18th EBF Open Symposium

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Science in High Definition

Emerging Small Molecule Modalities – Bioanalytical Challenges and EBF Perspectives

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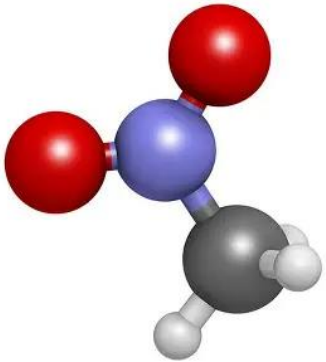
The Challenge

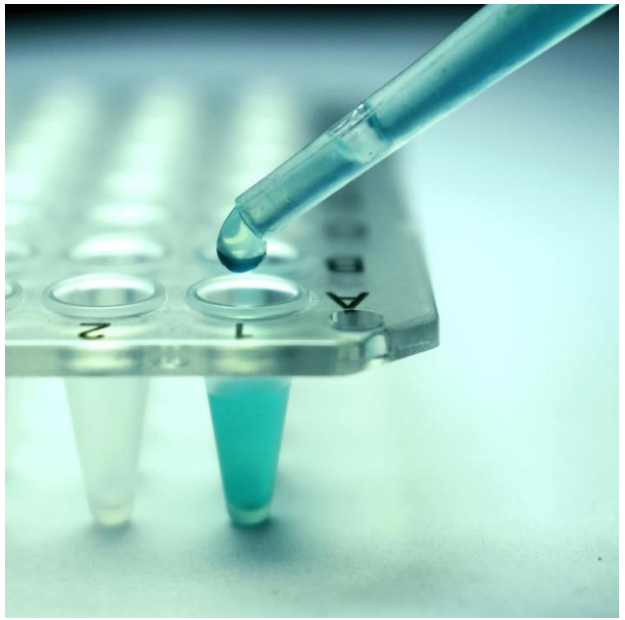
The field of small molecule bioanalysis

- A mature filed with most major hurdles resolved ☐
- A field that continues to evolve and face new difficulties ☑



Why Emerging Modalities Challenge Bioanalysis

- Emerging Modalities Complexity
  - New drug types like oligonucleotides, peptides, prodrugs, lipid nanoparticle formulations, excipients, small molecule biomarkers, and covalent inhibitors add analytical challenges to traditional bioanalysis.
- Unique Properties and Challenges
  - These modalities have distinct physicochemical and immunogenic characteristics requiring innovative analytical strategies and cross functional expertise



EBF Oligonucleotide, Peptides and New Modalities Team

EBF

- **Collaborative Industry Effort**
  - The European Bioanalysis Forum formed a cross-industry team with 19 member companies represented to tackle these evolving challenges collaboratively.
- **Adapting Bioanalytical Practices**
  - Work to systematically identify and categorize key challenges from seven modality types
  - Preliminary mitigation strategies to provide an early roadmap to adaptive bioanalytical practices for new modalities
  - Reinforcing the need for collaboration and regulatory dialogue as bioanalysis continues to evolve alongside innovative drug development.

Seven Modalities – with Shared Challenges

- **Seven Emerging Modalities**
  - Seven small molecule modalities present unique bioanalytical challenges in drug development.
- **Distinct Analytical Issues**
  - Each modality faces unique stability, selectivity, sensitivity, and quantification problems.
- **Shared Bioanalytical Hurdles**
  - Common hurdles include matrix effects, poor recovery, and ADA interference across modalities.
- **Tailored Analytical Approaches**
  - Customized methods and flexible guidelines are essential for reliable quantification and validation.

Overview of team practice

- For each of the seven modalities:
  - Oligonucleotides
  - Peptides
  - Formulations and Lipid Nanoparticles
  - Excipients
  - Small Molecule Biomarkers
  - Covalent Drugs
  - Prodrugs
- The team has documented recurring challenges and started to consolidate emerging strategies under consideration across companies to address them.

These preliminary solutions, while not yet standardized, reflect the collective thinking and current practices being tested across the team.

Oligonucleotides – Complex and Critical

- **Bioanalytical Challenges**
  - Their size, strong negative charge, high polarity, and significant protein binding affinity pose substantial challenges in bioanalysis
- **Common Analytical Issues**
  - Non-specific binding, sensitivity problems, chromatographic retention, carry over and ADA interference.
- **Improvement Strategies**
  - Use of DNA LoBind tubes, detergents, BSA, and enzymatic digestion to enhance analyte recovery.
- **Advanced Analytical Workflows**
  - Optimized LC–MS with ion-pairing reagents, fully inert chromato-graphic stationary phases and ADA-tolerant methods improve sensitivity, carry over and quantification.

Peptides – Diverse and Demanding

- **Peptide Diversity**
  - Peptides include cyclic, lipophilic, and highly cationic molecule types with varied structures.
- **Bioanalytical Challenges**
  - Challenges include enzymatic degradation, non-specific binding, poor LC retention, and low MS sensitivity.
- **Mitigation Strategies**
  - Use enzyme inhibitors, low-binding tubes, tailored columns, and dynamic wash protocols to improve analysis.
- **Optimized Detection and Screening**
  - Optimize MS detection and address ADA interference with digestion agents and immunogenicity screening.

Formulations – Free vs. Encapsulated

- **Chromatographic Challenges**
  - Lipid nanoparticles and PEGylated drugs complicate chromatographic separation due to high lipophilicity and co-elution with lipids.
- **Advanced Separation Techniques**
  - Alternative column chemistries and supercritical fluid chromatography improve separation for complex formulations.
- **Matrix Effect Reduction**
  - Matrix-matched calibration and phospholipid removal protocols reduce ion suppression in quantification workflows.
- **Reliable Quantification of free and total forms**
  - Through rigorous control of the processing conditions and with stabilizers to preserve the in vivo equilibrium.

What We Learned Across Modalities

- **Hybrid Analytical Approaches**
  - Combining LC–MS, ligand-binding assays, and immunogenicity assessments is increasingly essential.
- **Tailored ADA Strategies**
  - ADA strategies must be customized to each analytical modality
- **Standardized Tissue Protocols**
  - Standardization is critical for tissue analysis protocols to ensure consistency.
- **Context-of-Use Framework**
  - CoU guides assay validation and regulatory communication effectively.

From Initial Insights to Action

- **Initial Foundational Efforts**
  - The EBF team has established the foundation for future actionable steps for bioanalysis of new modalities
- **Future Initiatives**
  - EBF paper to share modality-specific guidance and common best practices for better outcomes.
- **Collaboration and Innovation**
  - Encouraging community engagement and sharing strategies to promote further harmonization and refinement.

Thank You & Let's Keep the Dialogue Going!!

- EBF Oligonucleotide, Peptides and New Modality team
- EBF Core Community

This work is **not a final answer but a starting point**. The insights and preliminary strategies shared will evolve into more detailed, harmonized approaches through ongoing community engagement, targeted discussions with regulators, and future EBF initiatives

We **encourage individual bioanalytical teams/companies** to use this initial categorization to further **share the experiences and best practise** to address the various bioanalytical challenges with these modalities.

EBF Oligonucleotide, Peptides and New Modality team

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