ChromSoc

The Chromatographic Society - founded 1956


# PROGRAMME, ABSTRACTS AND DELEGATE INFORMATION

## 25th INTERNATIONAL REID BIONALYTICAL FORUM

The Cambridge Belfry
Cambourne, Cambridge, UK
*4th – 7th September 2023*

Presented by
the Forum Syndicate of the Chromatographic Society

**Domestic Arrangements**

All meals are included for residential delegates. Lunches and refreshments are included in the registration fee. Conference badges will need to be shown for all meals as well as for conference attendance.

Breakfast for residential delegates is in The Bridge Restaurant.

Buffet lunches will be served in the College Suite. Tea and coffee will be served in the College Suite.

**Monday** – The evening buffet meal will be served in the University Suite from 18:00. There will be a European themed quiz night hosted by Ludo and Tim.

**Tuesday** – We will be heading into Cambridge for dinner at Peterhouse College, University of Cambridge.

Coaches will depart from outside the Cambridge Belfry at 16:30. The reception begins at 18:45, leaving a little time for delegates to explore Cambridge on their own. Dinner will be served at 19:30.

Return departures from Peterhouse College are at 22:15. Please be prompt.

The Tuesday evening event is sponsored by VRS Recruitment.

**Wednesday** – The evening Conference Dinner will be held on-site at the Cambridge Belfry in the University Suite.

Pre-dinner drinks are served from 19:30.

## Chromatographic Society

The Chromatographic Society is an international organisation devoted to the promotion and dissemination of knowledge on all aspects of chromatography and related separation techniques. The Society was founded in 1956 as the Gas Chromatography Discussion Group. Over the years it expanded, and its name was changed to the Chromatographic Society in 1984. The Society runs several one day and longer short courses and symposia. Members are entitled to a discount at most of the Society's events.

The first Forum met in 1975 organised by the late Eric Reid, then Director of the Wolfson Bioanalytical Unit at the University of Surrey. It has run every two years since (with a little wobble for COVID), making this the 25$^{th}$ meeting. Now run under the auspices of the Chromatographic Society, it retains much of its original character, encouraging young practitioners to learn from more experienced ones and appreciating the sharing of challenges as much as successes.

## Organising Committee

The organising committee is a sub-committee of the Chromatographic Society. Its members are Jon Bardsley, Rebecca Mogey, Sankeetha Nadarajah, Timothy Sangster, Ludovicus Staelens, Neil Spooner, Elizabeth Want and Amanda Wilson.

## Exhibitors and Sponsors

The organisers would like to express their thanks to exhibitors and sponsors, who contribute to covering the cost of the meeting. The exhibition will be held on Tuesday 5th and Wednesday 6th September in the College Suite.

## Oral Presentations

All oral presentations will take place in the University Suite. A computer and projector will be available. Presenters should contact the Chair for their session and load their presentations from memory stick in the break before their session begins at the latest. Use of your own laptop is not allowed.

## Posters

Posters will be displayed in the exhibition area in the College Suite. Posters will be on display for the full duration of the meeting, with selected posters having the opportunity to give a 5 minute presentation of their work on Tuesday afternoon. A list of posters can be found at the end of this programme booklet. If you have brought a poster but it is not listed, then please see one of the Conference Organisers for help.

The poster boards are set in portrait and are suitable for posters of size A0 portrait.

# Conference Programme at a Glance

## Pre-Conference Training Course – Monday 4th September

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|---|---|
| **11:30** | **Registration**<br>*Hotel entrance foyer* |
| **12:30 – 17:00** | **A Working Guide to Immunogenicity Training Course**<br>*Magdalene Room – Veerle Snoeck (UCB, Belgium) Lysie Champion (Celerion, UK), John Cook (Charles River Laboratories)*<br>Lunch will be served in the Restaurant for training course attendees |
| **18:00** | **Networking Reception & Quiz for Forum Attendees**<br>*University Suite* |

## Day 1 - Tuesday 5th September

| | | |
|---|---|---|
| **08:00 – 09:00** | **Registration**<br>*Hotel entrance foyer* | |
| **09:00 – 15:40** | **Conference Oral Sessions**<br>*University Suite* | |
| **09:00 – 09:05** | **Welcome**<br>Tim Sangster & Neil Spooner (Co-Chairs International Reid Bioanalytical Forum) | |
| **Bioanalysis of the Future**<br>*Session Chairs – Jon Bardsley (Thermo Fisher Scientific) & Neil Spooner (Co-Chair International Reid Bioanalytical Forum)* | | |
| **09:05 – 9:25** | **1H NMR screening of modifications to the metabolic profiles of human saliva following food consumption: Implications for the use of this biofluid for metabolomics analysis** | Georgina Page (De Montfort University) |
| **09:25 – 09:45** | **All in One: High Throughput LC/MS-Based Bioanalysis, Metabolite Identification, Imaging and Omics From Small Studies** | Ian Wilson (Imperial College) |
| **09:45 – 10:05** | **Driving more sensitive and selective bioanalysis using accurate mass spectrometry** | Heather Jane Hughes (Sciex) |
| **10:05 – 10:25** | **Optical photothermal infrared spectroscopy: A novel solution for rapid identification of antimicrobial resistance at the single-cell level via deuterium isotope labeling** | Sahand Shams (Liverpool University) |
| **10:25 – 10:55** | **Break / Vendors / Posters**<br>*College Suite* | |

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|---|---|---|
| **Regulated Bioanalysis**<br>*Session Chairs – Ludo Staelens (UCB) & Philip Timmerman (EBF)* | | |
| **10:55 – 11:15** | **Overview of EBF strongholds for 2023** | Philip Timmerman (EBF) |
| **11:15 – 11:35** | **Singlicate analysis for immunogenicity assays** | Johannes Stanta (Celerion) |
| **11:35 – 11:55** | **Overview of a MHRA Laboratory Inspection and case study** | Michael McGuiness (MHRA) |
| **11:55 – 13:25** | **Lunch / Vendors / Posters**<br>*College Suite* | |
| **13:25 – 14:25** | **Poster Blast –** *A number of our poster presenters will each give a brief presentation on their work*<br>*Session Chairs – Elizabeth Want (Imperial College) & Rebecca Mogey (Chester Zoo)* | |
| **14:25 – 14:55** | **Break / Vendors / Posters**<br>*College Suite* | |
| **14:55 – 16:00** | **Career Stories**<br>*Session Chairs – Sarah Palfrey (VRS) & Amanda Wilson (AstraZeneca)* | |
| **16:00** | **Off-Site Dinner –** Coaches depart from the hotel at 16:30. Reception starts at 18:45. Dinner at 19:30. Return coaches at 22:15<br>*Peterhouse College, University of Cambridge* | |

## Day 2 – Wednesday 6th September

| | | |
|---|---|---|
| **Open Session**<br>*Session Chairs – Tim Sangster & Neil Spooner (Co-Chairs International Reid Bioanalytical Forum)* | | |
| **09:00 – 09:20** | **Efficient and timely biotransformation support: Delivering impact at critical decision points** | Daniel Weston (GSK) |
| **09:20 – 09:40** | **Assessment and implementation of fast chromatography in a discovery DMPK workflow** | Ben Smith (Evotec) |
| **09:40 – 10:00** | **Pharmaco- and proteometabodynamics: Connecting pharmacokinetics to pharmacology via drug effects on metabolites and proteins** | Ian Wilson (Imperial College) |
| **10:00 – 10:20** | **Remote blood microsampling for research into lead toxicity** | James Rudge (Trajan Scientific and Medical) |
| **10:20 – 10:50** | **Break/ Vendors / Posters**<br>*College Suite* | |

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|---|---|---|
| **10:50 – 11:10** | **Quantitative dried blood spots (qDBS) and dried urine spots (DUS): Applications for the accurate determination of biomarkers** | Olga Angeliki Begou (Aristotle University of Thessaloniki) |
| **11:10 – 11:30** | **LC-MS/MS method development of a New Chemical Entity (NCE) to evaluate in vivo chiral inversion in a pharmacokinetics (PK) study** | Syeda Shah (UCB Pharma) |
| **11:30 – 11:50** | **Strategies for measuring oligonucleotides using Ligand Binding Assays and other techniques** | John Chappel (Gyros Protein Technologies) and Issa Jyamubandi (Resolian) |
| **11:50 – 12:10** | **Antisense Oligonucleotide plasma protein binding determination in multiple species using a single LC-MS/MS assay** | Philip Waghorn (Charles River Laboratories) |
| **12:10 – 13:40** | **Lunch / Vendors / Posters**<br>*College Suite* | |
| **How I Learned from Things That Didn't go to Plan**<br>*Session Chairs – Ian Wilson (Imperial College) & Elizabeth Want (Imperial College)* | | |
| **13:40 – 14:00** | **Is small molecule bioanalytical method development always straightforward? Pitfalls and lessons learnt from a recently established LC-MS/MS assay** | Sergio Menta (Roche) |
| **14:00 – 14:20** | **Don't trust the darkness, darker is always possible** | Svenja Stiltz (Nuvisan) |
| **14:20 – 14:40** | **Development of rat ex-vivo whole blood assay to assess off target effects of AZ modalities** | Rajit kolamunne (Astra Zeneca) |
| **14:40 – 15:00** | **Peak tales in bioanalysis - Causes, fixes and a case study** | Pou I Long (Resolian) |
| **15:00 – 15:30** | **CAMS (Community for Analytical Measurement Science) and the state of the nation for separation sciences initiative** | Ali Salehi-Reyhani (Imperial College) |
| **15:30 – 16:00** | **Break/ Vendors / Posters**<br>*College Suite* | |
| **16:00 – 17:00** | **Bridging the Skills Gap**<br>*Session Chairs – Tim Sangster (Co-Chair International Reid Bioanalytical Forum) & Sarah Palfry (VRS)* | |
| **19:00** | **Conference Dinner with Mr Flavour Cocktails**<br>Speaker Peter Van Eenoo (DoCoLab; Ghent University)<br>Toast – Tim Sangster (Celerion)<br>*University Suite* | |

## Day 3 – Thursday 7th September

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|---|---|---|
| **Bioanalysis Beyond Pharma**<br>*Session Chairs – Rebecca Mogey (Chester Zoo) & Jon Bardsley (Thermo Fischer Scientific)* | | |
| **09:00 – 09:30** | **An antibody-free LC-MS/MS method for the quantification of sex hormone binding globulin and its mutated form in human serum and plasma** | Bas Sleumer (ICON) |
| **09:30 – 09:50** | **Biosynthesis of natural sunscreen compounds from cyanobacteria** | Afeefa Kiran Chaudhary (Liverpool University) |
| **09:50 – 10:10** | **Development and comparison of bioanalytical methods to measure free analyte** | John Chappel (Gyros Protein Technologies) |
| **10:10 – 10:30** | **Method development strategies to overcome selectivity issues using Gyrolab technology for PK analysis of new modalities** | Tom Wilfor (Resolian) |
| **10:30 – 11:00** | **Break**<br>*Lounges* | |
| **11:00 – 11:20** | **Targeted and untargeted metabolic profiling of mice liver extracts using a hybrid zwitterionic hydrophilic interaction liquid chromatography column** | Artemis Lioupi (Aristotle University of Thessaloniki) |
| **11:20 – 11:40** | **A simple robust method for synthetic therapeutic RNA. New chemistry, new quantitation** | Ken Cook (Thermo Scientific) |
| **11:40 – 12:00** | **Metabolic fingerprint and profiling analysis of cytochrome b5-producing E. coli N4830-1 using FT-IR spectroscopy and GC-MS** | Thanyaporn Tengsuttiwat (University of Liverpool) |
| **12:00 – 12:20** | **Seeing red - a suitable LC-MS/MS extraction for studying ADC exposure in in vitro bone marrow models containing phenol red** | Rachel Foreman (AstraZeneca, UK) |
| **12:20 - 12:30** | **Closing Remarks**<br>*Tim Sangster & Neil Spooner (Co-Chairs International Reid Bioanalytical Forum)* | |

# Full Programme and Speaker Abstracts

## Pre-Conference Training Course - Monday 4th September

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| **12:30 – 17:00** | ***A Working Guide to Immunogenicity Training Course***<br>*Magdalene Room – Veerle Snoeck (UCB, Belgium) Lysie Champion (Celerion, UK), John Cook (Charles River Laboratories)*<br>Lunch will be served for training course attendees in the Hotel Restaurant |
| **18:00** | **Networking Reception & Quiz for Forum Attendees**<br>*University Suite* |

**A Working Guide to Immunogenicity Training Course**

*Veerle Snoeck (UCB, Belgium) Lysie Champion (Celerion, UK), John Cook (Charles River Laboratories)*

In this training course we will discuss 3 specific areas what is Immunogenicity, the implication for bioanalysis and drug development for both clinical and non-clinical programs and then how to develop and validate the required assays.

In the first section we will go over the immune responses that generate antibodies in response to external stimulus from foreign substances (antigen). The aim is to give the delegates a working understanding of the biology in this area as this underlines the challenges of immunogenicity.

In the second section we will discuss the impacts of immunogenicity on drug development. The aim of this section will be to explain wanted and unwanted immunogenicity and also explain the concept and impact of antidrug antibodies on drug development and will discuss both the preclinical and clinical aspects.

In the final main section we will discuss the particular bioanalytical aspects in 3 sections:

- Assay development – including reagent generation, sensitivity and drug tolerance.
- Assay validation – reviewing the current requirements and discussing the learnings from the tutors.
- Sample analysis – discuss the application of these assays to sample analysis and discuss the challenges and specifically study specific cut points and highlight with case studies.

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## Day 1 Abstracts

**1H NMR screening of modifications to the metabolic profiles of human saliva following food consumption: Implications for the use of this biofluid for metabolomics analysis**
*Georgina Page, Martin Grootveld, Mark Edgar, Kayleigh Hunwin, Wyman Chan & Mohammed Bhogadia (De Montfort University, UK)*

Introduction: It has been previously demonstrated that there is a lack of standardization regarding the fasting/oral abstention periods required when human saliva is collected for analysis. Without standardized fasting, the 1H NMR profiles of human saliva are often contaminated by a range of dietary carbohydrates, organic acid anions and lipids. Therefore, 1H NMR-determined concentrations of such agents fail to reflect the actual, baseline levels of these agents found in this biofluid. The aim of this study is to establish a standardised fasting period for the collection of saliva samples for NMR-based metabolomics analysis.

Methods: Whole mouth saliva (WMS) samples were collected from n = 7 healthy human participants (mean±SEM age 22.57±1.05 years). Overnight fasted samples (≥8 hr) were primarily obtained, with subsequent samples collected 2, 30, 60, 90 and 120 min. following consumption of a breakfast meal. Saliva samples were centrifuged, and supernatants subjected to 600 MHz  1H NMR analysis.

Results: Subsequent to meal consumption, dietary-ingested citrate persisted at markedly increased levels than those found in pre-meal baseline samples for durations of ≥2 hr. in 6/7 participants. As expected, salivary glucose and sucrose concentrations substantially increased immediately after eating but returned near pre-meal levels within 2 hrs. for all participants. Further endogenous metabolites affected by meal consumption were lactate, trimethylamine-N-oxide, pyruvate, histidine, phenylalanine, succinate, formate and alanine.

Conclusions: We recommend that an absolute minimum of a 4 hr. (but preferably 8 hr.) fasting/oral abstention period is required for all future 1H NMR-linked metabolomics studies prior to WMS sample collection.

**All in one: high throughput LC/MS-based bioanalysis, metabolite identification, imaging and omics from small studies**
*Ian Wilson (Imperial College, UK)*

Advances in microsampling, LC, MS and techniques such as ion mobility (IM), both as separation technique and a further means of characterizing analytes through the determination of collision cross section (CCS) values, increasingly offer the potential to obtain comprehensive DMPK and biomolecular "omics" information from single, relatively small scale, rodent studies.

Here investigations aimed at returning the maximum amount of information describing the properties and effects (pharmacological or toxic) of drugs from small animal studies are described. In all of this work a tiered approach to sample analysis was taken with the determination of the PK of the dosed compound prioritized, followed by the characterization of the circulating and urinary excreted drug metabolites. Further biofluid analysis was then performed to determine effects on the metabolome (urine) and lipidome (plasma). Finally selected tissues were then analysed for parent drug and metabolites, lipids, proteins and finally by DESI/MS imaging. This workflow will be illustrated using studies on the TKI inhibitor gefitinib and the antidiabetic drug fasiglifam administered to the mouse and rat respectively.

The ability to use state of the art instrumentation to extract all of this information from the same microsamples, rather than having to perform individual "bespoke" studies, has obvious benefits with respect to the "3Rs", but arguably also produces better science.

Whilst the approaches that will be outlined were designed to be applied to rodent samples in the "drug discovery" phase of development it has not escaped our attention that they could easily be translated into later studies, including the clinic.

**Driving more sensitive and selective bioanalysis using accurate mass spectrometry**
*Heather Jane Hughes (Sciex, UK)*

Quantitation of small and large molecule therapeutics in biological matrices is important during drug development. Quadrupole-based liquid chromatography-mass spectrometry (LC-MS) has been routinely adopted for quantitation of therapeutics in bioanalytical laboratories. Advances in accurate mass spectrometry have allowed it to become a complementary option for quantitative bioanalysis.

These advances include greater selectivity, improved mass resolution and the flexibility of time-of-flight (TOF) MS/MS for data analysis. Here, quantitative analysis was performed on the ZenoTOF 7600 system, where the application of the Zeno trap enhanced overall MS/MS sampling efficiency and boosted quantitative sensitivity for bioanalysis.

**Optical photothermal infrared spectroscopy: A novel solution for rapid identification of antimicrobial resistance at the single-cell level via deuterium isotope labelling**
*Sahand Shams (Liverpool University, UK)*

The rise and spread of antimicrobial resistance (AMR) has become a growing concern, and a threat to the environment and human health globally. Most current antimicrobial resistance (AMR) identification methods in clinical settings rely on time-consuming and expensive culture-dependent techniques. Consequently, appropriate antibiotic stewardship is often provided retrospectively, with the initial treatment relying on the hope that a broad-

spectrum antibiotic will be effective. Hence, culture-independent and single-cell technologies are needed to allow for rapid detection and identification of antimicrobial-resistant bacteria and to support a more targeted and effective antibiotic therapy preventing further development and spread of AMR. In this study, for the first time, a non-destructive phenotyping method of optical photothermal infrared (O-PTIR) spectroscopy, coupled with deuterium isotope probing and multivariate statistical analysis was employed as a metabolic fingerprinting approach to detect AMR in Uropathogenic Escherichia coli at both single-cell and population levels. Principal component-discriminant function analysis of FT-IR and O-PTIR spectral data showed clear clustering patterns as a result of distinctive spectral shifts (C–D signature peaks) originating from deuterium incorporation into bacterial cells, allowing for rapid detection and classification of sensitive and resistant isolates at the single-cell level. The C–D signature peak at 2,163 cm−1 in the single-frequency images indicated diminished deuterium incorporation by the trimethoprim-sensitive strain when exposed to the antibiotic, in comparison to the untreated condition. Hence, the results of this study indicated that O-PTIR can be employed as an efficient tool for the rapid detection of AMR at the single-cell level.

**Overview of EBF strongholds for 2023**
*Philip Timmerman (EBF, Belgium)*

In his presentation, Philip will provide an overview of the recent, current and future discussion in the EBF related to the challenges in regulated bioanalysis of NCE, biotherapeutics and other modalities. This includes the ongoing efforts to support a harmonised implementation of the ICH M10 guideline, discussions related to hybrid assays, challenges for bioanalysis labs related to GCP and Biomarkers/CoU.

In addition, Philip will provide more detail on EBF's initiatives to develop the future generation of bioanalytical leaders as part of the EBF university and young scientist community (connecting at the yearly young scientist symposium).

**Singlicate analysis for immunogenicity assays**
*Johannes Stanta (Celerion)*

The traditional approach for ligand-binding assays has been to generate multiple measurements of the same sample from adjacent wells on a 96-well plate. There is no regulatory or statistical requirement to follow this practice and we questioned the true benefit of this ‘duplicate analysis’ by interrogating previously generated ligand binding based anti-drug antibody (ADA) data to assess the implication of this traditional assay set-up.

We looked at ADA data from 5 studies from different modalities with duplicate measurements. We determined the impact singlicate analysis would have on the results and what that means for the overall immunogenicity interpretation. We developed a pre-clinical ADA assay in singlicate and analyzed study samples with a streamlined singlicate approach.

We found that singlicate analysis for ADA assays has no impact on the overall interpretation of immunogenicity. We show how it can be implemented in the Bioanalytical laboratory without reducing scientific integrity and quality and how much resource can be saved.

**Overview of a MHRA Laboratory Inspection and case study**
*Michael McGuiness (MHRA, UK)*

An overview of what to expect when you are being inspected, aiming to de-mystify the process. The session will also include a case study highlighting issues seen during a bioanalytical inspection promoting where lessons can be learned.

## Day 2 – Wednesday 6th September

| | | |
|---|---|---|
| **Open Session** | | |
| *Session Chairs – Tim Sangster & Neil Spooner (Co-Chairs International Reid Bioanalytical Forum)* | | |
| **09:00 – 09:20** | **Efficient and timely biotransformation support: Delivering impact at critical decision points** | Daniel Weston (GSK, UK) |
| **09:20 – 09:40** | **Assessment and implementation of fast chromatography in a discovery DMPK workflow** | Ben Smith (Evotec) |
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| **14:00 – 14:20** | **Don't trust the darkness, darker is always possible** | Svenja Stiltz (Nuvisan, Germany) |

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| **14:20 – 14:40** | **Development of rat ex-vivo whole blood assay to assess off target effects of AZ modalities** | Rajit Kolamunne (Astra Zeneca, UK) |
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| **16:00 – 17:00** | **Bridging the Skills Gap**<br>*Session Chairs – Tim Sangster (Co-Chair International Reid Bioanalytical Forum) & Sarah Palfry (VRS)* | |
| **19:00** | **Conference Dinner with Mr Flavour Cocktails**<br>Speaker Peter Van Eenoo (DoCoLab; Ghent University)<br>Toast – Tim Sangster (Celerion)<br>*University Suite* | |

## Day 2 Abstracts

### Efficient and timely biotransformation support: Delivering impact at critical decision points

*Daniel Weston (GSK, UK)*

This presentation will outline the use of mass spectrometry for biotransformation, specifically the detection and structural elucidation of drug metabolites within DMPK in support of projects, whilst focusing on the impact of these data upon critical decisions. Biotransformation support is employed across the drug discovery and development continuum, with varying levels of resource, rigour and turnaround time to best fit the question at hand.

Firstly, a novel strategy to support discovery metabolism and aid chemical design will be presented, showing increased compound throughput and translatability by analysing residual in vitro clearance samples at drug concentrations of 0.5 µM, bolstered by in silico predictions. Evolving strategies for software-assisted data mining, structure elucidation and automatic reporting are also shown, to help increase efficiency and decrease inherent prediction biases for data interrogation, rationalisation and forming initial hypotheses.

In terms of maximising data impact – whilst high-resolution mass spectrometry is our primary tool – the added leverage when used in concert with other key analytical techniques (e.g. NMR, amongst other novel approaches) to help provide critical data ahead of key decisions points (de-risking early) will also be highlighted. Aside from the technical focus, the impact of these biotransformation data will be discussed, along with the current challenges – delivering more relevant data faster, the use of software for data interrogation, changing culture – and future directions.

**Assessment and implementation of fast chromatography in a discovery DMPK workflow**
*Ben Smith (Evotec)*

In the current rapid nature of drug discovery there is a growing need for fast chromatography. Small molecules make up a large proportion of drug discovery workloads allowing rapid generic approaches to increase throughput and instrument availability while also decreasing solvent usage. Bioanalytical methods play a vital role in the drug discovery process, making them important targets for fast chromatography development.

Here we present the assessment and implementation of a fast chromatography setup as the generic approach in a bioanalytical lab which sits within drug discovery DMPK analysis. This includes: an overview of the fast chromatography setup and accompanying LC conditions as part of our routine LC-MS/MS analysis, chromatographic data showing the peak shape, separation and matrix effects of the fast chromatography system and comparative analysis of bioanalytical PK samples demonstrating the suitability of fast chromatography versus our previous method. The overall benefits of reduced acquisition times and costs as well as any limitations such as sensitivity will also be discussed.

**Pharmaco- and proteometabodynamics: Connecting pharmacokinetics to pharmacology via drug effects on metabolites and proteins**
*Ian Wilson (Imperial College, UK)*

In the same way that pharmacokinetics and pharmacodynamics have been shown to be related to drug concentration vs time profiles (PKPD) we can clearly demonstrate a similar linkage to drug effects on the metabolome/lipidome and the proteome.

That such effects exist is not a surprise but does represent a currently unexploited source of information on the properties of drugs. In particular the ability to see effects on the biochemistry of animals (and humans) may provide insights into pharmacology, mechanism of action, and “off target” effects such as toxicity and unexpected pharmacology. The latter may provide alternative targets or opportunities for drug repurposing that, if discovered at an early stage, may maximize the potential value of new compounds.

However, at its most basic, the ability to compare the system-wide effects of candidate drugs at an early stage will enable compounds with the lowest potential to interfere with pathways that are not directly related to the intended pharmacology to be identified thereby ensuring that compounds with the fewest unwanted side effects in patients are selected.

These concepts will be demonstrated based on studies in rats and mice designed to obtain the maximum amount of information using the minimum number of animals.

**Remote blood microsampling for research into lead toxicity**
*James Rudge (Trajan Scientific and Medical, UK), Ciprian Cirtiu & Anthony Breton (INSPQ, Canada)*

In 2021, the World Health Organization (WHO) reported that approximately one million global deaths were from lead exposure annually. Additionally, exposure to this trace metal accounted for 30% of the global idiopathic intellectual disability, 4.6% of cardiovascular disease, and 3% of renal diseases. Lead contamination is found in many sources, including food, drinking water and some manufactured products, such as old plumbing pipes and paint products [1]. Currently, blood lead levels (BLL) are measured through venepuncture

(5-10 mL). However, this sample collection method can be stressful and challenging for vulnerable study cohorts, particularly in paediatrics where lead toxicity is of particular concern. Moreover, wet blood samples typically require cryo-transportation and storage, making logistics challenging for epidemiological studies and others.

To overcome these challenges, a capillary dried blood lead assay, using ICP-MS/MS, was successfully developed and validated employing 30 µL Mitra® devices with VAMS® technology. To minimize any trace lead contamination, the VAMS tips on the Mitra devices were washed using a novel approach prior to sampling. Results obtained from the dried VAMS extracts were then compared to samples collected using traditional venepuncture. Within the initial cohort (n=29), VAMS samples showed an average 40% higher BLL compared to venepuncture. However, the use of handwashing prior to sampling a second cohort (n=28) vastly reduced this observed bias (>75%). Moreover, blood collected using Mitra-VAMS showed good stability for Pb at room temperature.

1. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

**Quantitative dried blood spots (qDBS) and dried urine spots (DUS): Applications for the accurate determination of biomarkers**

*Olga Angeliki Begou, Olga Angeliki Begou, Stelios Papazoglou, Helen Gika, Georgios Theodoridis (Aristotle University of Thessaloniki, Greece)*

Dried Matrix Spot is a sampling technique that involves collecting a small volume of biological fluid, using a specialized filter paper. It offers several advantages compared to traditional liquid sample collection methods, in terms of simplicity, stability, and cost-efficiency. Thus, DMS gained popularity in various fields, including metabolic profiling applications. The storage and transportation of liquid specimens constitutes a major concern, especially in cases of sampling from remote areas. DMS is an easy and safe sampling technique, less invasive in comparison to conventional venipuncture, making it particularly suitable for sample collection, among neonates, the elderly, and patients requiring frequent and repetitive testing. Urine amino acid analysis has been proven essential for clinical or nutritional studies. Their determination from dried urine spots is of high potential for biomarker quantification, as it eliminates the need for cold sample transport. In that manner, an efficient method for the quantification of 14 amino acids was developed and validated, in order to evaluate its clinical applicability.

Moreover, two validated methods were developed for the accurate and reproducible determination of 4 different ceramides and 12 acyl-carnitines in qDBS. Both methods provided robust results for metabolites implicated in regulation of diverse cellular processes. Different extraction solvents and solvent ratios were tested to reach optimum analyte extraction. Extraction recovery, matrix effect and short- and long-term stability were also evaluated. DMS microsampling provides an attractive alternative for the quantitation of a plethora of metabolites and can be widely exploited in the field of bioanalysis for health and wellness monitoring.

**LC-MS/MS method development of a New Chemical Entity (NCE) to evaluate in vivo chiral inversion in a pharmacokinetics (PK) study**

*Syeda Shah (UCB Pharma, UK)*

A quantitative, enantioselective liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for a small molecule with a single chiral

centre. The purpose of this work was to assess a suspected in vivo metabolic chiral inversion of the molecule in an in vivo pharmacokinetic (PK) study. Chromatographic screening of authentic standards of the enantiomers was carried out using a range of chiral columns under various isocratic conditions to achieve optimum chromatographic resolution. Protein precipitation was used for analyte extraction from 40 µL of dog plasma. The subjects were given a po dose of either 1, 3 or 10 mg/kg followed by blood sample collection at timepoints over a 72-hour period. For the analysis, a Shimadzu liquid chromatography system was coupled to a Sciex QTRAP 5500 mass spectrometer operated using multiple reaction monitoring (MRM) in positive ion mode. The analytical method was able to successfully separate the two enantiomers. A significant level of chiral inversion was observed by quantitation of the individual enantiomers. This work helped in establishing routine chiral bioanalysis capabilities within the group.

**Strategies for measuring oligonucleotides using Ligand Binding Assays and other techniques**

*John Chappel & Frida Löthberg (Gyros Protein Technologies, Sweden) & Issa Jyamubandi (Resolian, UK)*

Oligonucleotides are a growing class of therapeutics with FDA approved therapies primarily for genetic diseases, but with many clinical studies underway for use in treating cancers. Development of these therapies has traditionally been challenging from a bioanalytical perspective with different and diverse methods for quantitation such as ELISA hybridization assays, LC-MS/MS and capillary electrophoresis being used to support preclinical and clinical studies. Each method has advantages and disadvantages in terms of relative sensitivity as well as challenges with the quantitation of metabolites.

This presentation will display how ELISA based hybridization assays can be adapted onto an automated microfluidic immunoassay platform and compared to other techniques. Various oligonucleotide chemistries and different assay formats were evaluated. The effect of n-1 – n-3 metabolites were evaluated to assess interference.

The aim was to develop a fast, efficient, and precise ligand binding assay that used small sample volume and delivered results quickly.

**Antisense Oligonucleotide plasma protein binding determination in multiple species using a single LC-MS/MS assay**

*Philip Waghorn (Charles River Laboratories, UK)*

Due to an increasing focus on gene therapies within the pharmaceutical market, there is a corresponding increase in demand for the efficient bioanalysis of oligonucleotides. Although these types of molecules are commonly analysed by both LBA and LC-MS/MS techniques, there are particular study designs where LC-MS/MS can offer a distinct advantage. A recent in-vitro plasma protein binding study in four species, for a 20-mer antisense oligonucleotide, provides a good example of the advantages that LC-MS/MS assays can provide.

By using LC-MS/MS a single assay was produced that enabled the quantification of buffer samples and both filtered and unfiltered plasma samples (from four different species) all against a single calibration line. This was accomplished despite the absence of a stable label internal standard. To achieve this, it was necessary to overcome the common challenges of oligonucleotide analysis such as their highly charged nature requiring ion-pairing LC conditions and their tendency to form adducts limiting sensitivity. This presentation will

discuss the measures taken to overcome these challenges, along with the additional challenges arising from combining multiple different matrices into a single assay, including interspecies variation in matrix effects and recovery, non-specific binding and stability issues in buffer and filtered plasma.

**Is small molecule bioanalytical method development always straightforward? Pitfalls and lessons learnt from a recently established LC-MS/MS assay**
*Sergio Menta (Roche, Switzerland)*

A good understanding of the intended use of the assay, knowledge of the analyte and proper experimental planning play a crucial role in bioanalytical method development. These are fundamental pillars in order to avoid unexpected findings during method validation and subsequent sample analysis.

In this presentation, a Roche direct experience on a recent method development will be described. The lack of information about analytical liabilities of the drug resulted in multiple bioanalytical challenges. These included an unpredictable analyte instability trend in biological samples, with lack of correlation between different matrices and across multiple species. In addition, the unavailability of in vivo biotransformation data resulted in a serious method specificity issue due to unstable metabolites interfering with the quantification of the parent compound. The bioanalytical strategy which was adopted to promptly mitigate them will be presented, along with an evaluation of the potential impact on the data generated using a developed method which was deemed appropriate and fully validated.

To conclude, the complexity of an additional method required to quantify an impurity of the same drug will be presented, describing how its tissue exposure could be demonstrated.

All the above clearly indicate that small molecule LC-MS/MS bioanalytical method development can be very challenging in the lack of key enabling information, with potential significant impact on the project progression.

**Don’t trust the darkness, darker is always possible**
*Svenja Stiltz (Nuvisan, Germany)*

There are various effects that can promote the degradation of substances in solution, in matrix or during sample preparation. Temperature, different pH values, adsorption as well as ultraviolet light can influence the stability of analytes. Therefore, it is important to protect compounds from degradation. For the quantitative determination of light-sensitive substances, it is important to avoid daylight during sampling, sample preparation and storage.

Here, we present the method development and validation of two assays for light sensitive compounds, namely, on the one hand, Doxorubicin, a cancer-fighting cytostatic drug that prevents DNA replication by inhibiting topoisomerase II. On the other hand, we developed different assays for Coproporphyrin I (CP I) and III (CP III). Coproporphyrins are metabolites arising from heme synthesis and can serve as biomarker for several pathologies. In addition, CP I and CP II are relatively selective substrates for OATP1B1 and OATP1B3 and can therefore be used as clinical biomarker to access OATP1B function.

Although a general light sensitivity was known for these analytes, inexplicable effects were observed during assay development. We describe our search for the reasons of these

effects, how they were identified and overcome. In the end, we were able to validate both methods successfully.

**Development of rat ex-vivo whole blood assay to assess off target effects of AZ modalities**
*Rajit Kolamunne, J. Basak, S. Marianna, L. Carrie & Amanda Wilson (Astra Zeneca, UK)*

In the whole blood multicellular environment, the interplay of plasma and red blood cells (RBS) has hindered the development of off-target safety screen. Since there are no validated methods, kinetic assays have been used to measure acetylcholine esterase (AChE) activity exclusively in the whole blood environment. It is not clear whether the activity of AChE is measured separately from the activity of other plasma-bound enzymes such as butyryl cholinesterase (BChE). We successfully developed ex vivo AChE whole blood assay based on the modified Elman-method to measure AChE activity exclusively using rat whole blood pre-treated with selective BChE-inhibitor, Ethopropazine. We defined Km of AChE kinetics with the substrate, acetylcholine iodide and validated the assay by inhibiting RBS-anchored AChE with Donepezil (IC50=22nM), a potent-selective AChE-inhibitor. From our late phase in vivo studies, we predicted that AZ compound-X, an inhibitor of innate immune system-associated kinases, may exert off-target effects against AChE activity. We tested AZ compound (X) (IC50 =1.6µM) to assess off-target effects against AChE and observed significant inhibitory effect against AChE. These data raise concerns about the safety of compound-X in development considering only its therapeutic benefits on a specific innate immune target. We conclude that this assay is an essential tool for understanding the off-target liabilities of the AZ small molecule portfolio. However, this assay can be further developed to assess off-target toxicity and pharmacological effects of different AZ modalities using whole blood from other species, including humans.

**Peak tales in bioanalysis - Causes, fixes and a case study**
*Pou I Long (Resolian, UK)*

Peak tailing is one of the most common chromatographic problems experienced during method development. Some of the reasons that can cause this peak distortion are deformation of the packing material in the column or an unwanted secondary interaction of the analyte with the stationary phase. Although there are many tips and guidance online on how to theoretically fix the problem from a chromatographic perspective, an addition consideration is needed for the bioanalytical samples which are complex in nature and can contribute to peak tailing.

Therefore, in this presentation I am hoping to share a case study of the peak tailing problems encountered when using a new column during the development of a method; from investigation on how we identified the root cause of peak tailing to how the problem was resolved in detail. This presentation is aimed to share the knowledge across the field that might have experienced the same issue, ran out of ways to fix peak tailing, or even new to chromatography, as peak tailing can be difficult to tackle.

**CAMS (Community for Analytical Measurement Science) and the state of the nation for separation sciences initiative**
*Ali Salehi-Reyhani (Imperial College, UK)*

The Community of Analytical Science (CAMS), is an industry led network across the UK and Ireland, whose purpose is aimed at promoting world-class analytical measurement science

training, research and innovation by bringing together a network of industrial and academic partners with interests in these fields across the UK and Ireland. In our first 3 years, CAMS has welcomed over 340 individual members into the network and 36 academic/industrial organisational memberships. We are funded by the Analytical Chemistry Trust Fund (ACTF), our industry memberships, academia programme co-funding, (former) BEIS and supported by the Medicines Manufacturing Industry Partnership (MMIP). As an industry led-network, CAMS works to address specific industry challenges in four priority areas: 1) Point of use sensors and photonics; 2) Complex mixtures, separations and detection; 3) Data analytics; 4) Novel instrumentation or techniques. Since launching, we are proud to have co-funded 41 programmes - including 14 academic co-funded positions in UK and Irish Universities, 8 of which are permanent (2 new chairs and 6 new lecturers in analytical science). The remaining funding has been directed towards postdoctoral fellowships and industry:academia PhDs. In our time as a network however, we have been less successful with engaging academic institutions to appoint separation scientists or commission separation science projects, and our industry members continue to report challenges with finding suitably qualified and / or experienced separation scientists. For this reason, CAMS is now co-ordinating a white paper for use with UK and Irish policy makers and funders and has catalysed the project by bringing together the leading Separation Science expert community groups - the Royal Society of Chemistry Separation Science Group (Chair John Langley) and The Chromatographic Society (Chair Tony Edge) to lead the charge on starting a conversation about the state of the Nation for Separation Science. The project began with a one day meeting in London on June 22nd - with many community experts in the UK and from the Netherlands and Belgium, and industry/instrument company representation and was recently discussed as a plenary session at the inaugural SiNS meeting in Cardiff (July 2023).This presentation will summarise the initiative output and invite further input from the REID forum community on this topic - please come along and have your say - is Separation Science on Life Support?

## Day 3 – Thursday 7th September

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| **Bioanalysis Beyond Pharma**<br>*Session Chairs – Rebecca Mogey (Chester Zoo) & Jon Bardsley (Thermo Fischer Scientific)* | | |
| **09:00 – 09:30** | **An antibody-free LC-MS/MS method for the quantification of sex hormone binding globulin and its mutated form in human serum and plasma** | Bas Sleumer (ICON, Netherlands) |
| **09:30 – 09:50** | **Biosynthesis of natural sunscreen compounds from cyanobacteria** | Afeefa Kiran Chaudhary (Liverpool University, UK) |
| **09:50 – 10:10** | **Development and comparison of bioanalytical methods to measure free analyte** | John Chappel (Gyros Protein Technologies) |
| **10:10 – 10:30** | **Method development strategies to overcome selectivity issues using Gyrolab technology for PK analysis of new modalities** | Tom Wilfor (Resolian, UK) |
| **10:30 – 11:00** | **Break**<br>*Lounges* | |
| **11:00 – 11:20** | **Targeted and untargeted metabolic profiling of mice liver extracts using a hybrid zwitterionic hydrophilic interaction liquid chromatography column** | Artemis Lioupi (Aristotle University of Thessaloniki, Greece) |
| **11:20 – 11:40** | **A simple robust method for synthetic therapeutic RNA. New chemistry, new quantitation** | Ken Cook (Thermo Fisher Scientific, UK) |
| **11:40 – 12:00** | **Metabolic fingerprint and profiling analysis of cytochrome b5-producing E. coli N4830-1 using FT-IR spectroscopy and GC-MS** | Thanyaporn Tengsuttiwat (University of Liverpool, UK) |
| **12:00 – 12:20** | **Seeing red - a suitable LC-MS/MS extraction for studying ADC exposure in in vitro bone marrow models containing phenol red** | Rachel Foreman (AstraZeneca, UK) |
| **12:00 - 12:10** | **Closing Remarks**<br>*Tim Sangster & Neil Spooner (Co-Chairs International Reid Bioanalytical Forum)* | |

## Day 3 Abstracts

### An antibody-free LC-MS/MS method for the quantification of sex hormone binding globulin and its mutated form in human serum and plasma

*Bas Sleumer (ICON, Netherlands)*

Sex hormone binding globulin (SHBG) is a hormone binding protein which plays an important role in regulating the transport and availability of biologically active androgens and estradiol to target cells and used to calculate free testosterone concentrations. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed, featuring

an albumin removal step followed by a tryptic digestion. After a reduction step with dithiothreitol and alkylation with iodoacetamide three signature peptides were used for the quantification of SHBG. The method was validated according to the most recent guidelines. This method was also applied for the semi quantitation of a regularly occurring mutated form of SHBG caused by the single-nucleotide polymorphism (SNP) rs6258, which differs in structure from wild-type SHBG by only one amino acid but shows a different binding towards testosterone.

During this presentation, different aspects from the method development will be discussed, like the optimization of the albumin depletion step and the digestion. Furthermore, the performance of the method in terms of sensitivity, selectivity, accuracy and precision will be discussed, including the semi quantitation of the mutated form.

**Biosynthesis of natural sunscreen compounds from cyanobacteria**

*Afeefa Kiran Chaudhary (Liverpool University, UK)*

The skincare-focused pharmaceutical industry is dedicated to developing environmentally friendly, cost-effective, and photostable sunscreen formulations to combat the increasing risk of ultraviolet (UV) radiation exposure. This research project seeks to explore nature-inspired molecules derived from cyanobacteria, particularly mycosporine-like amino acids (MAAs), known for their UV-absorbing properties and ability to protect against harmful radiation in high-light environments.

The study concentrates on investigating the production of MAAs in Chroococcidiopsis thermalis (7203), a cyanobacterium possessing the Ava gene cluster responsible for synthesizing the MAA Shinorine. Previous studies have revealed that even in the absence of one gene in the cluster, the remaining three genes can produce Mycosporine Glycine (MG), a universal precursor to various types of MAAs. The researchers identified three homologous genes (0778, 0779, and 0780) in Chroococcidiopsis thermalis that may have the capacity to produce MG.

To gain further insights into the potential variety of MAAs produced, cloned ten different genes that I got after blast search against the organism's genome. The outcomes of this investigation could significantly enhance our understanding of the synthesis and function of MAAs in Chroococcidiopsis thermalis and related microorganisms, as well as uncover the diverse applications of these compounds in various fields.

The research project utilizes High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LCMS) as analytical techniques for compound analysis and identification. Additionally, plans to incorporate Raman spectroscopy in the future aim to augment the depth and comprehensiveness of the chemical analysis. By combining these powerful methods.

**Development and comparison of bioanalytical methods to measure free analyte**
*John Chappel & Nena Lopez Lee (Gyros Protein Technologies, UK)*

While the measurement of free biotherapeutic in vivo during development is important to understanding its pharmacologic effects, using plate-based immunoassays to determine free analyte presents technical challenges related equilibrium shifts and overestimations from long incubation steps or when diluting samples.

In this study, free analyte for bevacizumab (Avastin®) and ranibizumab (Lucentis®) with vascular endothelial growth factor (VEGF) as capture reagent was measured using a microfluidic immunoassay platform to overcome these challenges and using ELISA for comparison.

This work will also evaluate the development of an assay to measure free IgE in human serum. One further issue with conventional plate-based immunoassay techniques sample dilution has the potential to impact accurate determination of the free molecule depending on the affinity of the interaction. For this reasoning the developed assay used 100% undiluted matrix to ensure that the assay procedure did not cause dissociation of the drug: IgE complex. More importantly, a microfluidic flow-through technique was used where binding interactions were extremely short, therefore minimizing dissociation or reassociation of any complexes during the assay procedure.

The results point to the importance of selecting the right analysis method for free analyte measurement considering the effect of incubation times and sample dilutions on shifting equilibriums and the affinity characteristics of the analytes.

**Method development strategies to overcome selectivity issues using Gyrolab technology for PK analysis of new modalities**
*Tom Wilfor (Resolian)*

Selectivity is the ability of a ligand binding assay to quantify accurate levels of drug in the presence of non-specific matrix components and is a requirement within the scope of the ICH M10 bioanalytical method validation guidelines adopted by regulatory authorities. Endogenous interferents such as heterophilic antibodies, drug ligand, degrading enzymes and more, can cause non-selectivity in ligand binding PK assays, leading to inaccurate quantification of drug concentrations.

This presentation describes the development of a Gyrolab method to quantify a novel cancer therapeutic from Crescendo Biologics, CB307, a trispecific Humabody® T-cell Enhancer, in patients with PSMA positive solid tumours in support of a first-in-human clinical study. It will explore how the molecule presented challenges in achieving assay selectivity in both healthy and disease state populations, as well discussing several bioanalytical strategies used to resolve selectivity failures. Approaches such as selection of the most specific antibody orientation, optimisation of sample minimum required dilution, assay buffer and assay sensitivity and appropriate selection of the solid phase will be presented. The strategies described resulted in a selective method for CB307 in disease state matrix that successfully met BMV acceptance criteria and is used to support clinical sample analysis.

**Targeted and untargeted metabolic profiling of mice liver extracts using a hybrid zwitterionic hydrophilic interaction liquid chromatography column**

*Artemis Lioupi, Christina Virgiliou, Georgios Theodoridis & Helen Gika (Aristotle University of Thessaloniki, Greece), Ian Wilson (Imperial College, UK) & Thomas Walter (Waters, USA)*

The chromatographic performance of a zwitterionic hydrophilic interaction liquid chromatography column was investigated for a wide range of small hydrophilic metabolites with varying chemical properties under different mobile phase compositions and pH values. A total of 89 hydrophilic metabolites were examined, including significant metabolic intermediates such as amino acids and their derivatives, amines, organic acids, sugars, purines, pyrimidines and derivatives. Chromatographic efficiency parameters such as peak resolution, peak shapes, selectivity and precision in retention and peak areas as well as characteristics that are critical for metabolic profiling analysis such as metabolite coverage and retention time distribution were studied. The optimal chromatographic conditions were then used for both targeted (UHPLC-MS/MS) and untargeted (UHPLC- QTOF MS) analysis of liver tissue extracts from two groups of mice (control vs treated). The capability of the chromatographic systems to acquire a comprehensive metabolic profile and extract meaningful metabolic information was demonstrated; the performance of the BEH Z-HILIC column in terms of sensitivity of detection and metabolite coverage was evaluated. Overall, the hybrid zwitterionic HILIC column provides an effective and complementary tool for exploring the polar metabolome, which also holds potential applications in lipidomics.

References - A. Lioupi, C. Virgiliou, T. H. Walter, K. M. Smith, P Rainvill, I. D. Wilson, G. Theodoridis, H. G Gika, “Application of a Hybrid Zwitterionic Hydrophilic Interaction Liquid Chromatography Column in Metabolic Profiling Studies” Journal of Chromatography A, 2022. DOI: 10.1016/j.chroma.2022.463013

**A simple robust method for synthetic therapeutic RNA. New chemistry, new quantitation**

*Ken Cook (Thermo Fisher Scientific, UK)*

Oligonucleotide analysis has gained considerable interest over the last few years with the successful introduction of mRNA vaccines using a lipid nano particle transport system. Modified synthetic oligonucleotides therapeutics however, have proven difficult to characterize. They form metal and amine adducts easily which splits the signal making simple quantitation difficult. Some modifications form stereoisomers which separate during the chromatography producing multiple unresolved peaks. The data presented here resolves these issues with chromatography and MS conditions that eliminate all these commonly found adducts. The amine adducts are removed with in-source collision energy. The optimised MS source conditions also prevent in-source generation of impurities which is another common problem. Quantitation and identification is simple with clean high resolution data devoid of MS adducts and MS derived fragmentation impurities. Relative abundance RSD values of the FLP are below 0.2% and low-level impurities all below 5%. Limits of detection for impurity analysis are below 0.05%. The same method can be used for bio-analysis including Met ID. All calculations are embedded in a specific report which delivers the results within 2 minutes.

**Metabolic fingerprint and profiling analysis of cytochrome b5-producing E. coli N4830-1 using FT-IR spectroscopy and GC-MS**

*Thanyaporn Tengsuttiwat (University of Liverpool, UK)*

The pharmaceutical biotechnology industry has been growing rapidly since the first approve of recombinant Humulin in 1982. The global recombinant protein market is expected to reach US$ 400 billion by 2025. To increase recombinant protein production, optimisation of production condition is important. Herein, Fourier transform infrared (FT-IR) spectroscopy has been applied as a high throughput metabolic fingerprinting approach to optimise and monitor cytochrome b5 (CYT b5) production in Escherichia coli N4830-1. This strain contains a plasmid with either 0-6 copies of the gene encoding CYT b5 under a heat-inducible promoter, λPL promoter. Multivariate analysis of these FT-IR results illustrated features correlating to the different numbers of cyt b5 genes as an increasing trend. This finding was confirmed by a colourimetric method and others such as SDS-PAGE and western blot. While these traditional methods required a cell extraction step, FT-IR spectral data allowed for the quantitative detection of CYT b5 directly inside the intact cell. Furthermore, GC-MS has been employed as an untargeted metabolic profiling method, to identify and highlight the important compounds and pathways that are most affected by CYT b5 production. The metabolic profiling data from GC-MS analysis is in agreement with the FT-IR findings and may allow to further define the metabolic changes related to the burden from CYT b5 production in the cells. For instance, some cellular energy requires to maintain the recombinant DNA and this may lower the host growth rate and the level of gene expression.

**Seeing red - a suitable LC-MS/MS extraction for studying ADC exposure in in vitro bone marrow models containing phenol red**

*Rachel Foreman, Richard Lucey, Adam Leaney, Humaira Naseer & Amanda Wilson (AstraZeneca, UK)*

Antibody drug conjugates (ADCs) are biopharmaceutical products in which a monoclonal antibody is linked to a small molecule cytotoxic payload drug. Physiologically-relevant bone marrow models are vital for the safety risk assessment of new ADCs, and cell culture in vitro systems are used to interrelate data for future clinical application. Phenol red is a useful acid pH indicator, and is used in many cell culture assays to monitor oxidative activity. However, there are some informal suggestions that this substance can be detrimental to LC-MS/MS bioanalysis of in vitro models, as it may cause suppressive matrix effects and affect accurate quantitation. Many researchers even do their final stage of cell culture in ‘phenol red free’ media, to reduce any bioanalytical interferences, but in collaborative work this is not always feasible.

In standard LC-MS/MS practice, samples are subject to chemical extraction from the original matricies, and the development of a suitable method for removing phenol red would allow for cleaner final samples to be analysed. Here we describe three extraction methods for the analysis of a free ADC payload in cell media containing phenol red. The recovery efficiency, matrix effect and chromatography of each assay were compared, to decide which was the most suitable for removing phenol red from the samples. The final method was proven to be precise and accurate over a suitable range and was successfully applied to samples from

an in vitro study, with confidence that phenol red was not causing any detrimental effects to the analytical system.

# Posters

**Simultaneous raman and infrared spectroscopy: a novel combination for studying bacterial infections at the single cell level.**
*Shwan Ahmed (University of Liverpool, UK)*

Sepsis is a life-threatening reaction to infection, leading to organ dysfunction and failure and is one of the foremost causes of mortality in both adults and children in the world. Therefore, developing accurate diagnostic and prognostic tools for the rapid diagnosis of sepsis and, perhaps most importantly, identifying antimicrobial resistance and susceptibility is urgently needed to improve sepsis recognition and management.

Despite numerous reports using Raman and infrared spectroscopies to analyse bacterial samples. However, to date no study has reported both Raman and infrared signatures being collected from the same clinical samples simultaneously and this is due to the difficulty of sharing light sources and optics in conventional Raman and Fourier transform infrared (FTIR) spectrometers. Here, we report for the first time the use of optical photothermal infrared (O-PTIR) and Raman spectroscopy for the simultaneous analysis of 12 bacterial strains: including six isolates of Gram-negative and six Gram-positive bacteria isolated from blood infection in children. Infrared signatures were collected from bulk samples via both FTIR and O-PTIR spectroscopies, whereas Raman signatures were acquired from bulk by using the O-PTIR spectrometer probe beam (532 nm) as excitation. Infrared data from individual single cells was also obtained by O-PTIR spectroscopy. Our findings showed significant similarity and high reproducibility in the infrared signatures obtained by all three approaches, whereas the discrimination patterns obtained by Raman and infrared data as input to clustering algorithms evidenced the superior ability of Raman spectroscopy to probe bacterial pigments.

**Increasing chromatographic quality, throughput and feature detection in DMPK, metabolomic and lipidomic studies with vacuum jacketed columns: Gefitinib a case study**
*Amy Bartlett (Waters, UK), Rob Plumb (Waters, USA) & Ian Wilson (Imperial College, UK)*

We describe the use of a vacuum jacketed column (VJC) assembly located directly at the MS source to minimize on-column and post-column band broadening. VJC enhances the performance of high throughput LC/MS-based analysis.

Throughput of results is key in high feature content LC/MS analysis, such as DMPK & metabolomics. Sub 2µm particle liquid chromatography (UHPLC) with accurate mass MS has become the mainstay of complex mixture analysis. However, absolute performance of sub 2µm LC has not been realized when connected to MS, due to dispersion in the connecting tubing post column and dispersion in the MS probe. Positioning the LC column directly at the MS source addresses some dispersion issues, however, without thermal regulation, frictional heating reduces performance through band broadening. LC/MS biological samples from IV and PO dosing of tyrosine kinase inhibitor to mouse were subjected to DMPK, omics analysis. A 1-minute quantitative VJC assay was developed for gefitinib and 4 metabolites with a longer 3-minute LC-MS(IMS-TOF) method for metabolite profiling. The 1-minute VJC-MS/MS DMPK assay gave an average peak width of 0.6 seconds versus 1.2 seconds for UHPLC, reduced peak tailing by 10%, increased sensitivity 5-fold. The pharmacokinetics of the gefitinib in the mouse gave a maximum plasma concentration of 4 & 7µg/mL (IV, PO), with T1/2 = 2.6 h, 14 drug metabolites were detected. VJC lipidomic analysis reduced

analysis time 4-fold and significantly improvement lipid resolution. Data analysis revealed LPCs, tryptophan, taurocholic acid, and lysyl-arginine concentrations increased with dosing while deoxyguanosine, 8-hydroxydeoxyguanosine, asparaginyl-histidine and PC lipids decreased.

**Use of a 2D LC-MS/MS system to enhance selectivity and sensitivity of biologics**
*Rebecca Dowell (BioApp Solutions Ltd, UK)*

There is an increase in the number of biological molecules in the drug development process. These molecules do not just differ by size but also in how they are made, mode of action and their behaviour in the body. There is a growing need for the capacity to analyse these molecules, especially peptides, and quantify them at very low levels for TK/PK purposes. The physico-chemical properties of peptides traditionally made them difficult to analyse intact using mass spectrometry. The analytical experience and approaches required to analyse these compounds have slowed down the method development process and timelines.

This poster will show the use of an online SPE (2D) System to develop high sensitivity assays, focusing on the determination of peptides in human plasma by LC-MS/MS. Giving a comparison between offline and online SPE (1D vs 2D), it shows how to manipulate the sample extraction and/or chromatography to achieve desired sensitivity levels.

**Achieving high recovery and reproducibility in high throughput sample preparation using silica/polymer composite 96 deep-well solid phase extraction plates**
*David Dunthorne (VWR, Avantor, UK)*

Achieving reproducible, high-recovery sample preparation is essential in high sample throughput laboratories, such as clinical, drug development and food analysis labs. In these areas, 96-Well Solid Phase Extraction (SPE) has become one of the main high-throughput sample preparation techniques, owing to its capability to selectively extract analytes from complex sample matrices, ensuring greater assay sensitivity and robustness, as well as reducing downtime with valuable instrumentation.

Typically, SPE sorbents use functionalised loose-packed silica particles secured by frits in a plastic housing, whereas utilisation of a solid silica/polymer-based composite media, removing the need for frits, enables higher analyte recovery at lower elution volumes, and greater repeatability. In addition to the cost-saving and environmental benefits of lower elution solvent usage, fritless composite SPE media enables analysts to greatly reduce or remove lengthy dry-down steps from their SPE workflows, saving precious time.

This poster showcases the benefits of fritless composite SPE media via a variety of application-based data that demonstrate the advantages that can be realised for analysts who operate with limited sample quantities, are looking to reduce workflow time, or choose a greener sample preparation technique than loose-packed sorbent SPE.

**Speciation of trace Nickel(II) ions in human saliva: A 1H NMR investigation**
*Kayleigh Hunwin, Georgina Page, Martin Grootveld & Mohammed Bhogadia (De Montford University, UK)*

Introduction/Objectives: Placement of nickel-containing metal alloy dental prostheses (NiC-MADPs) such as restorations and orthodontic appliances have caused much concern regarding the possibility of adverse hypersensitivity reactions arising from the release of Ni(II) ions from these materials via in vivo corrosion processes. Since the biological activities

and toxicities of ‘foreign’ metal ions is predominantly determined by their precise molecular nature, here we have explored the speciation of Ni(II) ions in human saliva, a rich source of biomolecular Ni(II)-complexants/chelators.

Methods: Unstimulated whole human saliva samples were obtained from n = 7 pre-fasted (≥8 hr.) healthy participants, and clear human salivary supernatants (HSSs) were obtained from these via centrifugation. Microlitre aliquots of stock aqueous Ni(II) solutions were sequentially titrated into HSS samples via micropipette. 1H NMR spectra were acquired on a Jeol JNM-ECZ600R/S1 spectrometer.

Results: Addition of Ni(II) to HSSs gave rise to its complexation/chelation by a range of salivary biomolecules, their relative efficacies being determined by the influence of increasing added Ni(II) concentrations on the line-widths and chemical shift values of their 1H resonances. Those most affected were lactate ≈succinate ≈ethanol >formate ≈5-aminovalerate ≈pyruvate. These orders demonstrate that such biomolecules compete for available Ni(II) on the basis of their variable Ni(II)-complex stability constants, and also their available HSS concentrations.

Conclusions: These observations provide valuable information regarding the identities of Ni(II) complexes in human saliva, which is relevant to trace metal ion speciation and toxicology, the in vivo corrosion of NiC-MADPs, and the molecular fate of ingested Ni(II) ions in this biofluid.

**RNA quantification via nucleic acid nanorobotics**
*Esther May, Roma Galloway, Alexander Jackson, Mark Chadwick, Jurek Kozyra, Hemaprakash Nanja Reddy, Jasmine Bird, Tarin Taleb, Harold Fellermann & Maria Oranges (Nanovery, UK)*

Nanovery introduces a nanorobotic approach for precise detection and quantification of nucleic acid targets from a diversity of biological matrices in bioanalysis. Current methods suffer from slow speeds, high costs and errors, but Nanovery's easy-to-use, high-throughput platform delivers reliable results within hours. Our proprietary nanorobots come in reagent form and can detect sequences in complex biological environments like cell cultures, tissue extracts and serum, providing streamlined workflows and robust data that can be automated at scale.

Nucleic acid nanorobotics (NANs) is enzyme free and offers unparalleled target specificity, emitting a fluorescent signal for direct and absolute concentration measurements using a simple plate-reader. This poster highlights NANs' characteristics, sensitivity, selectivity, and multiplexing capabilities. Our adaptable and cost-effective technology opens doors for various sectors, including bioanalysis and diagnostics.

**The journey for efficiency in a high throughput discovery DMPK laboratory**
*Sam Morris (GlaxoSmithKline, UK)*

Generating in vitro Tier 1 DMPK data to support Lead Optimization of the GSK small molecule portfolio requires a vast amount of data to be generated quickly. Embarking on a journey to find efficiency through automation and employment of software and hardware solutions for request management, assay execution, bioanalysis and data processing. Using metrics to identify bottlenecks and novel technologies to overcome these has been the focus of the discovery DMPK organisation, a highlight of this process has been the

deployment of a bioanalyical workflow utilising the LS-1, a trap and elute system to provide rapid MS sample analysis, the first use of this in Europe.

**Current use, 3Rs benefits and barriers for microsampling in toxicology programs: 2021 cross-sector survey results**

*Helen Prior (National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs, UK), Elisa Passini (NC3Rs, UK), Amanda Wilson (AstraZeneca, UK) & Neil Spooner (Spooner Bioanalytical Solutions, UK)*

The NC3Rs actively promotes the 3Rs, scientific and business benefits to microsampling, through an international expert working group with representatives from 24 organizations.

Data were collected on the current use of microsampling in toxicology studies between December 2020 and April 2021 with 54 surveys returned, mainly from pharmaceutical (25), agrochemical (5) or CROs (8) located in the UK/Europe or North America. 87% of the surveys indicated that microsampling was used. Microsampling was used most for TK within small molecule and agrochemical toxicity studies (84% of 44 surveys; 82% of 11 surveys respectively), but less frequently within large molecule, cell/gene therapies or industrial chemical studies (60% of 37 surveys; 29% of 21 surveys; 25% of 8 surveys respectively). Microsampling was used most in rodent non-GLP studies: PK studies, discovery pharmacology/efficacy and/or toxicology < 4 weeks. Microsampling was also routinely used in GLP rodent studies such as regulatory toxicology < 4 weeks and > 4 weeks, reproductive, juvenile and carcinogenicity studies by 35%, 55% and 80% of surveys from pharmaceutical, CRO or agrochemical organizations respectively. The refinements most frequently reported were reduced blood loss, less invasive technique/more accessible sampling site and shorter procedure time. Barriers to wider adoption included needing multiple samples for different purposes, or not validating another bioanalytical method.

The data indicates that although some organizations have adopted microsampling routinely across many/all rodent toxicity studies, there are opportunities to further reduce and refine animal use by wider adoption for molecule types outside of small molecules and for more GLP studies.

**A sensitive, simple and efficient LC-MS/MS method for the bioanalysis of human IgG-1 in pre-clinical matrices**

*Patrick Stephenson (Charles River Laboratories, UK)*

With an increasing number of therapeutic monoclonal antibodies (mAbs) undergoing testing, there is a growing requirement for bioanalytical methods for their quantification. The size of these proteins prevents their intact analysis by QqQ MS, and HRMS currently doesn't provide the required sensitivity for intact analysis, hence these types of analyte have routinely been quantified by LBA techniques. However, the use of tryptic digestion and surrogate peptides, which enables quantification of these analytes by LC-MS/MS, is becoming a common approach. The faster method development, enhanced selectivity and increased dynamic range, often makes this approach more appealing, particularly in the early stages of drug development. As most mAbs are IgG based, this provides the opportunity to utilise the same surrogate peptide for the analysis of a variety of mAbs in preclinical matrices, further accelerating and simplifying method development.

This poster illustrates an efficient and cost effective method for the quantification of human IgG1 based mAbs in rat plasma by LC-MS/MS, via the monitoring of the

'VVSVLTVLHQDWLNGK' peptide. The approach uses the latest Sciex QqQ MS technology to boost sensitivity, enabling an LLOQ of 0.500 µg/mL, whilst also maintaining linearity across a >2 orders of magnitude range. The simple and efficient digestion procedure enables the extraction of 96 samples in < 3 hours, enabling the fast turnaround of data required in early drug development. All of this is achieved using commonly available and affordable reagents, ensuring the assay remains cost effective and unaffected by the increasingly common supply chain limitations.

**Development and validation of an MSD method for determination of a soluble TCR bispecific in human serum**

*Katie Tetlow (York Bioanalytical Solutions, UK)*

A novel bispecific molecule is being developed using an immune-mobilising monoclonal T-cell receptor against Human Immunodeficiency Virus (HIV) and an anti-CD3 effector function. HIV attacks the immune system causing chronic infection and if not treated it can lead to Acquired Immunodeficiency Syndrome (AIDS). The World Health Organisation (WHO) estimates there were more than 38 million people living with HIV in 2021. This approach is immunotherapeutic and designed to specifically eliminate CD4+ cells that are persistently infected with HIV. The drug targets a peptide derived from the Gag protein that is presented by HLA* A02 on the surface of HIV infected cells. Killing HIV reservoirs is one way to potentially achieve a state of viral suppression in the absence of anti-retroviral medicines.

Determination of the drug within human serum requires an analytical method that is specific, sensitive and robust. The developed method employs immobilisation of a biotinylated peptide HLA complex on an MSD streptavidin plate pre-blocked with casein. Diluted serum samples are then incubated on the plate to capture drug. Bound drug is detected using Sulfo-tag conjugated CD3 and an electrochemiluminescent readout is obtained. Optimisation of the critical reagents was assessed within the development process, as well as the accuracy and precision of the assay. Within validation stability at -20°C and -80°C, selectivity with healthy and disease state individuals and the potential effects of lipaemic and haemolysed samples were assessed. The method is sufficiently sensitive and selective, and demonstrated acceptable precision and accuracy, to be validated to internationally accepted levels for use in clinical sample analysis.

**Metabolomics of age and diversity: a step closer to achieving healthy ageing, globally.**

*Dakshat Trivedi (University of Liverpool, UK)*

With increasing life expectancy in most developed countries, the promotion of healthy ageing is a significant public health objective in this decade of healthy ageing (2020-2030). Although a global problem, the changes in metabolome during this phase in today's ethnically diverse populations are relatively under-studied. We studied metabolic profiles from serum of 572 men based in the United Kingdom. These men were from White European, South Asian and African-Caribbean ethnic backgrounds between 40 to 86 years of age. Data on alcohol intake, physical activity and co-morbidities were collected and used for this study.

Liquid-chromatography mass spectrometry (LC-MS) and gas-chromatography mass spectrometry (GC-MS) were employed for collecting metabolic profiles of these individuals. Cross validated PLS-DA based classification models reported average correct classification rate (CCR) of 90% and 91% for age and ethnicity predictions respectively. Pathway

enrichment analysis revealed metabolic features linked to ageing to be perturbing Porphyrin metabolism, biosynthesis of unsaturated fatty acids and the tricarboxylic acid (TCA) cycle in these men. The N-glycan biosynthesis pathway was significantly impacted by metabolic features linked to ethnicity. Pearson's correlation analysis showed no confounding effects from the pre-existing co-morbidities or influence of age in ethnicity classifications and vice-versa.

We found serum metabolic profiles can predict age and ethnic origin in men. We report novel ethnicity-specific metabolic features and perturbations in metabolic pathways with potential implications in epidemiological metabolomics, and translational medicine. These findings take us a step closer to achieving healthy ageing in diverse, global populations.

**Visualization of drug/metabolites and metabolism with DESI TQ and DESI Q-ToF mass spectrometers**

*Ian Wilson (Imperial College, UK)*

Typically DMPK studies are performed using LC/MS with tissue distribution studies made using tissue homogenates where molecular localisation is lost. Here gefitinib was dosed IV at 10 mg/kg via the tail vein to male C57BI6 mice with DESI MSI directly on tissue to image gefitinib (and/or its metabolites) directly on liver. DESI MSI was performed on livers collected at 0.5, 1, 3, 8 and 24h post-dose using both multi-reflecting Q-Tof (MRT) and tandem quadrupole mass spectrometers in +ve ESI with imaging at sizes of 50 and 100 µm pixels Using full scan MS for untargeted MSI with the MRT (at mass resolution >200,000 FWHM, mass accuracy <500ppb) detected protonated gefitinib (but no metabolites) in the 0.5-8h but not the 24h livers, with a mass accuracy of 179ppb. Effects on endogenous lipids were noted with potassiated PC (34:1) the most abundant lipid at 0.5h whilst potassiated PC (34:2) was the most abundant lipid at all other times. Further MSI experiments were performed using targeted MRM mode using the DESI TQ MS with MRM transitions obtained from UPLC MS/MS studies. Gefitinib, and 16 metabolites were imaged, as well as potassiated lipid PC (34:1) and ten were detected. Gefitinib, M7, M9, M10, M11, M12 and M14 concentrations were maximal at 0.5h, declining thereafter, whereas metabolites M1, M2, M6 and M13 reached their maximum concentrations at 3h post dosing. A workflow that combines targeted DESI MSI based on data from LC/MS based DMPK offers a more complete picture of drug/metabolite distribution than untargeted studies.

**Rapid HDMSe blood product lipidomic screening using a DESI inlet**

*Ian Wilson (Imperial College, UK)*

Here we have examined the feasibility of using DESI – an imaging source typically used to investigate compound localization within tissues - as an alternative rapid direct analysis method for performing lipid profiling on blood products. The technique has been applied to screen sera obtained from a UK human population as part of the Human Serum Metabolome study (HUSERMET). 2 µL of IPA extracted serum samples (n=500) were spotted onto PTFE locators on a glass microscope slide. A single line pass through the center of each spot was performed by the DESI and the data was acquired on a QToF mass spectrometer in HDMSe acquisition mode.

The results demonstrate the potential of DESI to be used as a rapid basic screening technique for the lipidomic analysis of small volumes of blood products. Individual sample analysis was achieved at a rate of <15 seconds per sample, representing a significant reduction in instrument time compared to typical LC/MS analyses. The data gave an overview of sample quality, revealed fold changes in lipid profiles and could be used to facilitate a more in-depth subset analysis by LC/MS. If performed using a high mass resolution or ion mobility capable instrument improved separation/identification of lipid

species could be possible, enabling greater information extraction from each sample. DESI pre-screening can also be used to indicate potential issues such as contamination or the presence of incorrect sample type, which could have devastating effects on subsequent LCMS analysis.