EBF

14th EBF Open Symposium

Science – Our Universal Language

Status Update From The EBF Protein MS Team

Matt Barfield, on behalf of the EBF

24-26 November 2021, Barcelona

Evolution of EBF Influence and Leadership

Focus Meeting
2011- large meets small

Publication
2014 – LC-MS of LM in a regulated BioA environment – which acceptance criteria to apply?

Focus Workshop
2017 – BioA strategies for LM: LBA/LC-MS United

Publication
2018 - Towards decision-based acceptance criteria for Bioanalytical Method Validation: a proposal for discussion from the EBF

2020 – A new dawn

- Creation of a new focus group
- Protein analysis by LC-MS
- Continue the journey and keep the discussion ongoing. The discussion's don't just impact Protein LC-MS but all new future technologies that support PK/safety
- We need to have a simple solution, fit for the future

Name	Company
Jason Cook	Alderley Analytical
Mike Blackburn	Quotient
Amanda Wilson	AZ
Mark Jean Gnoth	Bayer
Benno Ingelse	Byondis
Matt Ewles	Covance
Iain Love	CRL
Richard Welford	Idorsia
Sune Hove Sporring	Novonordisk
Nico van de Merbel	ICON
Robert Wheller	LGC
Fabrizia Fusetti	QPS
Gregor Jordan	Roche
Stéphane Muccio	Sanofi
Matt Barfield	Roche

Even the Pandemic didn’t stop the discussion

EBF 2021 Cyberconnect Events:

Focus Workshop: Peptides & Proteins with (LC-)MS

17-18 June 2021

Scientific Challenges (e.g. reagents, free total, intact vs digested)

Scientific developments (new tools)

How to interpret the data (e.g. biological specificity vs. analytical specificity, which data are ‘true’?

Defining a bioanalytical strategy for peptide/protein: which assay when and why?

Regulatory challenges – experience and industry recommendations

Current industry experience with measuring large molecule by LC-MS - A finger on the pulse survey outcome

- 2/3 companies that responded are using LC-MS for protein analysis
- Protein analysis by LC-MS is used across all phases of R&D
- The most used application is for PK assays
- Customers and stakeholder are not commonly involved in assay choice or acceptance criteria decisions
- It's a close call as to whether companies set acceptance criteria based on technology or by the scientific question being asked of the data
- The use of different acceptance criteria or technology in the measurement of the same molecule is rare
- Overall 4:6:15 criteria are applied however if immunoaffinity or digestion are applied then 4:6:20 is applied

Questions to Consider when Building a Bioanalytical Strategy for Proteins

- Sensitivity and Selectivity remain main drivers when selecting a bioanalytical platform though practical considerations also key
- Data generated on two platforms are both “true” – An understanding of why results are different is more important than any numerical difference. How is the communicated?
- Acceptance criteria for LC-MS assays of proteins: “it depends” – Depends on what? Can we standardise?

There is one consistent from 2010 until now

There remains confusion with acceptance criteria and different strategies are used across the community

We have heard this many times – I have presented this many times

- We discuss in detail…….
- We discuss a lot…….
- We have logical scientific rationales
- We often have agreements
- In reality do we live those agreements??
- If we don't - the regulators will and do decide for us

So what is the team doing?

- Understand the state of Protein MS in BioA
- Draw from the experience of the community to build a shared understanding and best practice
- Learn from each other
- Continue to challenge the community

- We asked ourselves a series of questions

Questions

Technology Platform

- Do you do large molecule quantitation using intact mass and what are the advantages and disadvantages?
- Do you use high resolution mass spectrometers for quantitative protein MS (intact mass and MRM)?
- Which approach do you use for top down quantification? XIC vs. de-convoluted mass?

Method Development

- Is it better to use expensive vendor supplied kits for enzyme digests or purchase enzymes and reagents separately?
- Pre-validation robustness testing of bottom-up assays – How do others test this? In line with small molecule assessments or include additional assessments?
- Standard and quality control preparation - Do you use serial dilution or spike solutions?
- Do you use IS SIL peptides or a SIL version of the target protein?
- How / why do you select the analytical approach: intact versus digested, which surrogate peptide(s), which type of extractio

Questions continued

Method Validation

- How do you perform matrix effect experiment for Protein MS (bottom up or top down)?
- How do you perform a recovery experiment for Protein MS (bottom up or top down)?
- How do you perform validation experiments for an immune-affinity-LC/MS method, especially for selectivity (6 versus 10 sources), specificity, and the number of A&P runs (3 versus 6)?
- In LBA, the concept of total error is used e.g. in P&A runs. Do we need to look at total error in IA-LC-MS methods?
- What are suitable acceptance criteria?
- Can regular LC-MS assay be used for stability determination?

More Questions

Regulatory Space

- Using LBA and MS technology at different stages – Do you perform a bridging between the technologies? If yes – how?
- What if the bridging does not show/give the same results? (e.g. lower MS response due to better selectivity or presence of metabolites co-measured in the LBA assay and not in the LCMS assay)
- Do you perform a bridging with respect to AUC coverage between preclinical and clinical if different technologies were used?
- What to do if you use other kinds of MS technology, e.g. Echo MS?

Functional Aspects

- Peptide analysis – How do you handle potential “metabolites” (both active / inactive)? (e.g. containing unnatural amino acids)?
- Affinity capture considerations (e.g. free vs bound, presence of ADA)?

An example - Technology Platform

Do you do large molecule quantitation using intact mass and what are the advantages and disadvantages?

- **Intact mass is the first choice for peptide bioanalysis**
- For proteins the choice between top-down and bottom-up is driven by the specific (bio)analytical question and mass cut-off
- Always applied in combination with sample preparation by (immuno) affinity capture/hybrid approach for larger proteins (in general)
- Distinguish between application in CMC characterization and bioanalysis, might requires different dedicated instruments and expertise
- **Limited experience present in the group on filing with intact MS data**
- *intact mass data for DAR or for protein biomarker relative quantitation : these studies can be performed in a regulated environment for example for an endpoint biomarker (method validation, qualified system, data integrity)*
- **Sensitivity has improved (state-of-the-art TOF instruments are almost as sensitive as QqQ)**
- State-of-the-art instruments and software have become more accessible and user friendly
- **Applicability and advantages of using top-down instead of bottom-up should be evaluated case by case, depends on the scope**

Do you do large molecule quantitation using intact mass and what are the advantages and disadvantages?

Top 3 advantages

1. **Addresses the whole protein, does not need proteolytic digestion**
2. **Can deliver a high degree of molecular detail.**
   - Presence of proteoforms* (modified versions of the same protein)
   - Identification of post translational modifications (PTMs)
   - Molecular heterogeneity glycosylation fingerprinting
   - Presence of (discrete) degradation fragments
   - Metabolism
   - Stability
   - DAR in ADC characterization
3. **Quicker and simpler sample preparation procedure**

Do you do large molecule quantitation using intact mass and what are the advantages and disadvantages?

Top 3 disadvantages

1. Sensitivity of top-down is still inferior to bottom-up approach
2. Need for appropriate (immuno) affinity capture materials
3. Software and instrument validation (GxP vs non-GxP compliant studies) - or is this a perception?

If you have a question but didn’t know who to ask

Reach out to the team

Acknowledgements

The team and all those involved over the many years and many discussions

Contact Information

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