EBF Experiences on LC-MS Analysis of Large Molecules

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on behalf of the EBF TT26 (Team B)

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Outline

- Background
- Survey results
  - Experience
  - Method validation
  - Internal standard selection
  - Acceptance criteria
  - Assay comparability, MS vs. LBA
- Towards a Recommendation
- Future work
- Acknowledgement

Background - EBF and LC-MS/MS of LM

Large interest from EBF member companies:

- EBF dedicated Focus Meeting “Large meets Small” in June 2011
  - Presentation, first EBF reflection (P. Timmerman, on behalf of EBF) - http://bru2011.europeanbioanalysisforum.eu/slides
  - Conference report - Bioanalysis (2012) 4(6), 627–631
- Identified as topic team on the EBF Strategy meeting in March 2012
- Many EBF members showed interest to participate in the topic team → 2 sub teams were formed in June 2012

Background - EBF TT-26

- Subteam A: Strategy for Peptides & Proteins
  - Analytical technologies
  - When to choose MS *vs.* LBA
  - Digestion procedures
  - Free *vs.* bound fractions

- Subteam B: Regulatory Expectations
  - Acceptance criteria peptides *vs*. proteins
  - Internal standard selection
  - Comparability of assays, MS *vs*. LBA
  - Complementarities of assays, MS *vs.* LBA

Background - EBF TT-26B

- Dietmar Schmidt, Sanofi, team leader

- Alberto Guenzi, Hoffmann – La Roche
- Carsten Krantz, Novartis
- Christoph Siethoff, Swiss BioQuant
- Gabriella Rohde, Bayer
- Magnus Knutsson, Ferring
- Michael Blackburn, Covance
- Michael Pilgaard Andersen, Novo Nordisk
- Paul Lassahn, SwissBioanalytics
- Richard Kay, Quotient Bioresearch
- Ronald Schmidt, Sanofi
- Simon Wood, Celerion

- Philip Timmerman, Janssen R&D, SC sponsor

Survey outcome - Experience

A high level survey was send out to all EBF members in Oct 2012

Full details of survey presented as poster

Q: Is your lab involved in regulated LC-MS/MS bioanalysis of large molecules?

Answer Options	Response Count
No	7
Yes	12
answered question	19

Survey outcome - Experience

Q: Which type(s) of large molecules do you analyse in your lab?

Answer Options	Response Count
Peptides	11
Proteins	7
Monoclonal ABs	4
Oligonucleotides	2

- Definition of a Peptide = no pre-treatment required before analysis; intact analyte
- Definition of a Protein = pre-treatment is required before analysis; digested analyte

Survey outcome – Method validation

Q: Do you perform additional validation experiments compared to a typical small molecule based method validation?

Answer Options	Response Count
Yes	4
No	8

- Digest efficiency
- Testing the cleavage from anti-drug antibody (positive control for ADA assay is used as “standard”)

Survey outcome – Method validation

Q: Do you investigate matrix effects for protein quantitation?

Answer Options	Response Count
Yes	10
No	0

- Majority uses “small molecule” approach on tryptic peptides

Survey outcome – Method validation

Q: Do you investigate recovery for protein quantification?

Answer Options	Response Count
Yes	7
No	4

- Ionisation and extraction recovery
- Digested spiked (protein) matrix sample vs. pure target peptide (Stable Isotope labelled IS) spiked into a digested blank matrix sample extract
- Synthetic ‘signature’ peptide to investigate extraction recovery

Survey outcome – Method development

Q: Do you experience adsorptive losses of your analyte?

Answer Options	Response Count
Yes	10
No	1

- Addition of BSA and/or surfactant to standard solutions
- Use Special plates
  - Low Binding plates
  - High quality/purity PP plates
- Use silanised glass ware
- Block surfaces with similar peptides
- Careful (or avoiding) evaporation

Survey outcome – IS selection

Q: Which kind of internal standard(s) do you use in your intact analyte assay?

Answer Options	Response Count
Stable labelled whole peptide	9
Surrogate peptide	3
Others – please specify	2

- Use halogenated peptides

Survey outcome – IS selection

Q: Which kind of internal standard(s) do you use in your digested analyte assay?

Answer Options	Response Count
Stable labelled whole protein	3
Stable labelled tryptic peptide	7
Others – please specify	0

Survey outcome – Protein quantification

Q1: If you perform tryptic digestion of a protein, how many peptides do you quantify?

Q2: How many transitions per peptide do you monitor?

Answer Options	Response Count	
	Q1	Q2
1	3	6
2	3	5
3	1	4
4 or more	1	1

Survey outcome – Acceptance criteria

Q: Which acceptance criteria do you normally use when performing LC-MS/MS analysis of

a) intact analyte?
b) digested analyte?

Answer Options	Response Count	
	Intact analyte	Digested analyte
15% (20% at LLOQ)	9	3
20% (25% at LLOQ)	3	5
other	0	0

- Includes hybrid assay, immunoaffinity sample prep. + LC-MS/MS

Survey outcome – Acceptance criteria

Q: According to your experience, what is the most important factor in setting the acceptance criteria for LC-MS/MS analysis of intact analyte?

- Availability of internal standard
- Behaviour in MS (fragmentation, charge-state)
- Immunocapture
- Performance and complexity of assay
- Variability in extraction procedure

Survey outcome – Acceptance criteria

Q: According to your experience, what is the most important factor in setting the acceptance criteria for LC-MS/MS analysis of digested analyte?

- Efficiency and reproducibility of digestion procedure
- Immunocapture
- Interferences
- Performance and complexity of assay

Survey outcome – Assay comparability

Q: What is your experience when LC-MS/MS results are compared with LBA in clinical and preclinical studies?

Answer Options	Response Count
Good correlation - Good agreement	5
Good correlation – Poor agreement	2
Bad correlation – Poor agreement	1

Commonly used definitions

Correlation: $R^2 > 0.8\text{-}0.9$

Agreement: Slope 0.85-1.15 or
RE: < ± 20-25%

Reasons

- Free *vs.* total
- Catabolism, metabolism
- IS working differently in spiked *vs.* incurred matrix

Assay comparability

In addition to assay set up, performance or acceptance criteria *(*where EBF can already provide some recommendations), we feel that **assay comparability** is an important aspect of peptide/protein analysis by LC-MS/MS (where EBF still has more questions than answers to date)

Assay comparability

- LC-MS/MS measures (very specific) MS(/MS)-transitions
- LBA measures a binding to a 3D/Van der Waals/other binding structural feature of a molecule….

?

Should we have full concordance of concentration data generated by an LC-MS(/MS) and a LBA assay?

NO, not necessarily

Concordance = **nice** ←→ difference = **interesting**

- LBA and LC-MS(/MS) may be orthogonal assays
- Different result can tell something on epitope/activity relationship, metabolism,….

Questions to the audience and panel discussion

- What is your opinion on ‘comparability’ of data from two (orthogonal) assays?

  - According to you, why should they match?
  - According to you, why is this not needed?

- Does anybody have experience in this field?

Towards a recommendation

- LC-MS analysis of peptides and proteins is scientifically challenging
  - Experience in industry is growing, and we are still learning
- LC-MS/MS acceptance criteria (4-6-15) in existing guidelines originates from SMOL, and should not be used by default for LC-MS analysis of LM
- 4-6-15 will work for some LM, but when scientifically justified, a priori set wider acceptance criteria should be OK
- A full concordance of concentration data generated by an LC-MS and a LBA assay is not necessary

Future work

- Get further input from
  - All of you in this session
  - Get input from EBF TT-26A and EBF members
- Continued discussions in topic team with focus on key scientific challenges
  - Scientific interface between LBA and LC-MS/MS technology in MVAL
  - Assay comparability
  - Acceptance criteria
- Publication 2013
  - Survey results + EBF recommendation

Acknowledgement

- EBF TT-26B
- Philip Timmerman, SC sponsor for TT-26
- EBF members given input to this survey