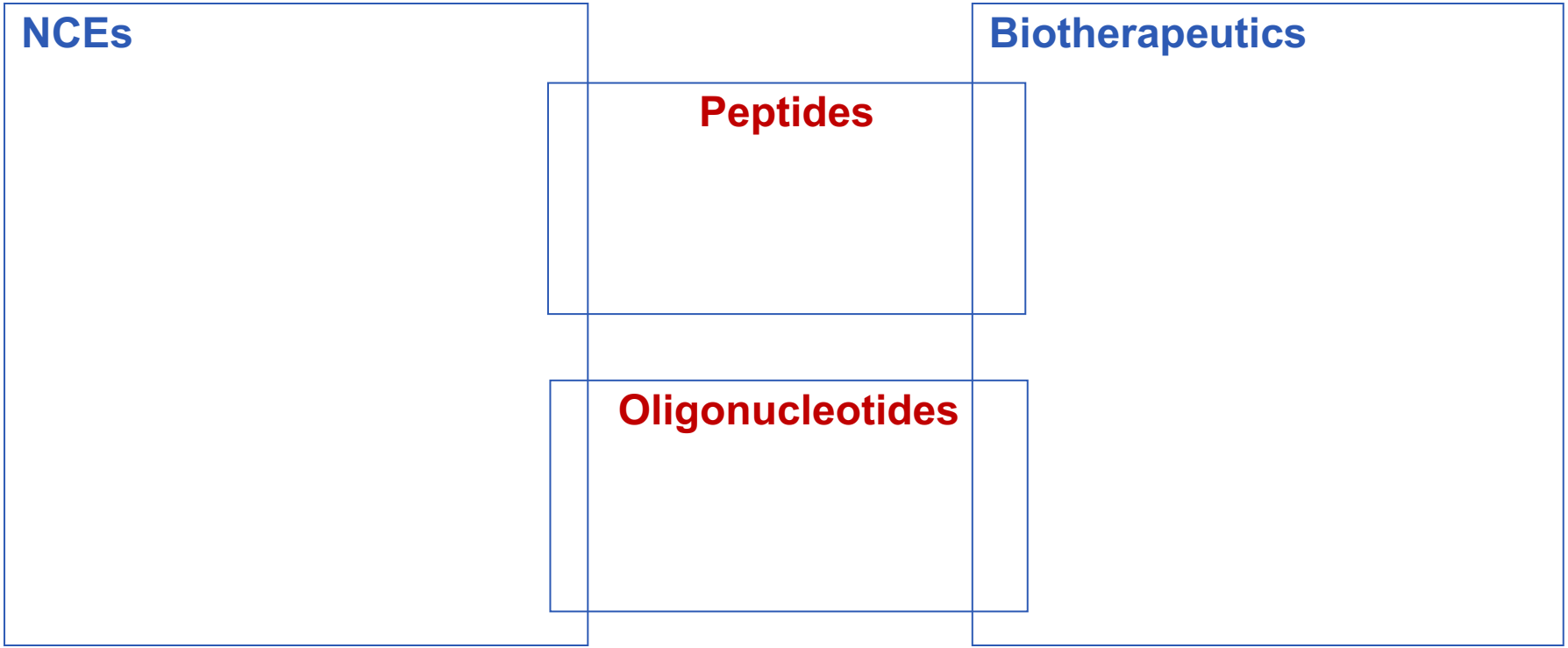
Metabolite quantification recommendation - fit for purpose

Philip Timmerman, EBF

The landscape



The landscape

NCEs

Well established but poorly understood

Metabolite ID and profiling`
Where do we start?…

Let’s start small…

Where do we start?…Let's start small…

A typical (non)-clinical protocol for *a novel chemical entity (NCE) pharmaceutical:*

5.2. Pharmacokinetics

Blood samples will be taken before dosing (0h) and 0h30, 1h, 2h, 4h, 8h, 24h, 48h, 96h and 168h after dosing on Li-heparin. The samples tubes need to be centrifuged within 30 minutes after sampling to prepare plasma. The plasma samples are frozen (> -18° C) within 30 minutes after centrifugation until analysis
In these plasma samples, the dosed drug ABC-12345 and *it's metabolites ABC-23456 and ABD-66523 will be quantified using a validated method.*

The Drug Research and Development Process

e.g. Study: 1m Tox study in rat and dog

What would be the rationale to include metabolites in a validated assay pre-FIH?

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The Drug Research and Development Process

e.g. Study: FIH in clinical Phase 1

What would be the rationale to include metabolites in a validated assay in an SAD/MAD study

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The Drug Research and Development Process

e.g. Study: phase 3 (patients)

What would be the rationale to include metabolites in a validated assay in BEQ study

e.g. Study: phase 1 (HV) DDI

What would be the rationale to include metabolites in a validated assay in BEQ study

e.g. Study: BEQ

What would be the rationale to include metabolites in a validated assay in BEQ study

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So we go to ICH M10 for guidance

What does ICH M10 say about metabolites

1.3. Scope

This guideline describes the validation of bioanalytical methods and study sample analysis that are expected to support regulatory decisions. The guideline is applicable to the bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in nonclinical toxicokinetic (TK) studies conducted according to the principles of GLP, nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies, and all phases of clinical trials, including comparative bioavailability/bioequivalence (BA/BE) studies, in regulatory submissions. Full method validation is expected for the primary matrix intended to support regulatory submissions. Additional matrices should be validated as necessary.

Looking at the bigger picture…

What does ICH M3(R2)* say about metabolites

3. Toxicokinetic and pharmacokinetic studies

In vitro metabolic and plasma protein binding data for animals and humans and systemic exposure data (ICH S3A, Ref. 7) in the species used for repeated-dose toxicity studies generally should be evaluated before initiating human clinical trials. Further information on pharmacokinetics (PK) (e.g., absorption, distribution, metabolism and excretion), in test species and in vitro biochemical information relevant to potential drug interactions should be available before exposing large numbers of human subjects or treating for long duration (generally before Phase III). These data can be used to compare human and animal metabolites and for determining if any additional testing is warranted.

Nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Such studies should be conducted to support Phase III clinical trials. For drugs for which the daily administered dose is <10 mg, greater fractions of the drug related material might be more appropriate triggers for testing. Some metabolites are not of toxicological concern (e.g., most glutathione conjugates) and do not warrant testing. The nonclinical characterization of metabolites with an identified cause for concern (e.g., a unique human metabolite) should be considered on a case-by-case basis.

**ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals - Scientific guideline*

Use the right map

DDI

EMA Guideline: Guideline on the Investigation of Drug Interactions (2012)

- Phase I metabs with both >25% of the AUC of parent drug and >10% of the drug-related exposure;
- use unbound concentrations, if PPB data not available, use (bound + unbound)
- Pharmacologically active metabolites based on AUC contributing *in vitro* activity ≥50% of the target activity identified
- Formation and elimination pathways should be determined

US-FDA: DDI studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (2012)

- DDI potential for metabolites ≥25% of parent drug (AUC) should be considered
- both metabolism-based DDI and transport-based DDI

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US FDA Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry (2023)

- a systematic, risk-based approach
- Scope limited to Proinflammatory Cytokine (modulators) which may affect CYP450 expression

MHLW (Japan): Drug interaction guideline for drug development and labeling recommendations (2014)

- Aligns with FDA / EMA

MIST

- US-FDA Guidelines: Guidance for Industry, Safety Testing of Drug Metabolites, (2020) → R2 from 2020 aligns with ICH M3(R2)
  - Metabolites with ≥10% of parent drug exposure (AUC)
  - Other metabolites also can elicit safety concern, (…) should be addressed on a case-by-case basis
  - Timing: toxicity assessment should be reported before beginning large-scale clinical trials.
  - No further testing required if human metabolite exposure is covered in toxicity assessment
- ICH Guidelines: included in ICH M3(R2), (2009) + Q&A (2012)
  - Metabolites with >10% of total drug-related exposure;
  - metabolites with an identified cause for concern (e.g., a unique human metabolite) should be considered on a case-by-case basis.
  - if dose/day <10 mg: greater fractions might be more appropriate triggers.
  - Waiver for phase-2 metabolites
  - Timing: to support phase 3 clinical trials

Industry papers

- Drug Metabolites in Safety Testing, Toxicol. Appl Pharmacol, 182, 188-196., 2002
- Seeing through the mist: abundance versus percentage. Commentary on metabolites in safety testing. Drug Metab Dispos. ;33(10):1409-17, 2005
- Drug metabolites in safety testing. Toxicol. Appl. Pharmacol. 190, 91–92. (5), 2003
- Metabolites and safety: What are the concerns, and how should we address them? Chem. Res. Toxicol. 19, 1570–1579, 2006
- Which Human Metabolites Have We MIST? Retrospective Analysis, Practical Aspects, and Perspectives For Metabolite Identification and Quantification in Pharmaceutical Development Chem. Res. Toxicol., 22 (2), pp 280–293, 2009
- A Decade in the MIST: Learnings from Investigations of Drug Metabolites in Drug Development under the “Metabolites in Safety Testing” Regulatory Guidance, Drug Metabolism and Disposition June 2018

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Bioanalysis (2016) 8(12), 1297–1305

Best practices for metabolite quantification in drug development: updated recommendation from the European Bioanalysis Forum

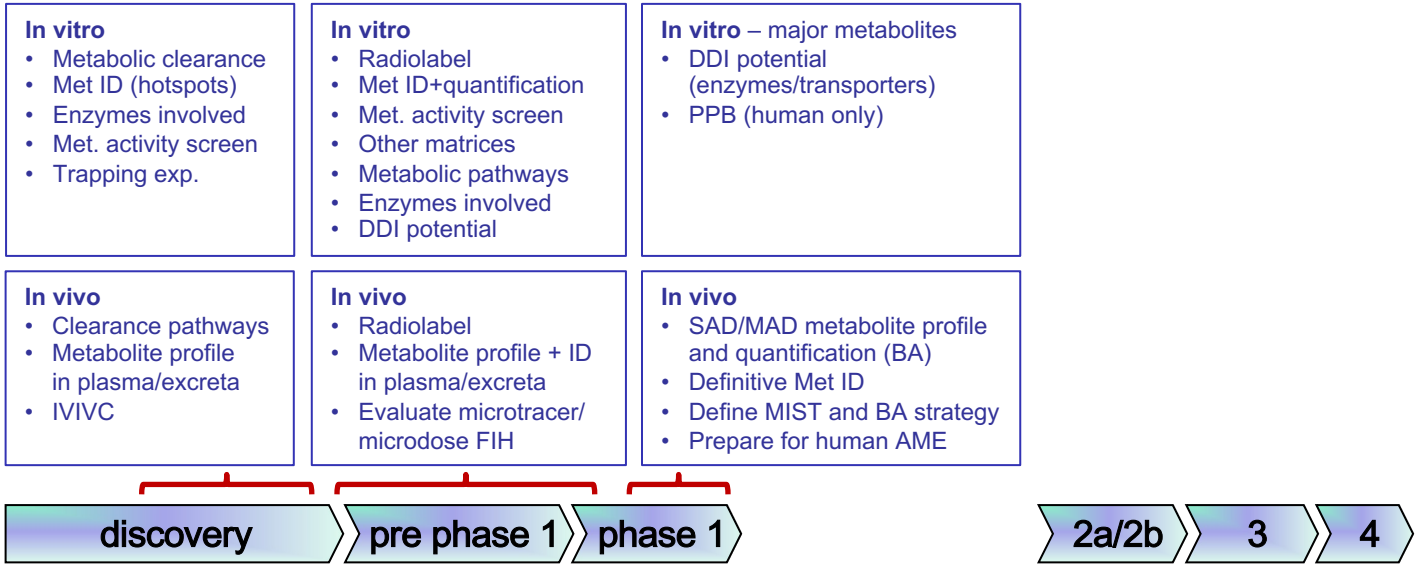
Metabolite quantification and profiling continues to grow in importance in today's drug development. The guidance provided by the 2008 FDA Metabolites in Safety Testing Guidance and the subsequent ICH M3(R2) Guidance (2009) has led to a more streamlined process to assess metabolite exposures in preclinical and clinical studies in industry. In addition, the European Bioanalysis Forum (EBF) identified an opportunity to refine the strategies on metabolite quantification considering the experience to date with their recommendation paper on the subject dating from 2010 and integrating the recent discussions on the tiered approach to bioanalytical method validation with focus on metabolite quantification. The current manuscript summarizes the discussion and recommendations from a recent EBF Focus Workshop into an updated recommendation for metabolite quantification in drug development.

First draft submitted: 26 January 2016; Accepted for publication: 27 April 2016;
Published online: 24 May 2016

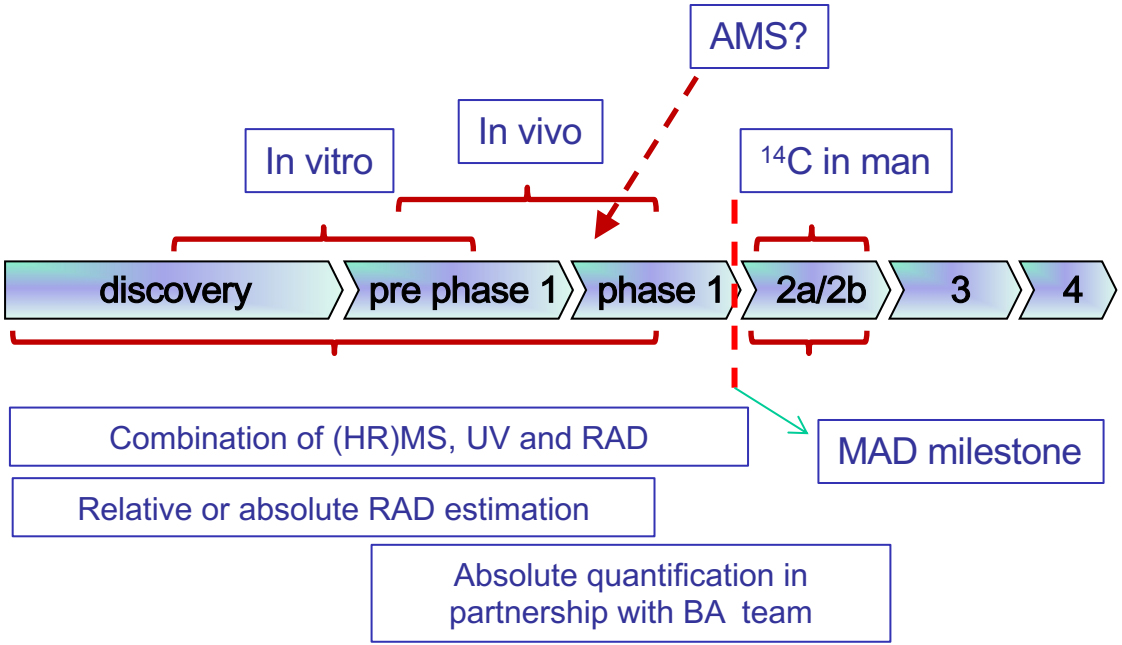
Philip Timmerman*[,1], Stefan Blech[2], Stephen White[3], Martha Green[4], Claude Delatour[5], Stuart McDougall[‡,6], Geert Mannens[1], John Smeraglia[5], Stephen Williams[4] & Graeme Young[3]

Written around NCEs

Metabolite profiling – *in vivo* & *in vitro* testing

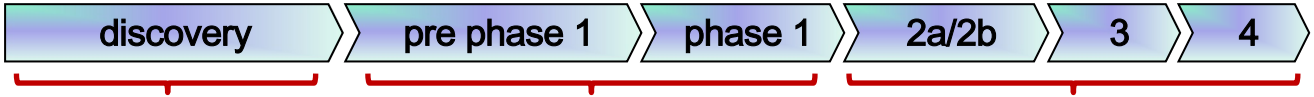


Metabolite profiling – how?



Metabolite quantification translated into bioanalytical work

When using AMS or ^{14}C, use scientific criteria relevant to the technology. Be cautious not to mix up profiling and quantification purposes



- Focus on *in vitro*
- Limit *in vivo* metabolite quantification
- Screening

Preclinical and clinical studies

- Plasma, serum or blood.
- Use **screening** (preferred for earlier phases) and **scientifically validated** methods (preferred @ SAD/MAD) to document the ICH M3(R2) coverage of metabolites.
- Consider **relative exposure** ratios in absence of reference standards
- Metabolite quantification in other matrices for profiling purposes

Clinical studies only

- Plasma, serum or blood.
- use **regulatory validated** methods to quantify only those metabolites contributing to >25% activity (not only AUC) relative to unchanged drug.
- No other metabolites require quantification
- consider selection of studies and/or selection of samples/subjects instead of all samples from all subjects in all studies

Milestone: around Multiple Ascending Dose (MAD) in human

- Document ICH(M3) coverage of metabolites (MIST perspective
- Ensure coverage of human disproportionate metabolites in animal studies (may require separate Tox study)
- Document PD activity (collaborate with clinical/pharmacology partners)

- Consider quantification in special studies (e.g. DDI) of other metabolites using **scientifically validated** methods

In simple words….

Not that many metabolites require a validated method as per BMV

Pre-phase 1:

- None, except pro-drugs
- Metabolites dosed as test article should not be considered as metabolites

Phase 1-2

- None, except pro-drugs
- Sponsors may want to consider to already include metabolites with documented activity > 25% (albeit, how to decide on '> 25%'?

> Phase 2, and considering metabolite profiling info

- UMM
- > 10% of drug exposure and not covered during tox studies
- Active metabolites as per FDA/EMA guidelines

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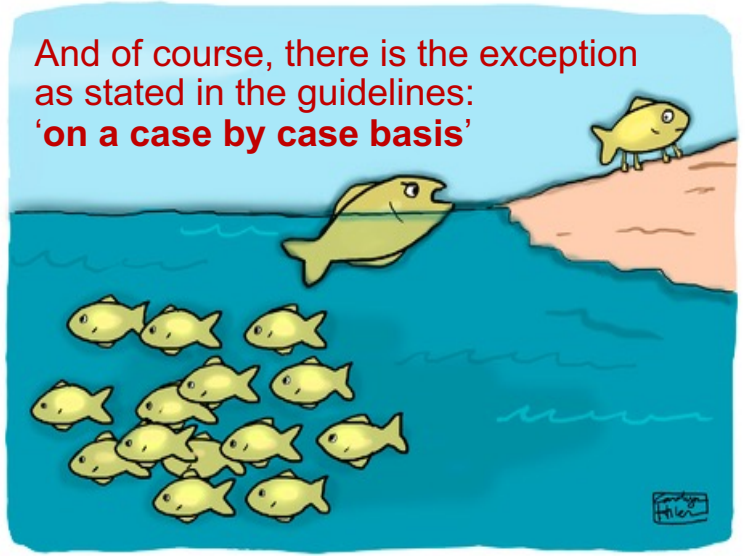
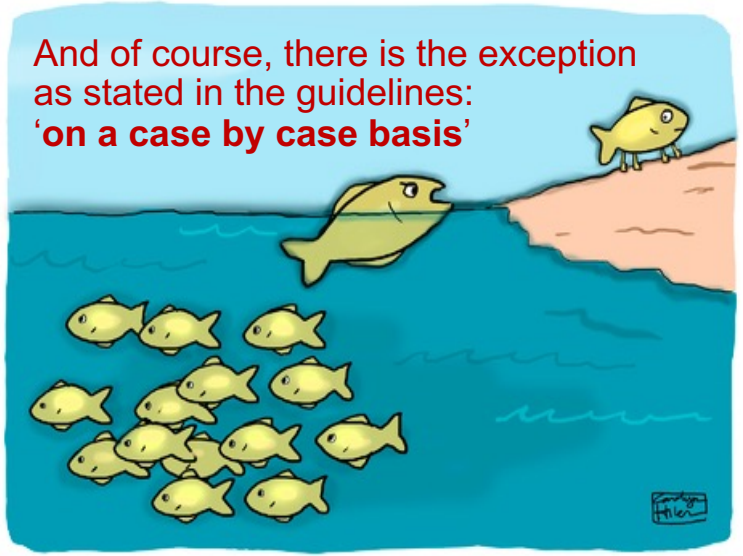


Paragraph 5.4.5 (EMA guideline) on how minor “active metabolites” should be treated under normal and abnormal (DDI) conditions, as this has an important impact on our business.

If there are active metabolites contributing to the efficacy and safety of the drug, the exposure to these metabolites should be evaluated in the interaction studies. Moreover, if there are pharmacologically active metabolites which do not contribute significantly to in vivo effects of an investigational drug during normal conditions, the need for determining the exposure of these metabolites should be considered as a marked increase in exposure resulting from the interaction could be clinically relevant.



In strategic words

- You can always do more, and it is likely desirable to get to know your project as early as possible…de-risking a project in phase 3 isn't smart…
- But don't inspire the regulators or our community that doing more for internal decision making is a regulatory requirement, because it may become one.

The landscape

Biotherapeutics

Biotherapeutics & metabolites

Not so much there…

➢ Not is scope of above

ICH S6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals - Scientific guideline

MIST and **DDI Guidelines – The details**

DDI

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ICH S6 (R1) says:

ICH S6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals - Scientific guideline

4.2.3. Metabolism

The expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood. Classical biotransformation studies as performed for pharmaceuticals are not needed.

Understanding the behaviour of the biopharmaceutical in the biologic matrix, (e.g., plasma, serum, cerebral spinal fluid) and the possible influence of binding proteins is important for understanding the pharmacodynamic effect.

US FDA Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry (2023)

- a systematic, risk-based approach
- Scope limited to Proinflammatory Cytokine (modulators) which may affect CYP450 expression

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

The landscape

Peptides

Oligonucleotides

FDA Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (draft guidance – 2022)

The landscape

FDA Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (draft guidance – 2022)

…Appropriate bioanalytical methods should be used to characterize the parent oligonucleotide and any relevant metabolites, including chain-shortened metabolites. Refer to the FDA guidance entitled Bioanalytical Method Validation (May 2018) for additional details….

What would this mean for

NCEs

FDA Safety Testing of Drug Metabolites Guidance for Industry (2020)

This guidance applies to small molecule nonbiologic drug products

***FDA In Vitro* Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry**

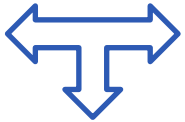
Only small molecule drugs are covered in this guidance. Interactions involving biologics (therapeutic proteins) are beyond the scope of this guidance.

ICH M3 R2

ICH M10?

Peptides

?



Oligonucleotides

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ICH M10?

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ICH S6 R1

ICH M10?

Bioanalysis (2016) 8(12), 1297–1305

Best practices for metabolite quantification in drug development: updated recommendation from the European Bioanalysis Forum

Likely, the thought process and analysis of the EBF recommendation from 2016 can be a starting point to prevent going overboard for Peptides/oligonucleotides

Acknowledgements

EBF community and EBF teams for discussion on the topic since 2010

Contact Information

Questions: info@e-b-f.eu

European Bioanalysis Forum vzw

www.e-b-f.eu