European Bioanalysis Forum

Regulatory Challenges and Acceptance Criteria

First EBF reflections on Method validation criteria for peptide/protein analysis with LC-MS based techniques

Presented by Philip Timmerman, on behalf of EBF

at the EBF Focus Meeting – Large meets Small

21-22 June 2011, Brussels, Belgium

Presentation outline

The changing environment

Historic perspective

Analytical challenges

Regulatory challenges

The Human Challenge

Conclusions

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The changing environment

1. Portfolios are changing

- Increased development times for small molecules, with increased pressure form generic market on NCEs
- Increased knowledge of peptide and protien chemistry and toxicology and pharmacology

2. Analytical tools are changing rapidly

- LC
  - Faster and better separations
- MS/MS
  - Higher sensitivity, also at higher m/z
  - Higher mass resolution
  - Diversification of interfaces
  - Faster ion optics (faster scanning)

1 + 2 = Great opportunities

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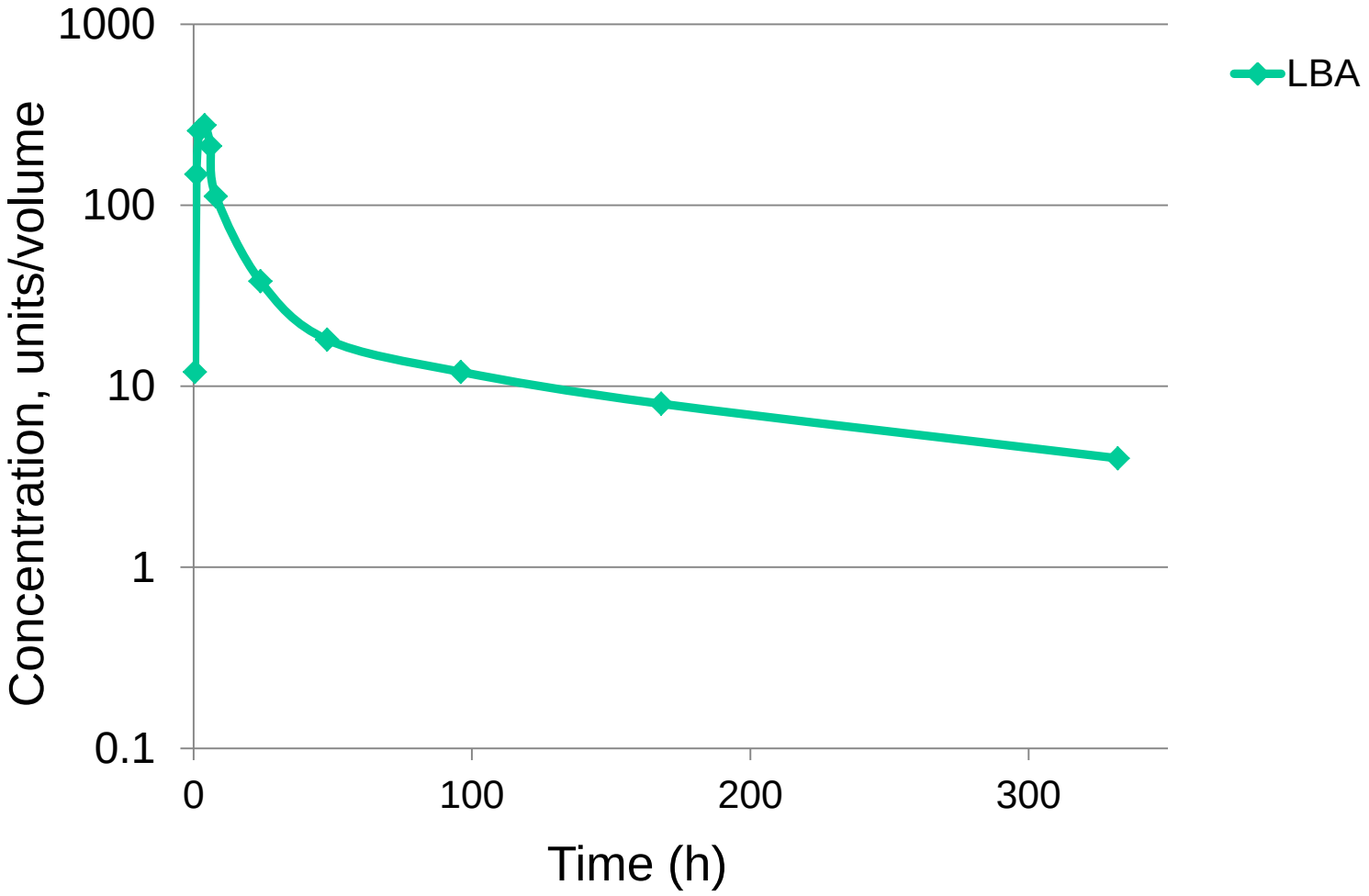
Who doesn’t remember this

LBA versus Chromatography

an history perspective on how “Small met Small” to sharpen our minds again

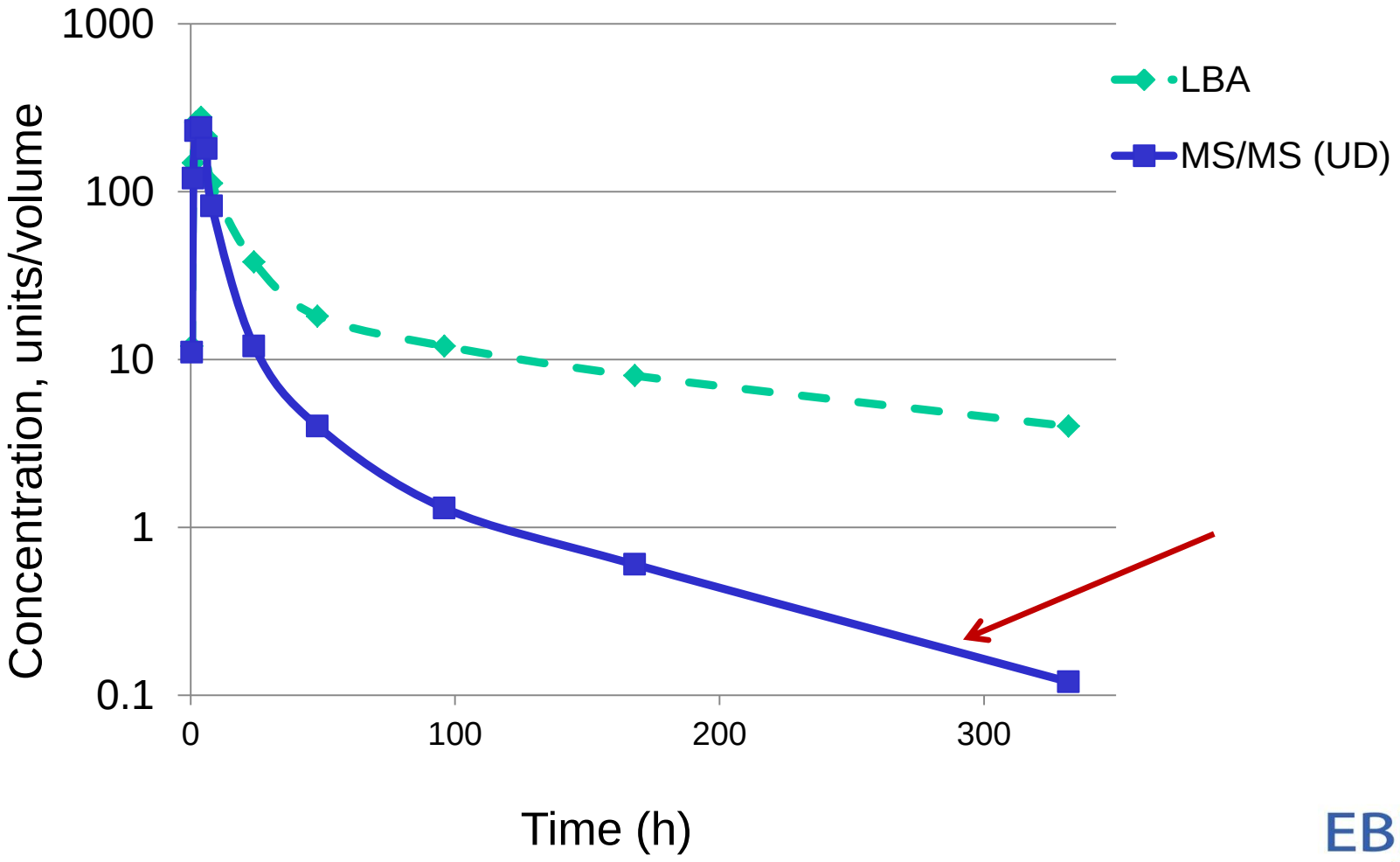
A simple example from the past

drug X (small molecule) measured in serum with Elisa



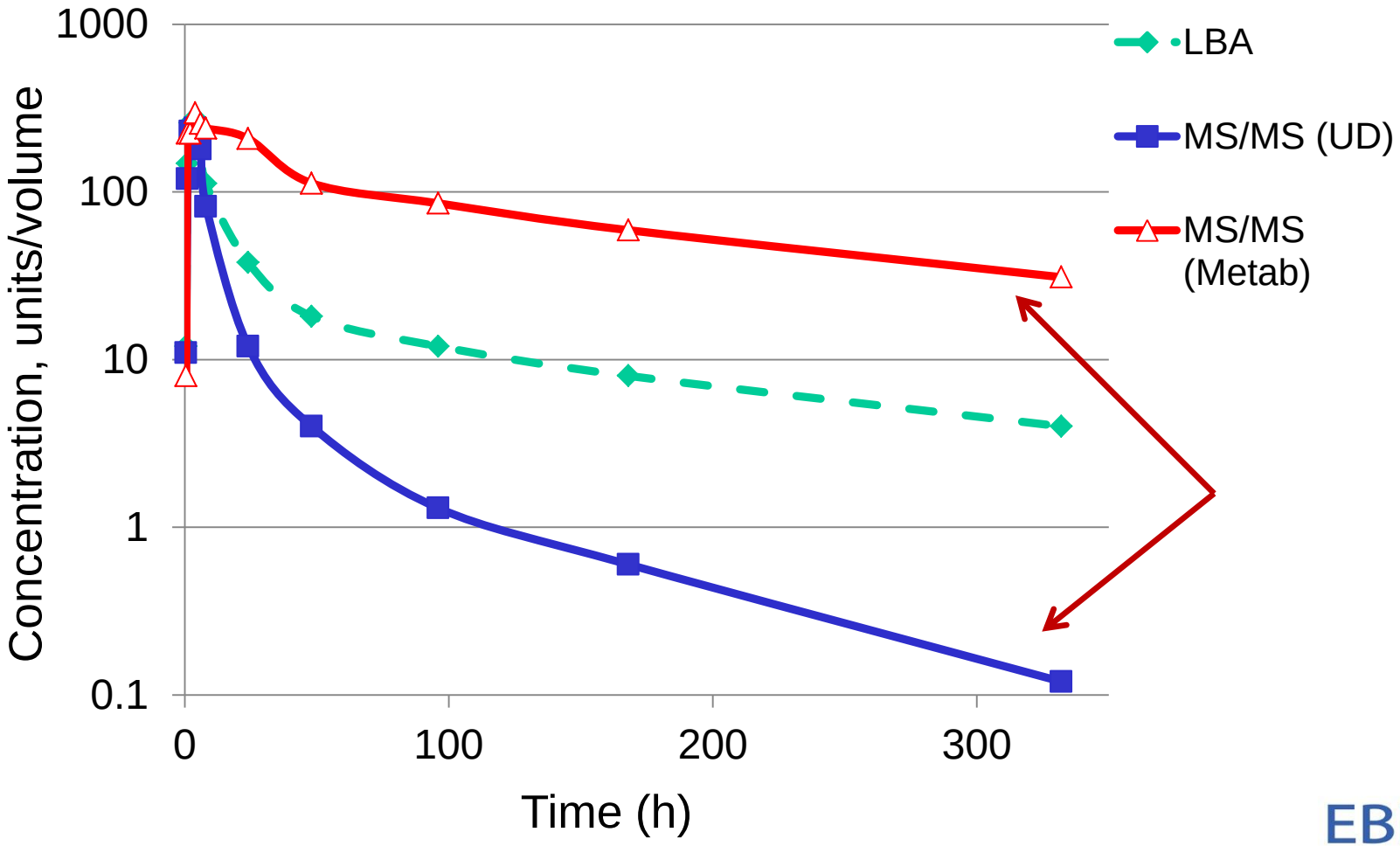
A simple example from the past

same drug X measured in serum with LC-MS/MS

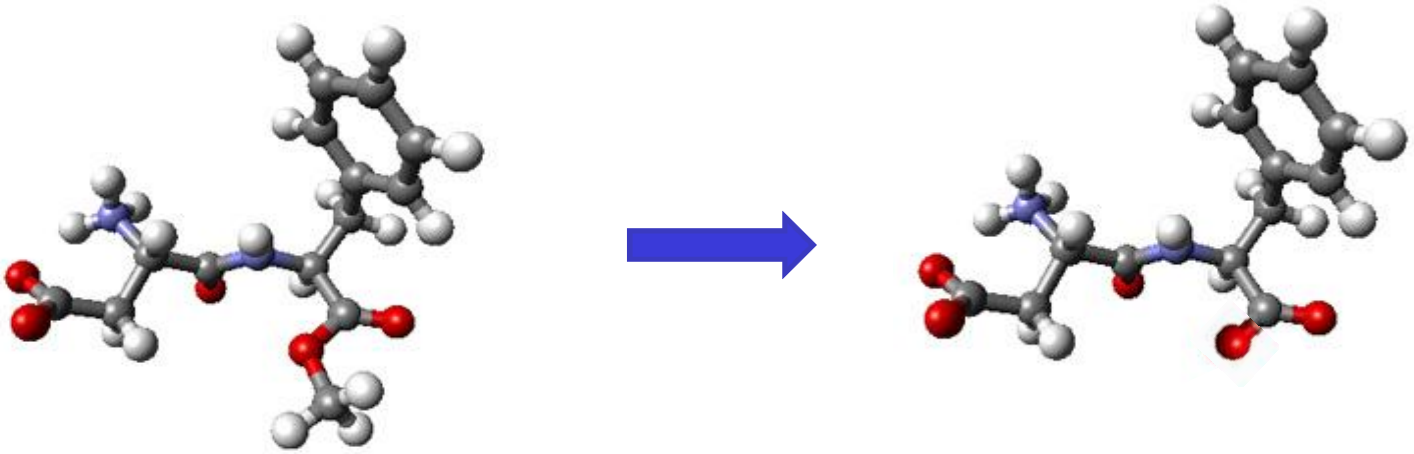


A simple example from the past

drug and metabolite measured in serum with LC-MS/MS

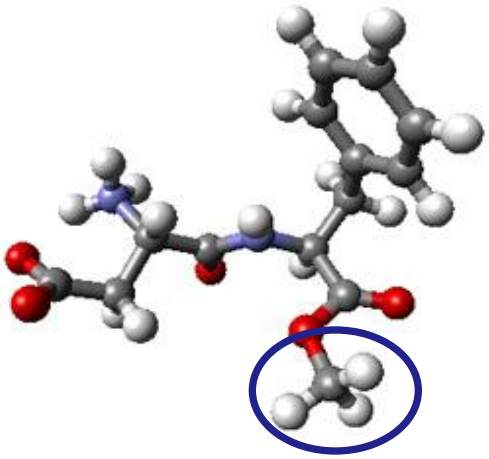


We all know what can happen...

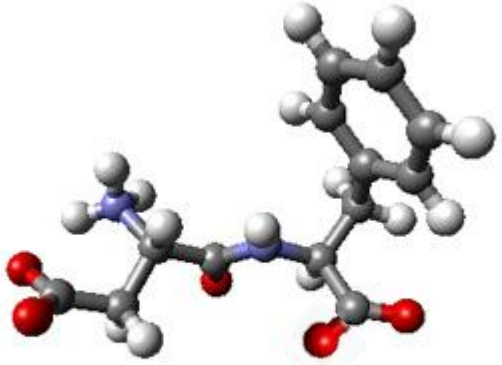


We all know what can happen...

Drug X



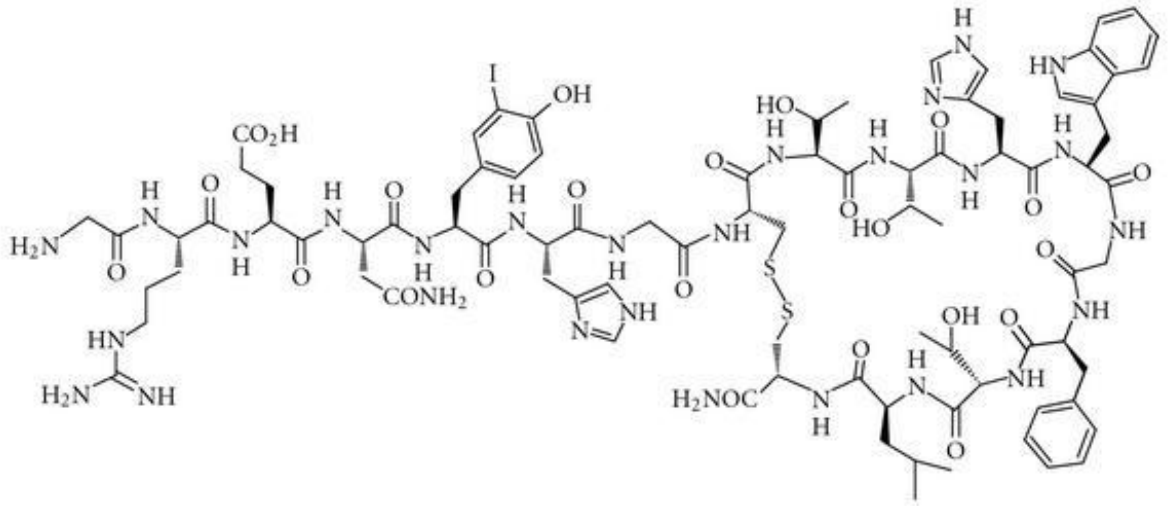
Metabolite of X



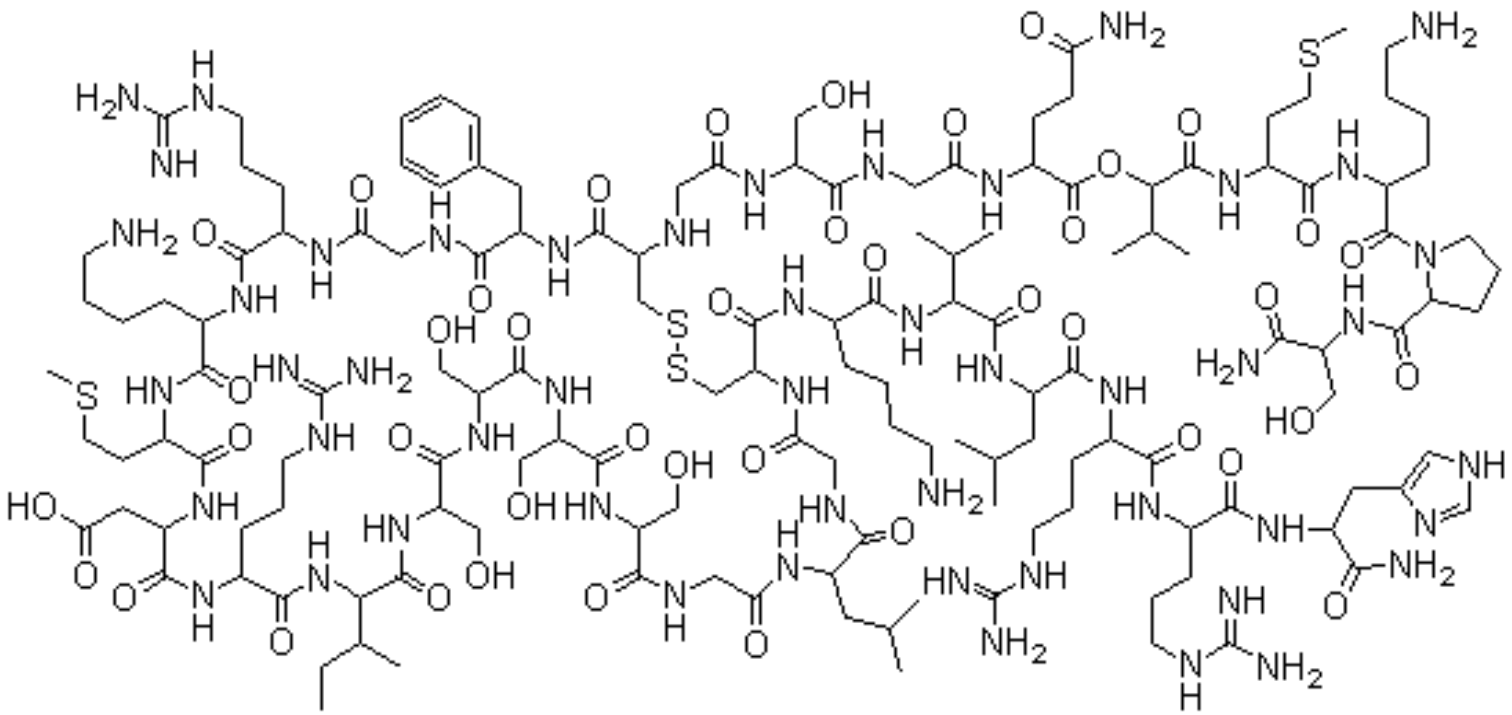
- Desmethylated metabolite
- Cross reactivity of metabolite = 1/8 in ELISA compared to UD
- $[\text{Metabolite of X}]_{plasma} >> [X]_{plasma}$
- $PK_{metabolites} \Delta\ PK_{Unchanged\ Drug}$

Additional attention needed to assess contribution of metabolite to pharmacological activity (PK/PD), toxicity,.. (+ MIST?)

But, do we already know what is going to happen here?

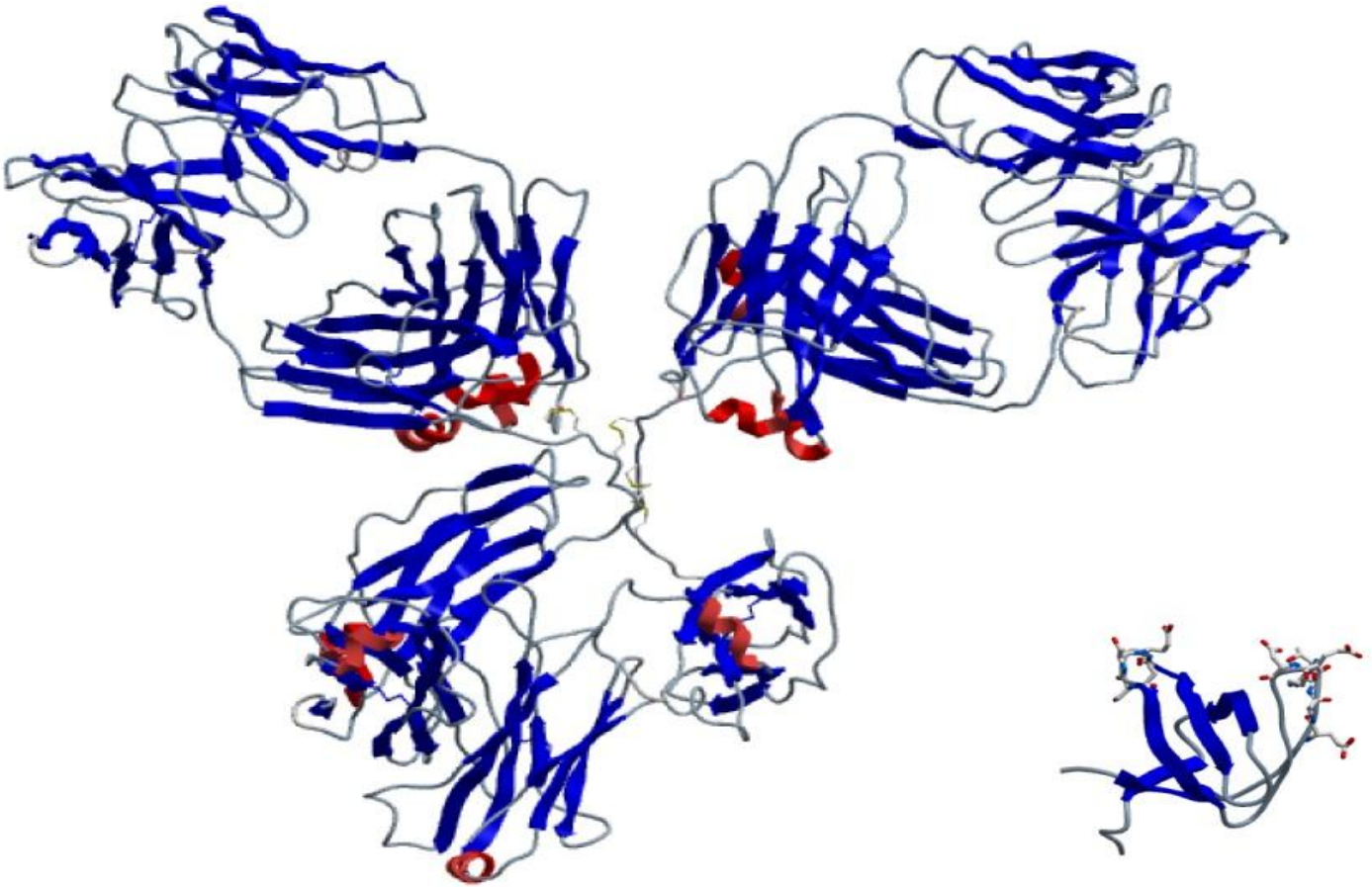


Or here?



Ser-Pro-Lys-Met-Val-Gln-Gly-Ser-Gly-
Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-leu-Gly-Cys-
Lys-Val-Leu-Arg-Arg-His

Or here?



Probably something similar….

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Points of attention – Analytical/Scientific

Heterogeneity of drug product/reference standard

- Impact of heterogeneity on MS/MS calibration (and differences with LBA)
  - (natural) Peptides
  - Proteins/Antibodies
- Differences in compound envelopes ‘incurred’ versus ‘spiked’ samples
  - For analytical reasons
  - Originating from PK
- 2003 FDA draft “*Guidance for Industry on Comparability Protocols - Protein Drug Products and Biological Products - Chemistry, Manufacturing, and Controls Information”* is good reading.

Points of attention – Analytical/Scientific

Metabolism

- Met. ID of peptides and proteins is a rapidly developing science
- Need to assess impact of metabolites on:
  - LBA vs. LC-MS/MS method comparisons
    - Probably more pronounced for peptides and oligonucleotides compared to the larger proteins or Abs
  - PK-PD and toxicokinetic evaluations
  - Selectivity of MS/MS
- Enzymatic digestions:
  - will enzymatic digestion differentiate for metabolites?

Points of attention – Analytical/Scientific

Physicochemical behavior

- Difference in overall physicochemical behavior of peptides/proteins *vs.* small molecules and impact on:
  - Solubility
  - Matrix effects
  - Sample prep
    - Incompatibility of MS technology with peptide/protein science?
  - Stability
    - Organic solvents *vs.* aqueous solvents
    - Strong acids or bases
    - Differences of impact tertiary/quaternary structure on stability
  - Adsorption/sticking

What happens when you move into ‘sticky things’ with limited experience

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With experience and SOP

Points of attention – Analytical/Scientific

Method comparisons / cross validations - do we anticipate:

- 1-1 relationship between LC-MS/MS and LBA assay and why?
- Differences between LC-MS/MS and LBA assay and why?
  - And how do we manage these differences from a PK, TK, PD perspective

Points of attention – Analytical/Scientific

Scenario building of strategic use of LBA *vs.* MS/MS

- Start with LBA and continue using LBA
  - Do we need to investigate specificity and selectivity better?
- Start with LBA and switch to MS/MS
  - Extend the cross validation to reevaluation of PK/PD,…?
- Start with MS/MS and remain on MS/MS
- Start with MS/MS and switch to LBA
  - Extend the cross validation to reevaluation of PK/PD,…?

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Method validation: acceptance criteria

- Do we have enough experience to judge?
  - Limited experience available to make a clear statement
  - A (potential) desire from the small molecule community to call LC-MS/MS of peptides/proteins 'the same' as LC-MS/MS of small molecules. But is this fair?
    - Who still remembers the origin of 4-6-15(20) or 4-6-20(25) and, more importantly, the rationale behind it?
      - Not that we want to challenge this again, but was 4-6-20(25) for chromatographic assays not good enough to document PK, safety and efficacy?
      - What drove/drives the difference in acceptance criteria for LBA vs. Chromatography?

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Points of attention - Regulations

Method validation: acceptance criteria

- Is ‘Size of molecule’ or ‘Technology’ the driver to define acceptance criteria?
  - Technology as driver: “its LC-MS/MS so LC-MS/MS rules apply”
    - Do we go back to pre-CCII criteria, e.g. because potential lack of Stable Isotope internal standards (resulting in pre-CC-II quality for MS/MS)?
    - What about ‘mixed technology methods’ (e.g. LBA sample prep combined with MS/MS detection?)
  - Size of molecule as driver: “it’s a large molecule, so LBA rules apply”
    - Can somebody give the definition of a Large Molecule?

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Points of attention - Regulations

Method Validation: experiments?

- Do we need to revisit relevance of the current validation experiments in the context of LmS?
  - Do we miss experiments/validation parameters?
  - Again, what about mixed technology methods (e.g. LBA prep + MS/MS detection?)
  - Or worse…..methods involving (enzymatic) digestion or tagging
- How to approach stability assessment and interpret them?

Study execution: acceptance criteria

- Similar discussion as for Method Validation
- Analytical design? Single or duplicate analysis? Etc…

So, still a lot of questions

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Please don’t get offended, this is only a teaser

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The Small folks

- A lot of analytical chemistry-genes
- Underdeveloped biology-genes
- Organic chemistry is a hobby
- Get all excited by high-end analytical instruments
- Thinks (s)he understands LBA …… but may underestimate the challenge
- Eager to start analyzing peptides/proteins with MS/MS because because because…
- Stubborn and sensitive, as all bioanalyst

The Large folks

- A lot of biology and biochemical-genes
- Underdeveloped analytical chemistry-genes
- Organic chemistry is a nightmare
- Convinced that automated pipettes are high-end analytical instruments
- Thinks (s)he understands chrom. Assays …… but may underestimate the challenges
- Not eager to dive into chromatography because because because….
- Stubborn and sensitive, as all bioanalyst

These challenges can only be overcome if we pool our experience and walk this trail together

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- Analysis of peptides and proteins with LC-MS(/MS) technology is a challenging scientific arena.
- Although our experience building, there is room for a lot of learning.
- There is a risk of undue ‘carbon copying’ current chromatography practices into LC-MS of peptides and proteins.
- Good science will help us to bring valid solutions.
- EBF wants to provide a home base for the continued discussion and connection in EU, feeding into GBC
- We will only be successful if we do this together

Acknowledgments

- All EBF members
- All of you

- And

and...Uderzo & Goscinny

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