutamate, γ-aminobutyric acid (GABA), cysteine, spermidine, lactate, butyrate, ornithine and kynurenic acid. For all these compounds, selected reaction monitoring (SRM) parameters were optimized, namely precursor- and product ion, cone voltage, and collision energy. A quantifier and qualifier transition were selected for each compound. For the LC gradient, methanol and ethanol were evaluated as greener solvents, but a negative effect on peak shape was observed. Currently, the autosampler stability at 8°C is being evaluated, and we are including an isotope labelled form of the derivatization reagent, to obtain an internal standard for all compounds. After optimizing these parameters, our compound list will be expanded to include additional brain metabolites. Furthermore, we will validate the quantification of these compounds in mouse brain microdialysis samples.

PS02-12

Monitoring of Leachable Compounds in Hospital Pharmacy-Compounded Drug Products by UHPLC-HRMS

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Hospital pharmacy-compounded drug products are crucial for patient care because they are time- and cost-effective in hospital pharmacy settings. They prevent waste, preparation, and dosage errors at patients' bedsides, ensuring microbial integrity, and increasing care givers security. Unfortunately, the drawback of this approach includes the risk of selecting unsuitable storage containers due to market availability or accessibility, cost constraints, and the lack of a regulatory framework. Indeed, plastic compounding materials could release plastic additives, i.e. leachables, when in contact with liquid drug solutions. These compounds are of major concern regarding the toxicity and safety in relation to the exposure of the treated population.

For this study, different important hospital pharmacy compounding's, i.e., vancomycin in prefilled syringe (PFS) made of polypropylene (PP) material, pediatric parenteral nutrition (PN) in ethylene vinyl

acetate (EVA) bag and diluted insulin in cyclic olefin co-polymer (COC) vial, were selected for a comprehensive leachable profile monitoring and risk assessment. A dedicated leachable monitoring workflow including a targeted approach was used. Samples were analysed on a UHPLC method using an Acquity™ BEH Phenyl column hyphenated to an Orbitrap MS operating in PRM mode. A post-column infusion setup was applied (2% NH4OH in methanol at 2 µL/min) to boost compounds' signal intensities (ensuring a signal intensity enhancement from 2 to 100 times).

An internal database (DELTA) was used and leachable compounds were identified. As a result, 17 leachable compounds for the vancomycin prefilled syringe (PFS), 25 for the pediatric parenteral bag, and 10 for the insulin COC vial were detected and their concentrations estimated, thanks to a semi-quantification approach. A toxicological risk assessment was further performed for each of the three investigated compoundings. The toxicological risk was obtained by calculating the permissible daily exposure (PDE) and investigate the risk of potential endocrine disruptors. The total daily exposure (TDE) of two compounds surpassed their estimative PDE threshold in the PFS, i.e. a flame-retardant and an oxidized fenozan acid derivative, which were of concern. PN EVA IV bag released the highest number of additives compared to the other two DPs. The compounds of major concern were mostly related to label-related compounds, which penetrated the polymer packaging into the final compounding solution. However, none of their TDE surpassed their PDE threshold. Lastly, the COC vial containing insulin exhibited the release of the fewest compounds at the lowest concentrations, all significantly below their PDE thresholds. This underscores the optimal potential of COC material for long-term storage. Additionally, all investigated drug products (DPs) were deemed safe for use based on their positive risk assessments. This suggests that containers designed for administration could be a viable alternative to those intended solely for storage, though a thorough evaluation of the risk impact on patients would always be necessary.

PS02-13

SPME as a novel extraction approach for in vivo analysis of phytocannabinoids in Cannabis spp. plants

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Solid Phase Microextraction (SPME) is an non-exhaustive extraction technique that enables the extraction low molecular weight compounds directly from the living system (in vivo-SPME) [1]. This technique is especially useful for the analysis of phytocannabinoids (PCs) in plant material, both for forensic purposes and for monitoring the PCs content in growing Cannabis spp. plants [2,3]. With the use of biocompatible SPME probes, it is possible to use this extraction technique in direct contact with a living organism, minimizing the impact on its vital processes. In contrast to traditional extraction techniques, the use of the SPME technique does not require the collection of material for extraction, enables monitoring changes in analyte concentrations over time in the same place (e.g. inflorescence) [1,3]. This is particularly important in the case of Cannabis spp. plants, since concentrations of PCs are dependent on external growing conditions (e.g. light intensity, fertilizer composition, temperature, humidity). The goal of our study was to optimize a method for the extraction of 5 acid forms of phytocannabinoids, namely Δ9-tetrahydrocannabinolic acid (Δ9-THCA), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabiviarinic acid (CBVA), and tetrahydrocannabivarinic acid (THCVA) in growing Medicinal Cannabis plants. In our study, we used the Direct Immersion–SPME technique

coupled with quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS). Considering that the SPME technique requires strict optimization for reliable results during our study, we selected the appropriate extraction phase (coating) of SPME probes, extraction temperature and time, desorption time and composition of desorption solvent. The linearity range of the method was 10-3000 ng/mL. The method was used to extract PCs from two types of Medicinal Cannabis (THC-dominant and CBD-dominant). SPME probes coated with C18 sorbent were placed in Cannabis spp. inflorescences cultivated under controlled conditions for 30 minutes. Best to our knowledge it was first in vivo extraction of PCs from Cannabis spp. plants. The results confirmed that the developed method can be applied as a new strategy for PCs extraction. Moreover, the SPME technique can be used in industry as an additional quality control system for cultivation of Medicinal Cannabis or in basic research to track metabolic changes in Cannabis spp. plants.

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PS02-14

LC-MS/MS targeted-metabolomics of kynurenines in cell supernatants: a reliable readout for assessing the in vitro potency of selective hIDO1 and TDO inhibitors

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1. Introduction

The kynurenine pathway (KP) is crucial for tryptophan catabolism, impacting immune regulation and cancer. It involves heme-oxidoreductases, like tryptophan or indoleamine 2,3-dioxygenases (TDO, IDO1, IDO2), yielding immunosuppressive kynurenines within tumors.[1]
Research centers on inhibiting human indoleamine 2,3-dioxygenase 1 (IDO1) for cancer immunotherapy. IDO1 exists in two forms: holo-IDO1 (active) and apo-IDO1. Although inhibitors like epacadostat (EP) and linrodostat (LIN) have advanced in clinical trials, further exploration is warranted due to results.[2,3]

2. Aim of the work

The primary objective of this study is to investigate changes in the KP following selective inhibition of TDO or hIDO1. Our specific goals include:
1. Identifying metabolic differences post selective inhibition.
2. Evaluating the pertinence of the cell lines involved in the inhibition assays.
3. Determining the half-maximal inhibitory concentrations (IC50) in preclinical drug research.

To achieve these objectives, we have optimized an LC-MS/MS method for the rapid quantification of KP catabolites in the supernatants of three cell lines: U87 (glioblastoma), MDA-MB-231 (breast cancer), and A375 (melanoma).

3. Experimental

External calibration utilized standard solutions of Tryptophan (TRP), L-kynurenine (KYN), xanthurenic acid (XA), 3-hydroxykynurenine (3OHKYN), kynurenic acid (KA), 3-hydroxyanthranilic acid (3OHAA), anthranilic acid (AA), 5-hydroxytryptamine (serotonin, 5HT), tryptamine (TRYP). L-tryptophan-(indole-d5) (d5T) served as the internal standard due to its structural similarity with the other kynurenines. Method validation followed FDA, EMA, and the ICH M10 guidelines in terms of linearity, accuracy, precision, matrix effect, carry-over, and stability.

Cell lines were cultured, treated with IFNγ, LIN, BL5, EP inhibitors, or TDO selective inhibitor 680C91.[3] IC50 values were determined using dose-response curves of Graphpad 9.1.0. Data underwent statistical analyses, including PCA (Principal Component Analysis), biplot, correlation, heatmaps via Metaboanalyst 5.0.

4. Results and discussion

The study successfully validated a method comparing neat solvent and matrix-matched curves in DMEM and EMEM, demonstrating negligible matrix effects. Each analyte exhibited R-squared values above 0.99. A375 cells treated with IFNγ and 680C91 displayed alignment, indicating minimal TDO expression. Conversely, MDA-MB-231 and U87 cells exhibited differences, suggesting basal TDO presence, with U87 consistently expressing TDO. This finding could be exploited for the IDO1 selectivity assessment. Multivariate analysis (MVA) explored differentiation between apo/holo mechanisms. PCA revealed trends: IDO1 inhibitors correlated with higher TRP and lower downstream metabolites, while IFNγ/680C91 condition displayed elevated KYN and downstream metabolites, signifying IFNγ-induced enhanced metabolism. Our LC-MS/MS method enables the determination of IC50 values for EP, LIN, and BL5 in cell lines. The KYN/TRP ratio served as an indicator of IDO1 activity. Finally, metabolomics and PCR highlighted the absence of TDO expression in the A375 cells.

5. Conclusions

The validated LC-MS/MS method quantified KP catabolites in A375, MDA-MB-231, and U87 culture supernatants, enabling exploration of selective IDO1 and TDO inhibition and their metabolic implications. The results suggest the method's potential as a screening tool for cellular models and IDO1 inhibitors.

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PS02-15

Hunting for oxidized lipids in complex biological matrices

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Lipids can undergo numerous modifications through the introduction of small chemical groups via enzymatic and non-enzymatic reactions, which ultimately affect their structure, function, and reactivity1. Among these, lipid oxidation has been extensively documented in the context of oxidative stress-related disorders, including cardiovascular and metabolic diseases2. Technically, the discovery

and structural elucidation of oxidized lipids is still lacking, as their identification in biological samples is hampered by their low abundance and structural diversity. To deal with this complexity, advanced analytical and computational tools are required.

We developed a workflow based on advanced LC-MS/MS approaches for the accurate annotation of oxidized lipids in plasma and liver samples. The method combines a rapid 2.5-minute untargeted lipidomic analysis with our newly assembled pipeline for lipid and 'epilipid' annotation to support high-throughput detection of oxidized molecular species. In addition, we applied electron-activated dissociation (EAD) fragmentation for comprehensive structural elucidation of the annotated oxidized lipids. Indeed, EAD has the capacity to fragment virtually any C-C bond in the acyl chain, allowing saturation and oxidation to be identified by mass shifts of H(1.00) and O(16.00), respectively. Overall, the proposed method provides its potential to address epilipidome changes in an untargeted manner and to guide future research into the role of oxidized lipids in biological systems.

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PS02-16

Early Biopharmaceutical Assessment and Formulation Development in Drug Discovery

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Only a small proportion of biologically active compounds possess optimal biopharmaceutical properties to become drugs. These properties include pharmacokinetics, ADME (administration, distribution, metabolism, excretion) profile, and toxicity. Early assessment of biopharmaceutical properties accelerates the discovery phase and helps generate high-quality preclinical candidates. Moreover, most of these candidates have poor absorption properties, necessitating the development of suitable formulations for preclinical or later phases.

The Biopharmacy Platform at the Faculty of Pharmacy, University of Montreal, provides research support services for academic and industrial laboratories. This platform evaluates the biopharmaceutical properties of drug candidates during the discovery phase. Services provided include:

- Bioanalysis: Development and validation/qualification of quantification methods using liquid chromatography-mass spectrometry (LC-MS/MS). Comprehensive biological sample analyses are performed to detect and quantify drugs and their metabolites in various biological matrices. Additionally, the identification of metabolites is conducted to understand the drug's metabolic pathways and potential interactions.
- DMPK/ADME: The study of drug metabolism and pharmacokinetics (DMPK) includes understanding how a drug is absorbed, distributed, metabolized, and excreted (ADME) in the body. Coordination of in vivo studies with the animal facility is provided, with experiments conducted on mice, rats, and other species upon request. Various administration routes are explored, including oral

(PO), intravenous (IV), intraperitoneal (IP), intramuscular (IM), and subcutaneous (SC). Using HPLC-MS/MS, samples from plasma, blood, feces, urine, and tissues (liver, kidney, heart, lung, etc.) are analyzed to determine key pharmacokinetic parameters such as clearance (CL), bioavailability (F%), volume of distribution (Vd), area under the curve (AUC), and half-life (T1/2).

- Permeability: Permeability studies using Caco-2 and MDCK-MDR1 cell models are conducted to assess drug absorption. In vitro passive diffusion is evaluated through the parallel artificial membrane permeability assay (PAMPA).
- Blood-Brain Barrier Permeability: In vivo studies are conducted to evaluate the ability of drug candidates to cross the blood-brain barrier. These studies involve administering the drug to animal models and measuring the concentration of the drug in the brain versus the plasma over time.
- Stability: The stability of drug candidates in various biological systems is evaluated, including microsomal stability, hepatocyte stability, and plasma stability.
- Enzyme Inhibition: The potential of drug candidates to inhibit enzymes is examined, focusing on competitive and irreversible inhibition of cytochrome P450 enzymes, as well as P-glycoprotein (P-gp) inhibition.
- Protein Binding: Studies on the binding affinity of drugs to plasma and microsomal proteins are conducted.
- Genotoxicity: AMES test is conducted to determine the mutagenicity potential of drug candidates.
- Pre-Formulation Development: Optimized formulations are designed based on the specific properties of active pharmaceutical ingredients (APIs) to improve their stability and bioavailability. To address bioavailability challenges, services for the development of nanoformulations are also provided. These approaches enhance solubility, which contributes to improved bioavailability and physical stability of the compounds.

This comprehensive range of services ensures a thorough biopharmaceutical evaluation facilitating the transition from discovery to preclinical stages.

PS02-17

Development and optimization of an SFC-MS method for the simultaneous analysis of anticancer drugs with a wide range of physicochemical properties.

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Context: Anticancer agents in conventional chemotherapy include compounds with a wide variety of physicochemical properties, making their simultaneous analysis by RPLC-MS/MS challenging without making compromises. In this context, Supercritical Fluid Chromatography (SFC) could appear as a promising alternative technique for separating a wide range of diverse compounds, as demonstrated in the field of pesticides or metabolomics. This is due to the specific retention mechanisms of SFC, which differ totally from that of classical RPLC. To our knowledge, no publication has proposed an SFC method capable of analyzing a wide range of anticancer agents with such a diverse physicochemical property. Existing SFC methods for anticancer agents have been limited to the simultaneous analysis of only up to three compounds. This project aims to develop a generic SFC-MS/MS method to simultaneously multi-target 23 anticancer agents.

Methods: The first part of method development focused on optimizing the chromatographic behavior of these 23 anticancer agents (5-fluorouracil, busulfan, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, ganciclovir, gemcitabine, idarubicin, ifosfamide, irinotecan, methotrexate, paclitaxel, pemetrexed, raltitrexed, topotecan, treosulfan, vinblastine, and vincristine), in supercritical conditions, using a generic gradient from 2 to 100% methanol in CO2. The optimized parameters included: i) stationary phase chemistry, with nine different columns tested; ii) nature and concentration of additives; and iii) organic modifier composition.

Results: Under the selected generic conditions, only five stationary phases allow the elution of all compounds. Among them, only two columns (Diol and Nucleoshell) provide a partial resolution of the isomeric pair doxorubicin/epirubicine. The Diol column was finally selected as it provides better peak widths. Regarding the individual effects of the various tested additives, the combination of 50 mM ammonium formate and 2% water was the most interesting one, considering the compromise between better peak shapes and overpressure system. In the end, methanol was mixed with 20% acetonitrile, ethanol and isopropanol. However, these mixtures of solvents did not result in significant chromatographic improvements, nor did them improve the separation of isobaric pairs such as Doxorubicin/Epirubicine and Cyclophosphamide/Ifosfamide.

Conclusion: This project highlights the potential of the SFC-MS/MS to simultaneously analyze a wide range of conventional anticancer agents. The Diol column proved to be the most promising for analyzing the set of 23 anticancer agents within 12 min, using a gradient from 2 to 100% methanol containing 2% water and 50mM ammonium formate.

PS02-18

Comparison of hemispherical directional reflectance and thermal emittance of metformin extended-release tablets exposed to sunlight

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The number of people with type 2 diabetes mellitus (DM2) is constantly increasing in all regions of the world, especially in developed ones (e.g. Western Europe). Metformin is the first-line drug in the treatment of DM2 showing pleiotropic effects on glucose metabolism. Patients, especially older ones, often do not pay attention to the manufacturer's storage recommendations and store drugs contrary to these. Thus, drugs are repeatedly exhibited in sunlight and at elevated temperatures. Photodegradation may occur at any stage of a drug's production, distribution, or storage in the patient's house and it ultimately may lead to the loss of the active ingredient or its transformation, its reduced efficacy, or the formation of new adventitious products. Literature data indicate metformin's sensitivity to oxidizing conditions and temperature. The study aimed to analyze the reflectance and emittance of the metformin extended-release tablets within wide spectral ranges when exposed to sunlight falling on the drug through the window.

The metformin extended-release tablets (expiration date 05.2027) were studied. Total hemispherical reflectance (THR) was measured within 7 spectral ranges (from 335 nm to 2500 nm) with a 410-Solar Reflectometer (Surface Optics Corporation, San Diego, CA, USA). In turn, the emittance of the tablets was measured using ET 100 emissometer (Surface Optics Corporation, San Diego, CA, USA) within 6 spectral ranges (from 1,5 to 21 microns). The ET 100 device measures: 1) directional thermal emissivity

(DTE) at two angles of 20° and 60°, and 2) hemispherical thermal emissivity (HTE). DTE and HTE analysis was performed by simulating temperatures from 300 K to 1200 K. The analyzed parameters were measured at the beginning of the study and after 20 days of storing one randomly selected blister from the main cardboard packaging (i.e. 15 tablets) on a window sill, which was exposed to sunlight during the day. The research was funded by the Medical University of Silesia in Katowice, Poland (project number BNW-1-062/N/3/F).

The THR values after 20 days of metformin tablets exposure to sunlight through the window significantly decreased compared to the beginning tablets in the following spectral ranges of 480-600 nm (0.953 vs 0.941, respectively; $p=0.007$), 590-720 nm (0.964 vs 0.950, respectively; $p<0.001$), and 700-1100 nm (0.939 vs 0.927, respectively; $p<0.001$). In turn, metformin tablets after 20 days of sunlight exposure on window sill had higher hemispherical reflectance at 20° within micron ranges of 1.5-2.0, 2.0-3.5, 3.0-4.0, and 4.0-5.0 compared to the beginning metformin tablets ($p<0.001$ each). Hemispherical reflectance at 60° was found to be significantly higher for tablets after 20 days of sunlight exposure in all analyzed emissometer ranges than in the beginning tablets ($p<0.001$ each). The ε H parameter for the beginning tablets was 0.918 while for 20 days tablets, ε H was 0.921 ($p<0.001$). In addition, the values of DTE at 20°, DTE at 60°, and HTE lowered with increasing temperature.

In conclusion, storing extended-release tablets with metformin in unfavorable conditions may affect the physical structure of the drug, which is reflected in changing reflectance and its directional and hemispherical thermal emittance.

PS02-19

Non-Invasive Quantification of Protein Glycation in Drug Development: A Time Domain NMR Approach

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Glycation, a reaction between a protein's primary amine and the aldehyde group of reduced saccharides, significantly impacts the efficacy of protein-based drugs by altering their structure and pharmacokinetics. Traditional methods for detecting glycation are invasive and destructive, limiting their utility for comprehensive analysis. This study introduces Time Domain Nuclear Magnetic Resonance (TD-NMR) as an innovative, non-invasive technique that measures proton mobility to observe molecular dynamics, offering a versatile solution that avoids the drawbacks of existing methods. The primary objective is to establish a robust, non-destructive TD-NMR methodology for accurately quantifying glycation levels in protein-based drugs, with bovine serum albumin (BSA) and glucose serving as the model system. This research aims to provide a valuable tool for drug development and quality assurance by facilitating detailed glycation analysis.

This investigation integrates the O-phthalaldehyde (OPA) method for free amino group quantification with advanced TD-NMR techniques, specifically T1 and T2 relaxation times and T1T2 mapping, to comprehensively assess glycation impacts. The use of elevated temperatures to accelerate the glycation process enables a more efficient analysis within the experimental timeframe. Progressive glycation is observed to decrease T1 and T2 relaxation times, following a mono-exponential decay pattern. T1T2 mapping, a two-dimensional NMR technique, reveals distinct proton populations

affected by glycation, providing insights into the molecular alterations. Correlation analysis between the T2/T1 ratio and OPA method results confirms the TD-NMR method's capability to non-invasively and accurately quantify glycation levels.

TD-NMR emerges as a highly promising technique for the non-invasive quantification of glycation in protein-based drugs. Its ability to provide detailed molecular dynamics information without sample destruction makes it an invaluable tool for enhancing drug development, quality control, and ensuring the efficacy and safety of protein-based therapeutics.

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PS02-20

Enhanced Insulin Encapsulation Using Core-Shell Natural Polysaccharide-Based Aerogels for Oral Delivery

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Protein and peptide-based therapeutic drugs can only be delivered through intravenous and subcutaneous injections. These methods cause significant discomfort to patients because they require frequent and repeated dose administrations. Therefore, the development of oral protein drug formulations is of great importance for biotechnological and pharmaceutical technologies. However, oral delivery of therapeutic proteins to patients is a challenging issue in the pharmaceutical industry due to their low bioavailability. The main reasons for this low bioavailability are an acidic environment in the digestive tract, degradation of the drug by proteases, and inability to cross intestinal membrane barriers. To eliminate these challenges, aerogels can be used as delivery and release agents for therapeutic proteins. They stand out as promising drug carriers and delivery systems thanks to their homogeneous, large porous structure, high surface area and industrial-scale production feasibility. In this study, both blank and insulin-loaded natural polysaccharide-based conventional and core-shell aerogels were produced. Humulin R® U-100 was used as insulin source for both types of aerogels. For conventional aerogels, the drug was added to 2.0% (w/v) alginate solution and hydrogels were produced by dropping the solution via syringe pump into a crosslinking bath consisting of 0.5 M $CaCl_2$ in a 0.4% chitosan solution. Subsequently, the drops were placed into ethanol baths with progressively increasing ethanol concentrations to solvent exchange for further supercritical CO_2 drying. As an enhanced alternative for the conventional aerogels, the drug was encapsulated in the core of the aerogels. In the core-shell aerogels, a 1.5% (w/v) alginate solution was used in the core part, while the shell was formed with a 2.0% (w/v) alginate solution using a co-axial nozzle system. The solvent exchange and drying steps were followed in the same way as conventional aerogels. Pore characteristics and surface area of core-shell and conventional aerogels were compared by using the Barrett-Joyner-Halenda (BJH) and Brunauer-Emmett-Teller (BET) methods, respectively. The morphologies of the aerogels were examined using Scanning Electron Microscopy (SEM) images, and structural differences in the aerogels were observed through Fourier Transform Infrared (FTIR) spectroscopy. Finally, the insulin encapsulation efficiencies of core-shell and conventional aerogels

were determined by reverse-phase high-performance liquid chromatography (RP-HPLC). Encapsulation efficiencies of 12% and 52% for conventional and core-shell aerogels, respectively, were obtained. The results demonstrated that core-shell aerogels exhibited enhanced morphological and pore characteristics, as well as a four-fold increase in insulin encapsulation efficiency.

PS02-21

Microsampling Meets Microextraction: Bridging the Gap for More Efficient Drug Analysis

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In contemporary preclinical research, there is an increasing focus on adopting the 3R principles (refine, reduce, replace). A significant advancement in this area is microsampling. This approach allows for repeated, minimally invasive sampling from a single animal, significantly reducing the number of animals required for studies.

Volumetric absorptive microsampling (VAMS) is a novel alternative to traditional dried blood spot. This device features a plastic handle and a hydrophilic polymeric tip capable of absorbing precise blood volumes (10, 20 or 30 µL). The key advantages of VAMS include improved volumetric accuracy and reduced reliance of the assay on hematocrit levels. Typically, conventional sample preparation techniques, such as direct extraction into an organic solvent, are used to extract analytes from VAMS tips. However, combining VAMS with microextraction techniques makes the process more environmentally friendly and significantly lowers the sample dilution compared to conventional methods, thus enhancing the sensitivity of the analysis.

In our study, we explored the direct treatment of VAMS with electromembrane extraction (EME) for the determination of doxorubicin (DOX) and its metabolite, doxorubicinol (DOXol), in blood. DOX is a cytostatic drug from the anthracycline (ANT) group, widely utilized in both clinical practice and preclinical cancer research. The optimized EME conditions for the extraction of DOX/DOXol from blood in VAMS were as follows: the donor solution - 0.2 M formic acid (205 µL), the acceptor solution - 0.5 M acetic acid (75 µL), and the supported liquid membrane - mixture of 1-undecanol and 1-ethyl-2-nitrobenzene (1:1, v/v). The extraction took 25 minutes at an agitation speed of 850 RPM, with the voltage starting at 20 V and increasing to 30 V after 1 minute. In addition to EME, we developed a conventional extraction technique involving the desorption of DOX/DOXol from VAMS tips into acidified water, precipitation with acetonitrile, and reconstitution into 50% methanol. All extracts were analyzed using a UHPLC-MS/MS instrument. Upon comparing both methods, EME demonstrated superior time efficiency and environmental friendliness, leading to its selection for further experiments.

The following pre-validation experiments were conducted for EME to evaluate their impact on the assay results: the effects of different anticoagulants, various hematocrit levels, and the formation of ANT complexes with multivalent ions. These experiments were followed by full validation of EME extraction with UHPLC-MS/MS assay according to EMA guidelines. The applicability of our validated method was further demonstrated by analysing samples from an in vivo study, where DOX (5 mg/kg) was administered to mice to determine the pharmacokinetic profile (n=5). This study represents a significant step forward in the development of microsampling techniques for preclinical research,

offering researchers a more ethical, efficient, and environmentally friendly approach for blood sample collection and analysis.

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PS02-22

Development of an in vitro BBB model to study the passage of magnesium taurate and related molecules in the brain compartment.

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Magnesium and taurine play crucial roles in brain health. On one hand, magnesium plays a role in the synaptic plasticity of neurons, regulating metabolism and harmonizing the transmission of nerve signals. Magnesium depletion causes low-level inflammation, which contributes to neurodegenerative diseases. On the other hand, taurine modulates homeostasis, and neuronal activity, and has neuroprotective capacities. In this context, numerous magnesium vectors have been developed in recent years. Among others, acetylated forms are expected to confer greater lipophilicity and permeability. The aim of the present study is to compare the permeability of these related compounds across the blood-brain barrier (BBB).

First, an in vitro BBB model was developed using a human Brain Microvascular Endothelial Cell (hBMEC) line seeded on Transwell-like cell culture inserts. The passage of the analytes was measured from the upper to the lower chamber of the inserts before, during, and 24 hours after model exposure. A permeability QC was developed to assess representative model permeability using Lucifer Yellow, a fluorescent lipophilic molecule. The permeability of each insert was assessed before and after each test to ensure the integrity of the cells and their tight junctions. Next, the passage of taurine and related compounds was evaluated by an UHPLC (HILIC) MS/MS method, and the passage of magnesium ions was evaluated by ICP MS/MS. Both methods and their sample preparation procedures were carefully optimized to handle the complex culture medium.

PS02-23

High Throughput FAMS – A Fatty Acid Mass Spectrometry Method for Monitoring Polysorbate Hydrolysis in QC

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Surfactants are an essential component in biopharmaceutical formulations. As such, polysorbates (PS) are commonly used in protein formulations. However, polysorbate degradation has been highly discussed in recent years, since product stability and quality can be impacted due to particle formation.

With this contribution, we present an end-to-end (E2E) process for the quantification of free fatty acids (FFAs). We provide a step-by-step protocol for both manual and automated sample preparation for high-throughput application. The sample measurement is based on a liquid-chromatography single quad mass detector (LC-QDa) method, which can be applied for the quantification of lauric, myristic and oleic acid as well as for the quantification of of the longer chain fatty acids (LCFAs), palmitic and stearic acid. The monitoring of LCFAs can give critical insight into process improvements in development, routine manufacturing and QC testing, since their release due to polysorbate degradation and particle formation correlate.

For better method transition, we highlight potential watchouts and give troubleshooting advice.

PS02-24

First steps in 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

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In the drug development cycle of dermal pharmaceutics, drug formulation studies constitute one of the most laborious steps. This crucial step implies the importance of in-vitro formulation studies which stands out for its ability to predict drug behaviour. Thanks to analytical techniques, drug penetration and diffusion can be estimated in the different layers of the skin [1;2]. The European Medicine Agency (EMA) currently recommends a reference analytical method for in-vitro permeation studies that involves the use of a Franz diffusion cell, coupled with a separation technique such as HPLC for the monitoring of an active pharmaceutical ingredient through a skin model. This widely adopted and straightforward technique comprises significant drawbacks, including being time-consuming and lacking spatial information of the permeated substances into matrix [3-5].

Surface Enhanced Raman chemical imaging (SER-CI) is a Raman derived chemical imaging method, exalting the inherent Raman scattering of an analyte close to or adsorbed on metallic nanostructures [2]. Silver and gold nanoparticles (NPs) commonly serve as SERS substrates. The aggregation of these SERS substrates is known to lead to the formation of 'hot spots', which are regions with significantly enhanced Raman signals [2].

Combining spatial and spectral information, SER-CI emerges as a promising technique for drug monitoring within the biomedical field. However, this analytical technique faces a major challenge related to the deposition of SERS substrates onto analysed surfaces. This limitation results in non-homogeneous NPs deposition, leading to spectral intensity variability [6] which compromises the reliability of quantitative analyses. Given the lack of SER-CI implementation in biomedical applications, the present project aims to address these issues for the first time within the framework of drug penetration studies through the skin. Solid polymeric hydrogels, mimicking the structural properties of biological tissue, will be developed to establish a model matrix ensuring a homogeneous distribution of a selected molecule. A Quality by Design approach, which systemically identifies and controls critical parameters throughout the development process to ensure quality and performance, will guide the robust optimisation of homogeneous NPs deposition using an automated spray-coating device for enhanced reliability and reproducibility. Parameters such as spray-coating distance, NPs and gas flow

rate, spraying time and nozzle design will be considered in the initial optimization phase. This specific step is essential before conducting SER-CI parameters optimization from the conventional point-by-point spectral acquisition mode to the global imaging mode to balance analysis time and spectral resolution. This optimized SER-CI methodology will be applied to drug permeation studies of a pharmaceutical and a cosmetic substance on reconstructed and real skin tissues in forthcoming investigations.

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PS02-25

Online and automated sample handling – The future of fast and efficient mAb characterization and quantification

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Monoclonal antibodies (mAbs) represent an important tool for the treatment of critical diseases. To accelerate and lower the cost of therapeutic antibodies approval, optimization of established processes is essential. Online LC-MS systems can contribute to this intent by fast and automated online characterization of mAbs and quantification of their modifications during multiple phases of drugs' life cycle. With this contribution, we present our latest developments in the field of online sample handling by LC-MS system. Our system overcomes recent challenges of online LC-MS instruments by accelerating analysis, increasing sequence coverage, improving chromatographic separation, and combining various levels of mAb characterization within one system. In addition, for peptide mapping, we present our novel on-column digestion setup that enables more reliable identification and quantification of post-translational modifications.

PS02-26

Pick your method - A Toolbox for LC-MS Peptide Mapping

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We present a toolbox for different approaches of peptide mapping by liquid chromatography - mass spectrometry (LC-MS) for both, characterization and quantification of post-translational modifications (PTMs) of biopharmaceutical products. Serving regulatory requirements, bottom-up approaches involving sample preparation (e.g. denaturation, reduction, alkylation and enzymatic digestion) and LC-MS based peptide mapping analysis are the methods of choice. Here, we present two generic, fast and robust methods for monitoring PTMs by LC-MS and highlighting their benefits compared to conventional procedures. First, an online multidimensional (mD)-LC-MS approach allows an on-column

reduction, tryptic digestion and subsequent peptide analysis by LC - high resolution MS for characterization. Here, a direct analysis of the product can be performed in a short time (< 2 hours) with a low amount of protein (10µg). Second, we offer an automated approach by using a pipetting robot that enables sample preparation in a high-throughput fashion. An optimized buffer system minimizes artificial artifacts, whereas different digestion procedures provide high flexibility. In combination with a Single Quad LC-MS system, this method can be used in a routine quality control (QC) environment for quantification. Both methods point out optimized conditions, so that the analyst can flexibly pick the approach from the "toolbox" depending on the analytical request.

PS02-27

Design of experiments-based sample-preparation optimization of plasma samples for targeted metabolic profiling of neurologically relevant amino acids

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Many neurological pathologies are still poorly understood. Targeted metabolic profiling allows to gain more insights in the pathophysiological state of an organism and/or to obtain relevant biomarkers for a specific neurological disorder. As mice are frequently used in preclinical neurological research, the goal of the present study is to perform targeted metabolomics on mice plasma. However, this poses some challenges. The volumes of plasma obtained from mice are limited to a few hundred microliter. Furthermore, neurologically relevant metabolites typically occur in low concentrations, more specifically in the pico- to micromolar range. Plasma is a complex biological matrix, with a variety of compounds that can interfere with the aimed analysis. This makes sample preparation a decisive step in the success of the analysis. The aim of this project is to develop novel sample preparation methods for a better clean-up and preconcentration of volume-restricted plasma samples without loss of information. As animal studies strive to be as minimally invasive as possible, the study will focus on minimizing the amount of mice plasma needed, with goals set on 10 µL. A high performance liquid chromatography with fluorescence detection (HPLC-FLD) system is used, after an initial o-pthaldehyde/2-mercapto-ethanol derivatisation of the primary amine groups. Fifteen neurologically relevant amino acids will be analysed simultaneously. The sample preparation focusses on decreasing interfering compounds and improving the response for the targeted amino acids. The most widely used sample preparation for plasma is protein precipitation and will be used as a reference method to evaluate the novel sample preparation method. Different variants of protein precipitation have been used depending on the compounds and the customs of the host laboratory. However, comparative studies between different protein precipitation protocols for the analysis of amino acids are lacking. To have an optimal reference method, an optimisation of the protein precipitation protocol is performed with a design of experiments. A fractional factorial screening design is applied to select the most influential factors on the outcome. These factors will then be optimized through a response surface design. Future steps include the development and comparison of novel sample preparation methods with the optimized protein precipitation.

PS02-28

Expediting Online Liquid Chromatography for Real-Time Monitoring of Product Attributes to Advance Process Analytical Technology in Downstream Processing of Biopharmaceuticals

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The application of Process Analytical Technology (PAT) principles for manufacturing of biotherapeutics proffers the prospect of ensuring consistent product quality along with increased productivity as well as substantial cost and time savings. Although this paradigm shift from a traditional, rather rigid manufacturing model to a more scientific, risk-based approach has been advocated by health authorities for almost two decades, the practical implementation of PAT in the biopharmaceutical industry is still limited by the lack of fit-for-purpose analytical methods. In this regard, most of the proposed spectroscopic techniques are sufficiently fast but exhibit deficiencies in terms of selectivity and sensitivity, while well-established offline methods, such as (ultra-)high-performance liquid chromatography, are generally considered as too slow for this task. To address these reservations, we introduce here a novel online Liquid Chromatography (LC) setup that was specifically designed to enable real-time monitoring of critical product quality attributes during time-sensitive purification operations in downstream processing. Using this online LC solution in combination with fast, purpose-built analytical methods, sampling cycle times between 1.30 and 2.35 min were achieved, without compromising on the ability to resolve and quantify the product variants of interest. The capabilities of our approach are ultimately assessed in three case studies, involving various biotherapeutic modalities, downstream processes and analytical chromatographic separation modes. Altogether, our results highlight the expansive opportunities of online LC based applications to serve as a PAT tool for biopharmaceutical manufacturing. [1]

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PS02-29

Optimizing solvent dilution for enhanced performance in online comprehensive two-dimensional liquid chromatography: theoretical and practical guidelines

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With the increasing complexity of samples in diverse application areas such as pharmaceuticals, environmental studies, energy research and food science, conventional one-dimensional liquid chromatography (1D-LC) can be limited in terms of speed, sensitivity and ability to resolve multiple components. To address these challenges, comprehensive two-dimensional liquid chromatography (LC x LC) has emerged as an attractive solution, particularly in the online mode (online LC x LC), which has grown significantly in popularity in recent years.

Among the different chromatographic modes that can be combined, hydrophilic interaction chromatography (HILIC) and reversed-phase liquid chromatography (RPLC) are particularly interesting because they offer a high degree of orthogonality. However, the combination of these two modes remains complex to implement due to the opposite solvent strength in both dimensions. The first dimension (1D) effluent may adversely affect the 2D-separation, leading to strongly distorted peaks. To avoid this problem, one solution is to dilute the 1D-effluent with (zdilution -1) volumes of a weaker solvent added to one volume of the 1D-effluent, where zdilution represents the factor by which the fraction volume has been multiplied. This can be achieved either by using active solvent modulation technology or by using an additional pump, prior to the second dimension analysis.

The aim of this study (1) was to develop theoretical models to predict whether or not dilution can be effective, and, if so, what is the minimum zdilution value required. Our approach is based on the calculation of the ratio (called xdilution) between the peak standard deviation due to the injection process and the peak standard deviation in the absence of extra-column dispersion. Xdilution is calculated for any compound of interest on the basis of theoretical relationships and plotted as a function of zdilution, to predict the dilution required to obtain a good peak shape for that compound, bearing in mind that the maximum xdilution value was found experimentally to be equal to about 1. The proposed theoretical approach was experimentally validated on a number of representative small molecules and peptides. The agreement between experimental results and theoretical models was very good, especially for small molecules. Finally, it is shown that this approach, combined with previously developed prediction tools (2) can predict, for given compounds, the most appropriate set of conditions in HILIC x RPLC. This predictive capability is significant because it is expected to make the method development process simpler and more systematic, thereby accelerating the progress of 2DLC method development in many application areas.

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PS02-30

Determination of Ethylene Oxide Residuals in Sterilized Active Pharmaceutical Ingredients via Full Evaporation Headspace - Gas Chromatography

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Ethylene oxide (EO) is, due to its high diffusivity, reactivity and material compatibility, the gaseous sterilant of choice for terminal sterilization of pharmaceuticals. Although it is removed as much as possible after the sterilization process, harmful residues may remain. These include EO, but also its reaction products ethylene chlorohydrin (ECH) and acetaldehyde (AA), the determination of which requires sensitive analytical techniques.

Static headspace (sHS) gas chromatography (GC) is the reference technique for the determination of residual solvents in pharmaceuticals. Although the sample treatment for sHS is rather simple, it requires the preparation of a homogeneous sample solution. The high reactivity of EO and AA limits the usable solvents, while the physical process of dissolving may result in loss of these volatile components. Furthermore, the possible presence of ECH requires a solvent with a boiling point above 131 °C.

In this study, the full evaporation technique combined with headspace gas chromatography and flame ionization detection (FET-HS-GC-FID) has been explored for the determination of EO residuals in seven common active pharmaceutical ingredients (APIs). It uses standard GC equipment and involves minimal sample preparation steps. Separation of AA and EO was a challenge, but this issue could be well resolved. Sample matrix effects (MEs) as well as time-mediated interactions of EO and APIs were also evaluated. An equilibration temperature of 105 °C for 20 min was found sufficient to evaporate all the analytes. The limit of quantification (LOQ) for EO was 0.05 ppm while for ECH it was 1.25 ppm. The method showed good linearity with r2 equal to 0.9996 for EO and 0.9992 for ECH. Precision was acceptable with relative standard deviation (RSD) values below 2%. The recovery ranged from 90-103%. A ME was present for one out of seven APIs. Sterilized samples were used to test the applicability of the procedure. The developed method offers enhanced sensitivity and selectivity, less interference by MEs and it saves solvent, cost, and time.

PS02-31

Analytical Characterization of Plasmid DNA Topological Forms

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Plasmid DNA (pDNA) is an essential tool in genetic engineering with increasing prevalence in cell and gene therapies. Plasmids exist as supercoiled (SC), open circular (OC), linear forms, and even multimers that could be produced during the manufacturing process and storage. Even though the SC forms are thought to provide optimal knock-in (KI) efficiency, there is no strong consensus on the effect of other topological forms on the functional activity. In this study, a workflow was developed for the analytical and functional characterization of plasmid DNA. An anion exchange chromatography (AEC) method was first developed to quantify the topological forms and multimers. Four AEC columns were first compared, with one of them proving to have superior chromatographic performance. The effect of mobile phase pH, various salts, column temperature and acetonitrile content on the separation performance was systematically studied. The method performance, including precision and accuracy, was also evaluated. The final AEC method was compared to orthogonal techniques, such as ddPCR, by analyzing representative pDNA from various manufacturing and purification steps. A forced degradation study revealed unexpectedly high degradation of the SC forms between different formulations. Finally, the KI efficiency was evaluated for the SC and OC forms.

PS02-32

Negligible depletion in-vivo SPME for determining free and total endocannabinoid concentrations in rat brain

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Endocannabinoids (ECs) are a family of lipid-derived endogenous compounds that act as retrograde neurotransmitters in signaling processes mediated by activating specific cellular receptors called cannabinoid receptors type 1 (CB1) and 2 (CB2). Alongside metabolic enzymes, ECs and CBs compose the endocannabinoid system (ECS). Among the ECs identified so far, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the best known and most widely studied. CB1 can regulate the transmission and metabolism of various neurotransmitters in Parkinson's Disease (PD) lesions, thus regulating abnormal signaling in PD. In addition, CB1 modulates neuroinflammation, oxidative stress, excitotoxicity, neurodegeneration, and changes in cortical striatum plasticity in PD. Therefore, developing new analytical methodologies to quantify ECs in biological samples is desirable.

Currently, AEA and 2-AG are determined in biological systems on the basis of ex-vivo methodologies, by using collected biofluids or dissected tissues. However, these methodologies cannot accurately indicate or predict the complex processes occurring in living organisms because sample collection can disturb the system and alter sample composition. In this context, we propose a new in-vivo SPME methodology, under negligible depletion condition, to determine the free and total AEA and 2-AG concentrations in rat brain.

Hydrophilic-lipophilic balance (HLB) was selected as extractive phase because it has adequate extraction efficiency, is reproducible, and has no carry-over effect. All the experiments were conducted by using 6-cm nitinol fibers coated with small extractive phase volume (5-mm length, 15-µm thickness), which maintained the extracted amount below 5 % (negligible depletion conditions). The desorption conditions were optimized by using lamb brain homogenate as surrogate matrix fortified with analytes, where the use of pure acetonitrile promoted higher desorption efficiency. The other desorption variables such as desorption solvent volume, agitation speed, and desorption time were also optimized.

Extraction time profiles were conducted in PBS and brain homogenate, which revealed that equilibration was achieved in 20 and 15 minutes, respectively. Apparent distribution constants (Kfs) of 10.500 and 7.300 were determined for AEA and 2-AG, respectively, by performing SPME extractions in PBS solutions spiked with the analytes at varying concentrations and by plotting the extracted amount as a function of the analyte concentration. The distribution constants will be used for calibration and determination of the free AEA and 2-AG concentrations. The matrix binding percentage (MBP%) was evaluated by spiking a series of brain homogenate samples with varying concentrations of AEA and 2-AG, individually, and plotting a curve correlating the extracted amount and spiked concentration. MBP% was calculated on the basis of the slopes of the curves constructed in the presence and absence of matrix. MBP% was as high as 99.9% for both analytes. Total AEA and 2-AG concentrations can be determined on the basis of their free concentrations and MBP%.

The developed methodology will be used for in-vivo AEA and 2-AG analysis in the brain of rats submitted to an animal model of PD and control animals to investigate the potential of ECs to act as biomarkers.

PS02-33

A rarely advertised approach to sensitize LC-sQMS methods

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Signal summing is a well-known technique for increasing signal-to-noise ratio in various analytical methods [1]. Sensitivity enhancement through the summation of multiple reaction monitoring (SMRM) transition pairs in liquid chromatography-tandem mass spectrometric (LC-MS/MS) assays has also gained considerable popularity [2-5]. Surprisingly, the utilization of stable isotopologue transitions in such assays, known as multiple ion transition summation / MITSI method remains sparesely applied and is an underutilized technique in quantitative mass spectrometry [6-7].

Even more surprisingly -according to our literature survey- liquid chromatography-single quadrupole mass spectrometric (LC-sQMS) assays with sensitivity enhancement via the summation of stable isotopologues has not yet been reported.

The poster will present LC-sQMS limit methods developed for the analysis of the potentional genotoxic impurities of drugs containing bromine atoms. In these cases, the desired limits of detection (LOD) and quantification (LOQ would be hardly achievable with the available instrument without summing the bromine isotopologues of the impurities.

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Keywords: bromine isotopologues, LC-sQMS, signal summing

PS02-34

Instant and non-destructive analysis of medicines using cloud-based portable NIR technology and machine learning

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Falsified medicines represent a significant illicit market that is often linked to organized crime activities. In addition to potentially endangering the lives of those who use them, they can lead to significant economic losses and a drop of confidence in the health system. Although the scale of the problem varies greatly from region to region, it shows no sign of slowing down. It is therefore critical to maintain, strengthen and diversify strategies to understand and disrupt this phenomenon.

In this context, cloud-based NIR technology can make a major contribution by providing a real-time, portable, non-destructive and highly informative approach to tackle various problems associated with medicines. This may include, for example, the control of authenticity, the establishment of links between seizures of falsified products, the screening of imported medicines and quality checks.

This poster showcases the results obtained with falsified Viagra tablets as a practical example. The instrument used is an OnSite-W MicroNIR from Viavi Solutions (Scottsdale, USA) coupled with a mobile application (via Bluetooth) that sends the NIR spectra to a server where the statistical data treatment takes place.

It specifically discusses the possibility of distinguishing between authentic and falsified Viagra, as well as obtaining information on the excipients present. It also addresses the possibility of linking different seizures of falsified Viagra. Finally, a model for quantifying sildenafil in these samples, based on machine learning algorithms, is presented. In this context, 48 falsified Viagra tablets (seized by Swiss customs at the Zurich-Mülligen postal centre) were quantified using UPLC-UV to obtain accurate reference values on the amount of sildenafil (Master's project from Stefan Stanojevic, Faculty of Science, University of Geneva, 2023). These reference values are then used to create a quantification model based on NIR spectral data with the help of machine learning algorithms. Despite the small

number of samples used, the results are very promising, showcasing an average error of prediction of 6 mg.

PS02-35

Is Transmission surface-enhanced Raman spectroscopy a good alternative to separative techniques for the nicotine dosage in e-liquid boosters?

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During the last decade, the use of electronic cigarettes grew in popularity. They became an alternative for smokers especially for young people due to their attractive various flavours1,2. Since 2014, electronic cigarettes must follow the European Directive on tobacco products. This new legislation called for the development of new analytical tools to control the quality of these products and more especially to determine the nicotine dosage in the e-liquid boosters since the nicotine concentration must not exceed 20 mg/mL. Chromatography techniques are commonly used in this context but the development of alternative techniques being faster, greener and compatible with in-field analyses are required. In this study, the feasibility of using surface enhanced Raman spectroscopy (SERS) as an alternative analytical tool was evaluated through the development and the validation of new methods. A transmission Raman scattering (TRS), enabling to improve the sample representativity, and a handheld Raman system were used to perform acquisitions aiming to compare the analytical performances of both instruments. As pioneers in surface-enhanced transmission Raman scattering (SETRS), a robust method validation, ICHQ2 (R2) compliant, was performed to evaluate the analytical performances. The SERS samples were prepared using lab-made gold nanoparticles in suspension (AuNps) as SERS substrate, diluted reconstituted e-liquid booster spiked with different nicotine concentrations and nicotine-d4, added as an internal standard. To reach a SERS signal stabilisation, the SERS samples were analysed after 15 minutes. The methods developed on both equipment were successfully validated involving 6 series using a total error risk-based approach for a range of concentration from 100 to 300 ppb. To answer to the main question, these promising results could pave the way to the implementation of SETRS in laboratory routine analysis while handheld Raman systems combined with SERS could be considered for in field analyses.
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PS02-36

Untargeted metabolomic analysis of hawthorn-based food supplements: study of circulating molecules for rationalizing phytotherapy practices

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Hawthorn is a phytotherapeutic plant renowned for its cardiovascular benefits and relaxing properties. On the European market, a wide range of food supplements and herbal teas based on hawthorn (Crataegus monogyna and C. laevigata) is available. These products primarily differ in the origin of the plant material, the plant organ used, the recommended dosage, and the formulation. Consumers are often confused by the lack of information on labels, which impacts their trust in these products. To rationalize phytotherapeutic practices and guide consumers in selecting hawthorn-based phytopharmaceutical products, the phytochemical composition of commercially available hawthorn-based products was compared. Additionally, the bioavailability of hawthorn molecules was investigated.

An HPLC-HRMS untargeted metabolomics study was conducted on 14 hawthorn-based products, including 12 food supplements and 2 herbal teas, both before and after in vitro static digestion and assimilation-mimicking systems using the INFOGEST protocol and the Caco-2 cell monolayer model. Furthermore, the commercially available products underwent an HPLC-HRMS/MS analysis to annotate hawthorn-specific metabolites. The results showed that the manufacturing extraction method is the main factor discriminating the commercially available products. Fourteen hawthorn-specific metabolites were annotated, including procyanidin B2, kaempferol-3-glycoside, 3-p-coumaroylquinic acid, isoorientin, chlorogenic acid, rutin, vitexin, vitexin-2''-O-rhamnoside, hyperoside, isorhamnetin-3-O-glucose, isoquercetin, isovitexin, caffeic acid, and p-coumaric acid. Herbal teas had the highest levels of hawthorn compounds, particularly vitexin-2''-O-rhamnoside, while extracts had the lowest levels.

Molecular network analysis highlighted the lack of hawthorn information in existing databases and revealed unidentified or unreferenced molecules in hawthorn. No circulating hawthorn molecules were identified. This study underscores the phytochemical variability among commercially available hawthorn-based products and highlights the necessity for regulation and transparency in product labeling to enable clear comparison between products. Moreover, further research is needed to optimize protocols for assessing circulating hawthorn molecules.

PS02-37

Metaboscan AI – the ML-based platform for full-Cycle management of metabolomic data

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Metaboscan AI is an ML-based platform tailored for comprehensive metabolomic data management throughout the entire data processing cycle. It showcases a unified web application designed to streamline metabolomic projects, emphasizing proper data preprocessing, analysis, systematization and archiving. The platform integrates a range of functional tools within a single interface, including: ML-driven automatic peak integration; quality control assessment; data preprocessing; advanced statistical analysis; interactive data visualization; LIMS management system and preliminary pathology diagnostics.

Using an ML algorithm, Metaboscan AI enables an automatic peak integration that enhances the efficiency of chromatographic raw data processing and minimizes human errors. The system automatically generates essential data matrices for further analysis. Quality control assessment

ensures the accuracy of instrumental analysis, offering detailed reports in PDF format to highlight any discrepancies.

The data preprocessing module empowers users to customize workflows for data cleaning, normalization, and scaling. The platform's data analysis section not only supports traditional univariate statistical analysis, but also features an autoML function for constructing diagnostic and prognostic models using diverse machine learning algorithms.

Interpretation of the results may be intuitively performed using the Metaboscan graphics suite, providing visualization with tools like boxplots, histograms, correlation networks, bubble plots, heatmaps, and AUCROC curves. Pathway analysis capabilities spotlight metabolites that exhibit significant differences within the broader biochemical context of human metabolism.

Metaboscan LIMS serves as an automated laboratory information management system, streamlining the organization, management, and storage of metabolomic project results. It combines information on patient data, metabolomic profiling results, experiment design, instrumental analysis details, and sample storage within the laboratory biobank of the projects.

Additionally, the Metaboscan diagnostics module empowers clinicians to estimate the likelihood of cardiovascular and oncologic diseases in patients based on diagnostic ML models developed within the platform.

Overall, Metaboscan AI offers a transformative solution to automate routine procedures in metabolomic analysis, ensuring a seamless workflow from raw file receipt to result evaluation and report generation in a user-friendly format.

PS02-38

An attempt to explain the mechanism of statin-induced muscle pain using a multiplatform metabolomics approach

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Objectives: Myalgia is one of the most common side effects observed in patients during statin treatment. Considering that statins are the most commonly prescribed medications worldwide, and side effects are reported by up to 25% of treated patients, this issue may affect a large number of people. The abortion of therapy is a common result when a patient experiences myalgia. However, the resignation from the therapy significantly increases the risk of cardiovascular diseases. Despite, the scale of the problem, and the efforts of many scientists, the mechanisms of myalgia are still unclear. To shed new light on this problem, multiplatform metabolomics was involved to search, at the molecular level, for the information that may help in explaining the myalgia process.

Materials and Methods: Plasma samples were obtained from statin-treated patients with myalgia episodes (n=23) and without side effects (n=39). Additionally, patients at the moment of the diagnosis of the need for LDL-decreasing therapy, but before starting statin treatment, were included in the study as the control group (n=18). From the same patients, skeletal muscle samples (vastus lateralis) were collected. Samples were analyzed using two analytical platforms, gas and liquid chromatography with mass spectrometry detection, applying an untargeted metabolomics approach in both cases.

Moreover, simvastatin and its hydroxy acid active form were determined in plasma and skeletal muscle samples.

Results: In plasma samples, no significant difference was detected between the metabolic profiles of statin-treated patients with and without side effects. Additionally, separated comparisons of metabolic profiles based on this biological material, obtained from patients with or without myalgia versus the control group, show mainly lipid-decreasing statin effect. Moreover, there were no observed significant differences in the plasma concentrations of simvastatin and its active form between myalgic and non-myalgic groups. Furthermore, the accumulation of statin in skeletal muscle tissue was not confirmed.

Conclusions: Plasma metabolites do not directly differentiate patients with and without myalgia. Statin accumulation in skeletal muscle tissue is not observed, so probably it is not a reason for muscle symptoms.

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PS02-39

From untargeted to targeted HRMS-based proteomics: study of a biotechnological drug in plasma and CSF for Lafora disease

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INTRODUCTION: High-resolution mass spectrometry combined with micro-liquid chromatography (microLC-HRMS) was applied for quali-quantitative analysis of the biotechnological drug, VAL-1221. This drug consists of a fusion protein combining the Fab portion of a cell-penetrating antibody with recombinant enzyme human acid alpha-glucosidase. The two parts are connected via GGSGGGSGGGSGG tag corresponding to a unique non-endogenous peptide sequence, used for quantitative purposes. VAL-1221, previously studied for Pompe disease, exhibits efficacy in clearing polyglucosans, representing a potential therapeutic approach for Lafora disease (LD) too. LD is a rare and progressive form of myoclonic epilepsy characterized by the accumulation of polyglucosan which forms Lafora bodies (LBs) within tissues, particularly in the brain[1]. There is currently no approved treatment, other than symptomatic therapy and assistive devices. As demonstrated in LD mouse models, targeting LBs through the direct delivery of polyglucosan-degrading enzyme to the brain improves neurological phenotype, and this could represent a potential therapeutic approach for LD patients[2]. This study aims to investigate VAL-1221 in plasma and cerebrospinal fluid (CSF) by microLC-HRMS/MS-based untargeted to targeted proteomics. To this end, CSF sample analysis was conducted to determine the drug's ability to cross the blood-brain barrier.

METHODS: Six LD genetically confirmed patients with mid-to-late stage were enrolled at IRCCS-ISNB. For compassionate use 3 patients received the biotechnological drug intravenously. Plasma from treated patients was collected in order to study drug pharmacokinetics: before infusion, straight after the end of infusion, after 30 min, 1 h, 4 h, and 24 h. CSF samples were also collected and timing was tailored for each patient based on their plasma pharmacokinetic profiles within 1 h and 24 h after the end of infusion. Biological samples were stored at -80 °C until analysis. A bottom-up untargeted approach was employed for drug identification, focusing on peptide fragmentation profiling. The

GGSGGGSGGGSGG tag was targeted for monitoring purposes. Method validation, on spiked plasma of non-treated patients, encompassed precision and accuracy assessment, matrix effect evaluation, and stability studies. The developed methodology was applied to evaluate drug presence in biological fluid samples. These samples were processed using standard procedures for protein extraction, digestion and peptide cleanup. Analysis was carried out using microLC-HRMS.

RESULTS AND CONCLUSIONS: Method validation demonstrated high precision, accuracy, good sensitivity, and stability under storage conditions (RSD%≤15, RE%≤15). Matrix effect was minimal in CSF while it was significant and patient-dependent in plasma. The drug was identified in plasma of treated patients. Pharmacokinetics analyses yielded reproducible results: drug signal decreased relevantly after 30 min. No drug detectable traces were found after 24 h. In CSF samples the drug was not detected, under the method limit of detection. Despite the drug's presence in systemic circulation, these data show a limited penetration through the blood-CSF barrier, challenging therapeutic efficacy.

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PS02-40

Strategy for Multiplexing Absolute Quantification in Metabolomics with SILs as internal calibrant: one to quantify them all !

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Absolute quantification in metabolomics is essential for comparing metabolite levels in diverse samples and conditions, offering insights into responses related to diseases and treatments. Quantification also plays a vital role in biomarker discovery, helping identify specific metabolites that act as indicators of physiological states or diseases, with potential applications in clinical research, prognosis and diagnosis. To determine the concentration of endogenous analytes, an external calibration function is often constructed using a surrogate matrix. Since this surrogate matrix differs from the actual sample, it is necessary to evaluate parameters such as matrix effects and extraction recovery.

In this context, multi-targeted internal calibration is an attractive and simple approach that avoids the need for in-depth matrix similarity assessment. The principle is to use stable isotope-labeled standards (SILs) as internal calibrants to simultaneously quantify the corresponding endogenous analytes using a calibration within the biological sample. Scientific interest in direct internal calibration with SIL is growing due to its straightforward analytical process and its ability to quickly provide accurate quantitative results. However, some limitations remain. Even if suppliers are manufacturing an increasing number of high-quality SILs, their commercial availability is limited to the most important compounds of any chemical family, and costs can be expensive. To develop a method involving a large number of compounds, it would be interesting to use one SIL to quantify several analytes.

Internal calibration with a SIL is based on the response factor (RF), which is the signal ratio between the SIL and the analyte. The efficiency of ionization depends on the properties of the compound, the

matrix and the instrumental conditions. The ionization environment for a given analyte depends on its retention time, which, in turn, is determined by the solvent composition at elution. When a SIL with a retention time far from an analyte is used, a constant ionization environment can be created to stabilize the RF during the analysis by applying a counter-gradient supplied by a secondary pump connected to the column outlet flow. A constant ionization environment then allows the generation of response factors independent of the mobile phase conditions, with improved stability and easier inter-laboratory comparison.

To evaluate the impact of the RF value on the quantification performance, non-matching pairs of analytes and SILs were created from a set of endogenous analytes of interest. Response factors were calculated for each pair, and their quantification performance was assessed using Standard Addition Method (SAM). All pairs demonstrated good accuracy (between 70% and 130%), indicating that a response factor different from 1 can be used for quantification, provided it is determined in the matrix after sample preparation. Using a single SIL to quantify several compounds of interest is a promising approach to extending absolute quantification in metabolomics.

PS02-41

Development of an optimised icIEF method for harmonising Quality Control of Monoclonal Antibodies by using an AQbD approach

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Monoclonal antibodies (mAbs) are becoming a commonly used pharmacological therapy for several oncological, inflammatory, and autoimmune diseases principally due to their high specificity in target antigen binding, reducing the need for frequent dosing. Today, more than 75 mAbs and antibody-drug conjugates (ADCs) have been approved as biotherapeutic products by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA). During their production in cell culture and storage, mAbs are prone to Post-Translational Modifications (PTMs) such as deamidation, glycosylation or oxidation which produce charge heterogeneities. Since some charged-based variants can have an impact on pharmacokinetics, biological activity, and long-term storage, charge heterogeneities are considered a Critical Quality Attribute (CQA) by regulatory authorities which must be monitored and evaluated during biotherapeutics life cycle. This quality control (QC) approach is commonly achieved using ion exchange chromatography (IEC) or isoelectric focusing (IEF) techniques.

The imaged-capillary IEF (icIEF) technique has gained wide applications in biopharmaceutical QC due to its improved sensitivity and robustness. Compared to conventional cIEF, icIEF allows faster separation, higher resolution, and a simpler method development procedure. These advantages offer in the regulatory context a potential analytical platform for an effective detection of several PTMs-related charge-isoforms. To accomplish this, icIEF utilizes whole capillary imaging at 280nm - without a mobilisation step - to separate analytes and allowing the determination of the isoelectric point (pI).
To strengthen their independence in QC activities, Official Medicines Control Laboratories (OMCLs) are currently involved in the effort to harmonize analytical procedures, trying to develop less product-specific method which still ensure their performance and reliability.

The pI value is not typically presented in the biotherapeutics release specifications as an identity parameter because of its variability depending on the instrument employed to conduct the analytical test. Instead, the comparison of the Drug Product (DP) isoform pattern is lead with the one of the Reference Standard (RS). However, in certain situations – for example, the fight against counterfeit drugs - when a RS for comparison is unavailable, the measure of an accurate, precise, and universal pI value can be crucial in distinguishing one biotherapeutic from another.

Firstly, following the ICH Q14 guideline on AQbD, we performed a DoE - tuning the icIEF fundamental parameters - using Infliximab as a mAb standard and obtaining also a good measure of the main peak pI value, according to those available on the literature.

As evidenced in our preliminary results, we are confident of the achievement of a new independent, transversal, and effective icIEF analytical method with an enhanced accuracy in the measure of the pI values of mAbs.

PS02-42

Targeted metabolomics as a tool for therapy efficacy monitoring of autoimmune and hematooncological diseases

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Objective: The monitoring the efficacy of pharmacotherapy assists in choosing the right treatment strategy and is an essential part of personalized healthcare. Any pathological process is associated with metabolic changes, which analysis is an additional source of valuable information about the patient's state. This is particularly relevant in the context of diseases with complex treatment regimens and associated with significant discomfort, such as multiple myeloma (MM) and rheumatoid arthritis (RA).
Methods: To identify metabolomic markers of pharmacotherapeutic efficacy, we developed a method of targeted metabolomic profiling based on high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS). The analysis includes more than 100 metabolites belonging to different biochemical classes: amino acids, acylcarnitines, tryptophan metabolism products, organic acids, nucleosides and vitamins.

Multiple Myeloma: Patients were classified into three response groups post-treatment: Very Good Partial Response, Stabilization, and No Response.

Rheumatoid Arthritis: Patients received a three-month course of therapy, with metabolite levels monitored pre- and post-treatment.

Metabolite levels in plasma were quantitatively analyzed and correlated with treatment responses.
Results: Multiple Myeloma: A correlation was found between changes in plasma levels of alanine, histamine, histidine, leucine, isoleucine, valine, serine and methionine and response to treatment. In addition, the levels of these metabolites were found to be higher in the group with a good response to treatment than in the other outcome groups. This suggests that these metabolites may have prognostic value in the selection of appropriate anti-tumor therapy.

Rheumatoid Arthritis: Notable increases in tryptamine, kynurenine, and the kynurenine/tryptophan ratio were detected in RA patients compared to controls, while levels of branched-chain amino acids (BCAAs), histidine, and histamine decreased. After therapy, significant reductions were observed in levels of ADMA, asparagine, short-acylcarnitines, cytidine, DMG, kynurenic acid, BCAAs, tyrosine, tryptophan, serine, threonine, and methionine, correlating with positive therapeutic responses.

Conclusion: Targeted metabolomics provides a promising approach for monitoring and predicting patient responses to therapy in rheumatoid arthritis and multiple myeloma. These findings could pave the way for personalized treatment plans based on metabolic profiling. Implementing targeted metabolomics in clinical settings could enhance therapeutic strategies and improve outcomes by allowing for tailored treatment adjustments based on metabolic changes.

PS02-43

Lipidomics and metabolomics to investigate synergistic effects of erlotinib-HCl and tubacin anti-cancer drugs within 3D spheroid cell model of kidney cancer

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Single-drug cancer chemotherapies may lead to the rapid emergence of tumor resistance as they are usually acting on a single pathway. Their high clinical dose can also compromise patients' quality of life. Thus, low-dose synergistic optimized drug combinations (ODC) can improve antitumoral activity by targeting various signaling pathways while minimizing resistance and side effects.

Synergistic drug-drug interactions of erlotinib-HCl and tubacin were identified in a proprietary four-drug ODC containing histone deacetylase inhibitors (tacedinaline and tubacin) and tyrosine kinase inhibitors (erlotinib-HCl and dasatinib). Metabolomic and lipidomic analyses were conducted to evidence the potential pathways involved using human kidney homotypic 3-dimensional spheroids exposed to erlotinib-HCl, tubacin or ODC for 72 hours. The aim is to identify the metabolic sub-networks and mechanisms driving the synergy of these drugs, in order to gain a better understanding of the toxic effects of mixtures.

Polar metabolomics was obtained with a zHilic stationary phase (pH=9.3) coupled to an Orbitrap Exploris 120 in negative mode, while lipidomics was obtained on a C18 column and positive mode, resulting in 118 (97 level 1) metabolites and 543 MS2-confirmed lipids, respectively. Multivariate analysis showed distinct alteration patterns following exposure to tubacin and erlotinib-HCl. Polar metabolomics revealed increased levels of metabolites linked to the purine pathway in tubacin-treated spheroids or to Warburg effect and Krebs cycle pathways in erlotinib-HCl-treated cells, while the leucine/isoleucine degradation pathway was shifted by both drugs. Lipidomics exhibited other compound-specific signatures. For erlotinib-HCl, triglycerides were increased, while most phospholipids (phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and phosphatidylserines) decreased. Conversely, for tubacin, several monohexosylceramides were increased, while trihexosylceramides decreased.

This preliminary study provides indications on the biochemical events that occur in kidney cancer cells following exposure to erlotinib-HCl, tubacin and an ODC and highlights their different mechanisms of action and their potential synergistic effect within the tested mixture.

PS02-44

Gas Chromatography-Tandem Mass Spectrometry Method for the Quantitative Determination of Ethylene Glycol and Diethylene Glycol in Pediatric Syrups

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Ethylene glycol (EG) and diethylene glycol (DEG) are two contaminants that are known to be toxic to humans. These contaminants are mostly associated with glycerol, sorbitol, and polyethylene glycol-based drug syrups. In late 2022, World Health Organization issued an alert regarding cough and antihistaminic syrups that were found to contain toxic levels of EG and DEG in multiple countries, which resulted in serious injuries and fatalities among children. From an analytical standpoint, several methods of glycol analysis in pharmaceuticals have been reported in the literature, with the majority focusing on raw material analysis. We sought to develop and validate a selective method for evaluating a wide range of pediatric syrups collected from the local market to determine their safety. In this study, we present a method for determining EG and DEG using gas chromatography tandem mass spectrometry (GC-MS/MS), which has significantly higher selectivity than traditional single quadrupole gas chromatography-mass spectrometry (GC-MS). The developed method complied with the current validation guidelines established by the International Council for Harmonisation. The selectivity of the method was demonstrated by the absence of interfering peaks in both the unspiked syrup sample and the reference standard solutions. The detection limit for EG and DEG was 400 ng/mL, with a quantification limit of 1 µg/mL. The calibration curves for EG and DEG were linear in the concentration range of 1-10 µg/mL. The recovery values for both EG and DEG met the accuracy acceptance criterion (80-120%). Furthermore, the developed method was successfully used to analyze pediatric syrups collected from the local market.

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PS02-45

A single LC-MS/MS method to quantify 8 cannabinoids in blood, breast milk and meconium

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Purpose: With cannabis legalization in Canada in 2018, its use by pregnant and lactating women is becoming increasingly prevalent, often sought as a remedy for symptoms like anxiety, nausea, and vomiting. The impact of cannabis on the developing brain of infants exposed during pregnancy and

breastfeeding remains poorly understood, with insufficient exploration of its correlation with pharmacokinetics. To address this gap, a clinical study in Montreal is underway to evaluate the pharmacokinetics of cannabinoids in meconium, infant blood, and breast milk.

Methods: To quantify cannabinoids in meconium, infant blood, and breast milk, a liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated. Three different extraction methods were used for these complex matrices. Linearity, accuracy, precision, selectivity, matrix effect, carry-over, recovery, and stability were evaluated to validate the LC-MS/MS method. The method was tested on 16 breast milk samples, 7 whole blood samples and 12 meconium samples collected from women using cannabis during pregnancy and/or breastfeeding.

Results: The LC-MS/MS method was able to quantify 8 cannabinoids, i.e., THC, CBD, CBN, 11-OH-THC, 11-THCCOOH, 11-THCCOOH glucuronide, 7-OH-CBD and 7-CBDCOOH. Cannabinoids were extracted from whole blood and breast milk by solid phase extraction with HLB Prime cartridges and from meconium with MCX Prime cartridges. Calibration curves ranged from 1.5 to 500 ng/mL in breast milk and whole blood and from 2.5 to 750 ng/g in meconium. Precision (2.3-19.2%) and accuracy (83.7-114.1%) complied with specifications from international guidelines (ICH). The relative standard deviation of matrix effect and recovery was less than 15%. The method was successfully applied to meconium, whole blood and breast milk samples. THC (2.3 to 16.0 ng/mL) and CBD (3.6-77.9 ng/mL) were the only cannabinoids detected in breast milk samples. Conversely, only 11-THCCOOH (2.8 ng/mL), and 11-THCCOOH glucuronide (16.2 ng/mL) were quantifiable in the blood sample of one infant. Finally, all cannabinoids except 7-CBDCOOH were detectable in meconium samples.

Conclusion: In conclusion, this LC-MS/MS method enables the quantification of cannabinoids in meconium, infant blood, and breast milk allowing for the correlation of cannabis exposure during pregnancy and breastfeeding with newborn brain development. In the long term, this will help update Canadian guidelines on cannabis use during pregnancy and breastfeeding.

PS02-46

DEVELOPMENT OF HPLC METHOD FOR THE DETERMINATION OF ABEMACICLIB FROM LIPID-POLYMER HYBRID NANODELIVERY SYSTEMS

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Abemaciclib (ABE) is a CDK4/6 inhibitor that has been approved for the treatment of metastatic oestrogen receptor-positive breast cancer patients. However, there is a paucity of studies investigating the effect of ABE on triple-negative breast cancer (TNBC) treatment, which is associated with more aggressive behaviours compared to other breast cancer subtypes and exhibits a poor prognosis.

Nevertheless, the development of drug resistance against ABE and the occurrence of serious side effects in patients limit the effectiveness of treatment.

Nanoparticulate drug delivery systems offer the potential to regulate drug delivery, circumvent acquired drug resistance, selectively target cancer cells and enhance the pharmacological properties of the drug through advances in nanotechnology.

In the presented study, we describe the development and characterisation of a nanoparticulate drug carrier system for ABE for the first time and determine its therapeutic efficacy in vitro. In this context, the objective was to produce and characterise ABE-loaded lipid polymer hybrid nanoparticles (ABE-N), develop a selective, precise and accurate analytical method for quantifying ABE concentration using HPLC-UV, and investigate the efficiency of the drug delivery system.

A novel HPLC-UV methodology was developed for this purpose, utilising a Kinetex C18 analytical column (Phenomenex, USA) with a 150 mm × 4.6 mm i.d. and 5 µm particle size. The flow rate was set at 1 mL/min. The mobile phase consisted of water containing 0.1% orthophosphoric acid (pH: 2.5) and acetonitrile (80:20, v/v), with the column oven set to 25°C and the detection wavelength adjusted to 320 nm. The injection volume was 20 µL.

The method was validated in accordance with the criteria for selectivity, linearity, LOD, LOQ, precision, and accuracy as specified in the International Council for Harmonisation (ICH) guidelines. The method was found to be linear in the concentration range of 0.1-20 µg/mL for ABE, with correlation coefficient (R^2) values exceeding 0.999. The precision was found to be below 1.91%. The limits of detection (LOD) and quantification (LOQ) for the method were established using signal-to-noise ratios. The LOD and LOQ were found 0.03 µg/mL and 0.10 µg/mL, respectively.

Following the completion of the analytical method validation studies, the optimised method has been demonstrated to be suitable for routine analysis of ABE from its pharmaceutical preparation and drug delivery systems in both the pharmaceutical industry and research laboratories.

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PS02-47

INVESTİGATİON OF THE MOLECULAR İNTERACTİON BETWEEN APROCLİNİDİN, AN α2-ADRENERGİC RECEPTOR AGONİST, AND BOVİNE SERUM ALBUMİN WİTH FLUORESCENCE AND MOLECULAR DOCKİNG TECHNİQUES

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Apraclonidine(APR), a potent and selective α2-adrenergic receptor agonist; It is used in the diagnosis of Horner's Syndrome, which is characterized by shrinking of the pupil and drooping of the eyelid in case of paralysis of the muscles that dilate the pupil. In the study, BSA, the only peptide biomolecule with strong fluorescence, was used as a protein model. BSA has a strong ligand-binding capacity and is a carrier protein for numerous drugs, metals, nutrients, metabolites, and other substances. This protein enables the binding of these molecules due to the presence of two well-differentiated ligand binding sites (Sudlow regions I and II). Ligand-protein complex formation causes fluorescence quenching. Fluorescence quenching was observed to monitor any structural changes and complex

formation that BSA may undergo with the addition of ligand molecules.The analysis of the interaction of APR with plasma protein is very important. In this study, fluorescence spectroscopy, which is often used to analyze ligand-protein interactions due to its high sensitivity and selectivity, was selected to comprehensively study the combination of APR with bovine serum albumin (BSA). In addition, the study was supported by computational docking. A binding affinity was obtained for the APR-BSA interaction. The thermodynamic results revealed that the APR-BSA bonding reaction is a spontaneous process governed predominantly by hydrogen bonds, van der Wals, and hydrophobic forces. The Ka values (ranging from 1.18 to 1.59 × 10^3 M^{-1}) indicate a considerable binding affinity between APR and BSA. The obtained findings suggest that the fluorescence quenching of BSA by APR involves a mix of dynamic and static quenching processes. The thermodynamic analysis indicated that the binding process is primarily driven by entropy, as seen by the positive ΔS^o value. Additionally, the system exhibits an endothermic character, as indicated by the positive ΔH^o value. The hydrophobic forces play a large part in the reaction. Furthermore, the presence of a negative ΔG^o indicates that the binding of APR to BSA occurs spontaneously. Based on the molecular docking research, it was shown that APR had a higher binding affinity for DS2 in comparison to DS1.This work investigated the spectroscopic methods and molecular simulations for evaluating the binding ratio, binding mode, binding locations, binding constants, important actions, and changes in the structure of BSA during its interaction with APR.

PS02-48

Design and Applications of Electrochemical Nanozyme Platform Consisting of Magnetic Nanomaterials and Inorganic Nanomaterials

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Although catechol is an industrial waste, it is a phenolic compound that has many harmful effects on human health, such as skin irritation and eye sensitivity. Catechol is also one of the main materials widely used in the chemical industry such as textile products, pharmaceutical industry, cosmetics, antioxidants, dye materials, and can also be found in water as waste. Many different methods have been developed to date for the determination of catechol. Among these methods, electrochemical techniques attract the attention of researchers due to their rapid results, low cost, high sensitivity, easy application and wide determination ranges. In this study, a nanozyme-based electrochemical sensor that mimics enzymes was developed for the determination of catechol. For this purpose, the GCE surface was modified with FeMNP/ZnCl (Iron magnetic nanoparticles/Zinc chloride) nanocomposite, which has magnetic properties and contains inorganic nanomaterials, synthesized from waste tea with green synthesis method, and MWCNT (multi-walled carbon nanotubes), which was prepared by dissolving it in chitosan. Plasma method was applied to modify the surface with magnetic nanomaterial. Autolab PGSTAT302N model potentiostat was used for all electrochemical procedures. The electrode surface was characterized using SEM (Scanning Electron Microscope), and the surface properties were studied in detail using EIS (Electrochemical Impedance Spectroscopy) and CV (Cyclic Voltammetry) methods. DPV (Differantial Pulse Voltammetry) method was used for catechol determination, catechol was determined in the concentration range of 1.0 x10^{-9} – 1.0 x 10^{-8} M, and according to the results, LOD was calculated as 2.59 x 10^{-10} M and LOQ was calculated as 7.87 x 10^{-10} M. In addition, the developed method allowed the successful determination of catechol in serum, green tea and black tea samples. According to the recovery studies performed in serum, catechol was determined with 100.3% recovery. The innovative and environmentally friendly electrochemical

detection platform has demonstrated a wide range of potential applications for routine, rapid and reliable catechol detection, and successful results have been achieved.

PS02-49

APTAMER-BASED HYBRID MONOLITHIC SORBENT FOR SELECTIVE EXTRACTION OF HOMOCYSTEINE IN BIOLOGICAL SAMPLES

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Homocysteine (HCY) is a sulfur-containing non-essential amino acid and an intermediate product of methionine metabolism in human body that presents itself at both free (thiol and disulphide) and protein-bounded forms [1]. Several studies reported that elevated levels of plasma total HCY (> 15 µmol L-1) are associated with increased risk of many disorders including Alzheimer's Disease (AD). Determining biomarkers in biological samples, including cerebrospinal fluid and plasma, represents the most direct means to study AD progression and to detect the AD preclinical and symptomatic stages. Sample preparation is the most important step during when applying LC-MS/MS methods to biological fluids because it removes endogenous macromolecules and pre-concentrates trace-level analytes. In this work, an aptamer-based hybrid monolithic sorbent for selective extraction of homocysteine has been developed to be used as extraction phase coupled online to the LC-MS/MS system. The hybrid organic-inorganic monolith was prepared via the in-situ sol-gel synthesis in a fused silica capillary (10 cm × 530 µm i.d.) using tetraethoxysilane and 3-aminopropyltriethoxysilane as precursors [2]. The permeability of the monolithic capillary was equal to 2.3 × 10-13 m². The synthesis procedure was followed by covalent grafting of two different aptamers (named HCy1 and HCy2) described in literature as able to specifically trap HCY (Kd in the order of hundreds nM) after their modification with 5'-amino-C12 spacer arm. A grafting yield of 96% and 82% were obtained respectively. The extraction procedure was first optimized in pure media using both monolith capillaries grafting with Hcy1 and HCy2 before being applied to biological fluids.

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PS02-50

Drug quality control in Senegal using capillary electrophoresis: retrospect on 12 years of work

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Medicines suitability and conformity controls are complex and different levels of action are needed. Among them, the quality control (QC) of different batches of drugs, although this strategy is often difficult to apply in developing countries due to (i) the lack of suitable analytical equipment, (ii) the high cost of say instruments, maintenance and consumables, and (iii) the low availability of reference substances and consumables. A large panel of techniques are used, and the gold standard is the HPLC which requires different type of column and huge amount of organic solvents.

Since 2012 Pharmhelp is helping Sénégal with the quality control of drugs using capillary electrophoresis. First by gifting the cheikh anta diop university's faculty of health of the ECB and afterwards by supplementing it by the WynSep WynCE®.

To analyze a high number of compounds and benefit from the devices, simple and generic methods were developed. Some of them were validated and some new methods were developed by the team in UCAD. About 15 molecules from the WHO list of essential medicines were analyzed in available drugs in Senegal, Benin and Gabon. More than 200 samples of manufactured drugs were collected and controlled.

Analyzed preparations have included quinine, furosemide, phenobarbital, sulfamethoxazole, trimethoprim, paracetamol, ibuprofen, diclofenac, metronidazole, amoxicillin and captopril, ramipril, Chlorpheniramine maleate, Salbutamol sulfate, and Insulin.

Results of these QCs were published then sent to the medical authorities of Senegal and developed method could be applied at the national control laboratory for drugs.

This analytical tool combined with method validation has a quantitative analytical robustness and accuracy and bring a reliable help for the QC of most medicines available in developing countries. Otherwise, this project comes to sustain education and competence transfer between University of Geneva and some developing countries.

One of the prospects is to introduce a portable device that will be designed and manufactured in Switzerland and then assembled in Senegal by local engineers. Seminars could be done to implement the skill transfer on building and maintaining a low-cost CE, and in other lab guidelines

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PS02-51

Developpement and validation of a capillary electrophoresis method for the control of salbutamol sulfate

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Salbutamol sulfate is a drug commonly used in the treatment of asthma. In this work, a fast, simple, sensitive, and financially accessible capillary electrophoresis (CE) method has been developed and validated for the identification and dosing of salbutamol-based drugs. The analysis was carried out using an CE WynSep. The separation took place in a silica capillary (total length: 33 cm; effective length: 25 cm; internal diameter: 50 µm), applying a voltage of 15 kV, with an absorption wavelength set at 200 nm. The analysis was carried out using a 50mM phosphate buffer at pH 5.3, ultra-pure water as a solvent and phenobarbital as internal standard. The samples were injected hydrodynamically for 5 s at 50 Mbar and the capillary temperature was kept at 25 °C.

The method has been validated following ICH guidelines on selectivity, linearity, fidelity, and accuracy. Under the best experimental conditions, the separation of the analyte and the internal standard takes less than 5 minutes.

The results obtained showed a good selectivity of the method with the non-interference of the peaks of salbutamol sulfate and phenobarbital. It also showed satisfactory linearity in the validity range (80 to 120 ppm). The statistical analysis of the results obtained under the conditions of repeatability and intermediate fidelity gave coefficients of variation that varied between 0.27 and 0.79% and 0.58 and 1.92%, respectively. About the accuracy, the recovery rates were between 99.94 and 102.21%. From the coefficients of variation of intermediate fidelity the tolerance limits Wele established, the lowest being at 96.20% and the higher at 98.87%, which are within the limits of acceptability set at 10% of the target concentration.

The results are found satisfactory, the method has been declared valid and reliable. It can be successfully applied for the dosage of salbutamol-based drugs.

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