WHAT METABOLITE TO QUANTIFY AT WHAT STAGE: FDA/ICH 代谢产物安全性评价和生物分析新指导原则下代谢产物分析的重新考量

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OUTLINE

- ❑ Introduction
  - ❑ Highlights of guidance: MIST and ICH M3 (R2)
  - ❑ Review of discussions at WRIB
- ❑ Workflow of metabolite monitoring at IND, FIH and beyond
  - ❑ Typical workflow
  - ❑ Changes and evolution in practice
  - ❑ Considerations in metabolite monitoring
  - ❑ Planning and logistic
- ❑ Case studies
  - ❑ Current workflow including additional requirements
  - ❑ Method validation under new bioanalytical method validation (BMV) guidance
- ❑ Conclusions

HIGHLIGHTS OF FDA GUIDANCE ON SAFETY TESTING FOR DRUG METABOLITES (2008; REVISED 2016)

- Applies to small molecule, non-biologic drug products
  - Risk-benefit assessment is considered for cancer therapies
- Encourages early evaluation of metabolism
  - Identify differences between human & preclinical species (ie, disproportionate metabolite exposure)
- Ph I metabolites more likely to be reactive or show pharmacological activity, and thus need evaluation
- Conjugated metabolites generally have decreased activity, eliminating need for further evaluation
  - However, some Ph II metabolites (ie, acyl glucuronides) show toxicity & require safety assessment
- Metabolites that can raise a safety concern are <u>human</u> metabolites that are <u>$\geq$10% of total drug-related material (DRM) exposure at steady state</u>

HIGHLIGHTS OF FDA GUIDANCE ON SAFETY TESTING FOR DRUG METABOLITES (2008; REVISED 2016)

- For human metabolites that are >10% of exposure to total DRM at steady state
  - Need to further characterize coverage in preclinical species
  - Evaluate on- and off-target activity
  - ADME liabilities (ie, CYP, transporter inhibition, etc.)
- If metabolite exposure in 1 tox species is >0.5 the human exposure, it can be assumed that the metabolite's contribution to overall toxicity has been established
- Options for any disproportionate human metabolites
  - Find new tox species that has exposure to the metabolite
  - Administer the metabolite directly in the tox species

ICH M3 (R2) & Q AND A (2010, 2013)

Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

Q3: *When characterization of metabolite toxicity is warranted, in what type(s) of in vivo nonclinical studies is it important that adequate systemic exposure to a metabolite be achieved?*

A3: It is important to have adequate exposure to the metabolite in one species used in the general toxicity evaluation, one species used in a carcinogenicity study when carcinogenicity evaluation is warranted (or one species used in an in vivo micronucleus study when carcinogenicity evaluation is not warranted), and one species used in an embryo-fetal development study.

FIT-FOR-PURPOSE (FFP) BIOANALYTICAL ASSAYS

- ❑ 2006 Crystal City Conference Report and FDA (2008) Guidance for Industry Safety Testing of Drug Metabolites recommended PK characterization of unique and/or major human metabolite as early as feasible.

- ❑ Characterization should proceed using a **flexible, "tiered"** approach to bioanalytical methods validations. The specifics of the tiered validation approach is driven by scientifically appropriate criteria. Validation effort increases as a product moves from early to late development.

DISCUSSION OF METABOLITE ANALYSIS AND MIST IN PAST WRIBS

- 2013 WRIB: Issues regarding MIST
  - A tiered approach is often employed in order to obtain relative exposure data in animal versus humans for MIST risk assessment in early drug development. A preliminary evaluation…samples pooled by AUC…

- 2015 WRIB: Unique/customized method development for metabolites
  - Validated, qualified, semi-quantitative methods
  - Only significant and unique human metabolites are followed throughout drug development
  - Stability of both parent drug and its metabolite(s) in the intended study matrix needs to be assessed and controlled.

DISCUSSION OF METABOLITE ANALYSIS AND MIST IN PAST WRIBS

- 2018 WRIB: Revisiting MIST following a decade of implementation
  - Understanding metabolism is important for pharmacology, PD responses and potential for drug-drug interaction
  - Neither practical nor required to implement fully validated methods for all possible metabolites during early development phases
  - Combining metabolite profiling and quantitative bioanalysis
  - Qualified methods can be useful when metabolite exposure is considered borderline
- Assessments of active and significant human metabolites should use fully validated methods. No regulatory expectations to assay major, inactive metabolites exist for those with adequate coverage in toxicology species unless a problem is anticipated in special populations.

INITIAL METABOLITE MONITORING IN IND TOXICOLOGY - FIH

Factors:

- % Exposure to parent
- Human exposure prediction
- Assay

Metabolite identified during discovery pre-clinical studies

Significant Exposure

Yes

Pharmacologically active
Known toxicology risk

Yes

Consider monitoring with a fully validated assay

No

Consider monitoring with a qualified assay

No

Pharmacologically active
Known toxicology risk

Yes

No monitoring

No

No monitoring

METABOLITE PROFILING TO ASSESS HUMAN SAFETY PER MIST GUIDANCE

Metabolite ID (high dose SAD & MAD)

Comparative biotransformation profiling (e.g., w/ representative samples from MAD & SS animal studies & screening/research-grade assays)

1) Determine relative exposure

2) Evaluate coverage

Estimate whether metabolite exposure is >10% relative to drug-related material (DRM) in human SS samples (MAD) (e.g., UV, or other exploratory assay, or BioA assay)

>10%

Compare metabolite exposure in animal (@ SS NOAEL doses) and human (MAD ~efficacious dose) using AUC-pooled samples or + BioA assay

BioA assay to confirm coverage in GLP tox studies

<10%

Metabolite peak area is comparable or greater in animal than human

NO

Selection of alternate/additional tox species or dosing metabolite to animals, if >10% in human & not covered by tox species

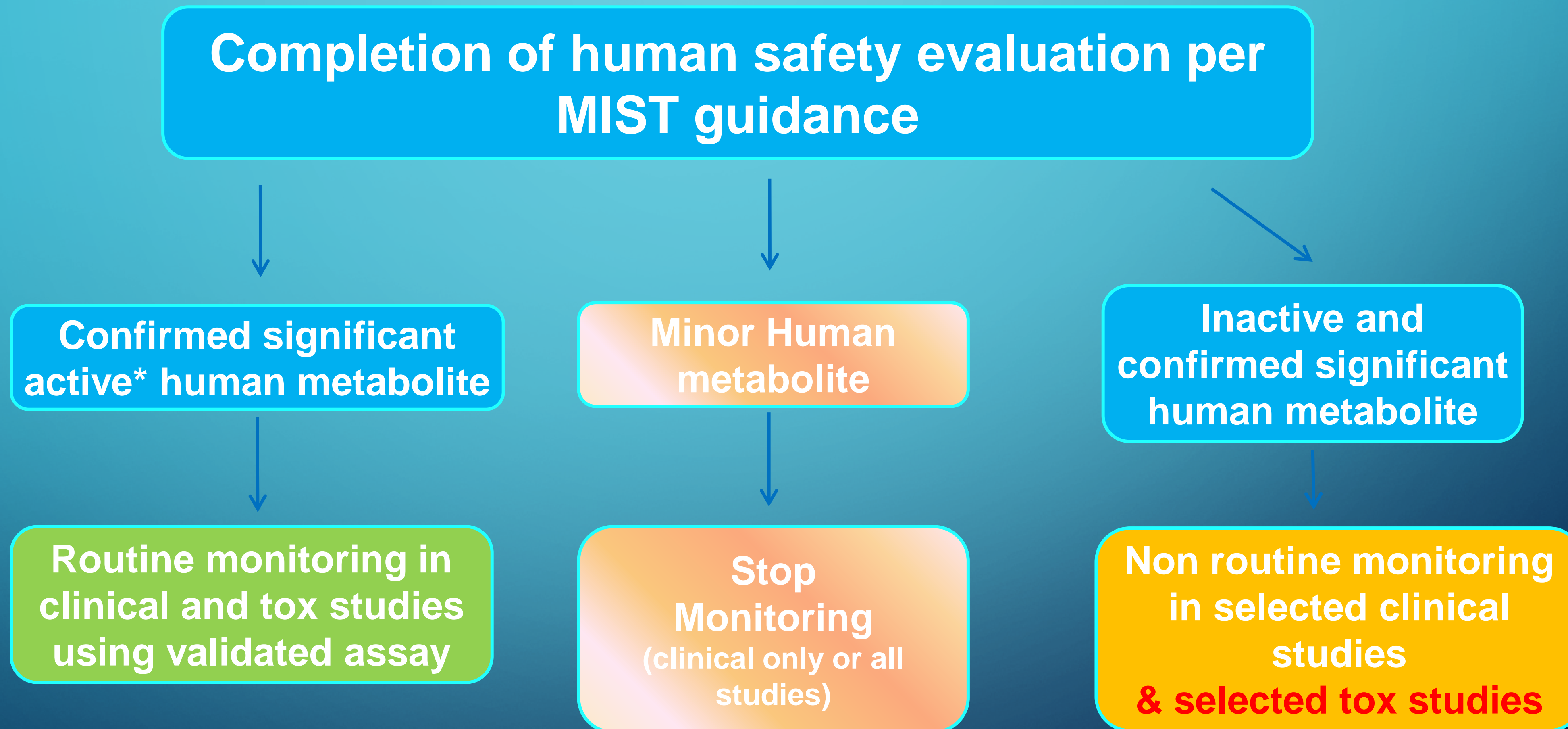
YES

Safety exposure coverage confirmed or safety not a concern

Post FIH

Consider need for further investigation: activity, off-target toxicity, to inform monitoring decisions

METABOLITE MONITORING POST FIH



*on or off-target activity

CONSIDERATIONS IN METABOLITE MONITORING

Before MAD:

- ❑ In vitro cross-species metabolite profiling suggest likely human generation
- ❑ Moderate/high exposure relative to parent (~ >25%" parent) in discovery tox studies; (leverage available data to put perspective on "%DRM")
  - ❑ *Regulatory Guidance does not define "significant" animal metabolite,*
- ❑ Pharmacological activity
- ❑ Structural alert or positive finding in off target screens

CONSIDERATIONS IN METABOLITE MONITORING

After MAD:

- Major human metabolite that was monitored in IND tox:
  - If safety coverage established: continue to monitor in long term (repeat dose) GLP studies only if exposure approaches/ exceeds parent drug
- Major human metabolite that was NOT monitored in IND tox:
  - Use comparative metabolite profiling of MAD vs SS animal samples (non-GLP) to support coverage for minor metabolites & (as preliminary) to support coverage for major metabolites until TK/PK data available
  - Identify one long term GLP study for each species to monitor

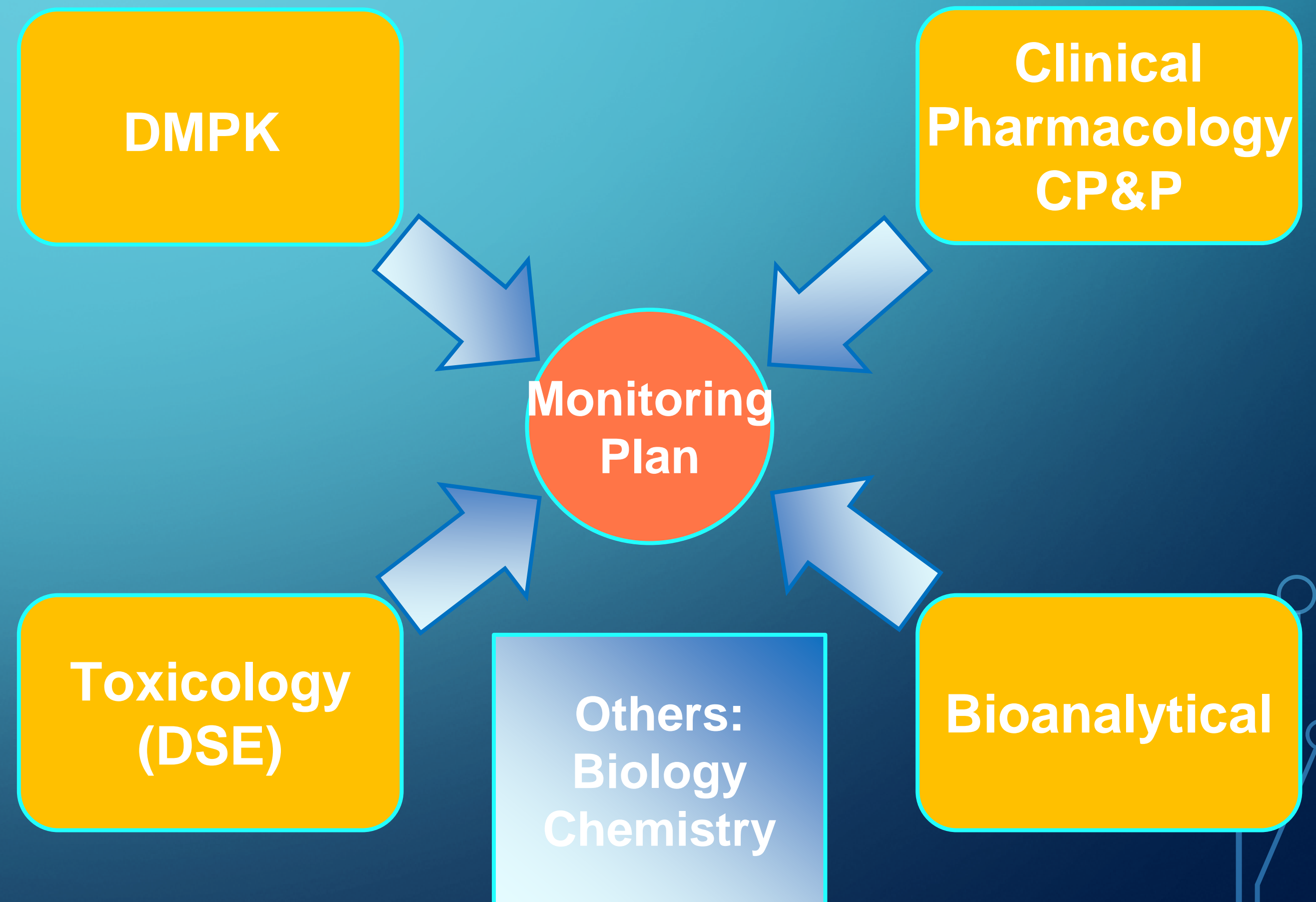
CONSIDERATIONS IN METABOLITE MONITORING (CONT.)

- Monitor major human metabolites in repeat-dose GLP rodent & non-rodent: to establish safety coverage
  - Ensure Met/P ratio is consistent across doses within a species.
  - If a new high dose is required, may need to monitor in a new study
  - If Met/P ratio is NOT consistent with dose or time, need to continue to monitor in additional studies
- Monitor major human metabolites in the following additional studies:
  - Embryo-Fetal Development (EFD) rodent and rabbit
  - Pre/Postnatal Development (PPND) rodent (lactation)
  - GLP Juvenile tox
  - Alternate rodent species for carcinogenic (CARC) tox (if 2 CARC species needed)

PLANNING AND LOGISTIC ISSUES IN ASSAY DEVELOPMENT FOR METABOLITE MONITORING

- Bioanalysis outsourced earlier at time of IND enabling tox studies
  - CROs generally requires more time to develop assays
  - Require larger amounts of metabolite reference standards
- Chronic tox studies may be initiated during FIH
  - Initiate monitoring in IND-enabling studies, if possible
- Obtaining metabolite reference standard can be challenging & time consuming
  - Chemical synthesis
  - Microbial or enzymatic synthesis
- How to define “significant pharmacologically active metabolite”?
  - Combination of exposure + efficacy?

Multiple Groups Are Involved in the Development & Execution of the Metabolite Monitoring Plan



CASE 1: COMPOUND-A IN FIH

M11 (des-methyl): active metabolite (>10%) in animal species

- ❑ Qualified assay in IND-tox, SAD and MAD
- ❑ Minor in SAD and MAD. Stopped after MAD in clinical, (included in chronic and repro-tox)

M12: major in SAD but minor in MAD

M17: major in SAD and MAD

7 days PK studies in tox species

- ❑ Objective: Comparison of exposure to the major human plasma metabolites in Tox species (rat and dog)
- ❑ Dose: 75 mg/kg for rat , 15 mg/kg for dog for 7 days. Plasma samples were collected day 7

Ratios (%) of metabolites to parent in plasma LC/UV profiles

Time	SAD 900 mg	
	M12	M17
2 h	17.78	15.39
4 h	15.36	16.23
8 h	17.44	16.67
16 h	11.63	12.78

Time	MAD 350 mg	
	M12	M17
2 H	5.19	27.64
4 H	3.54	23.11
8 H	3.03	19.95

CASE 1: COMPOUND-A: COMPARING LC/MS PEAK AREAS OF M12 AND M17 FROM AUC POOLED HUMAN, RAT AND DOG PLASMA SAMPLES

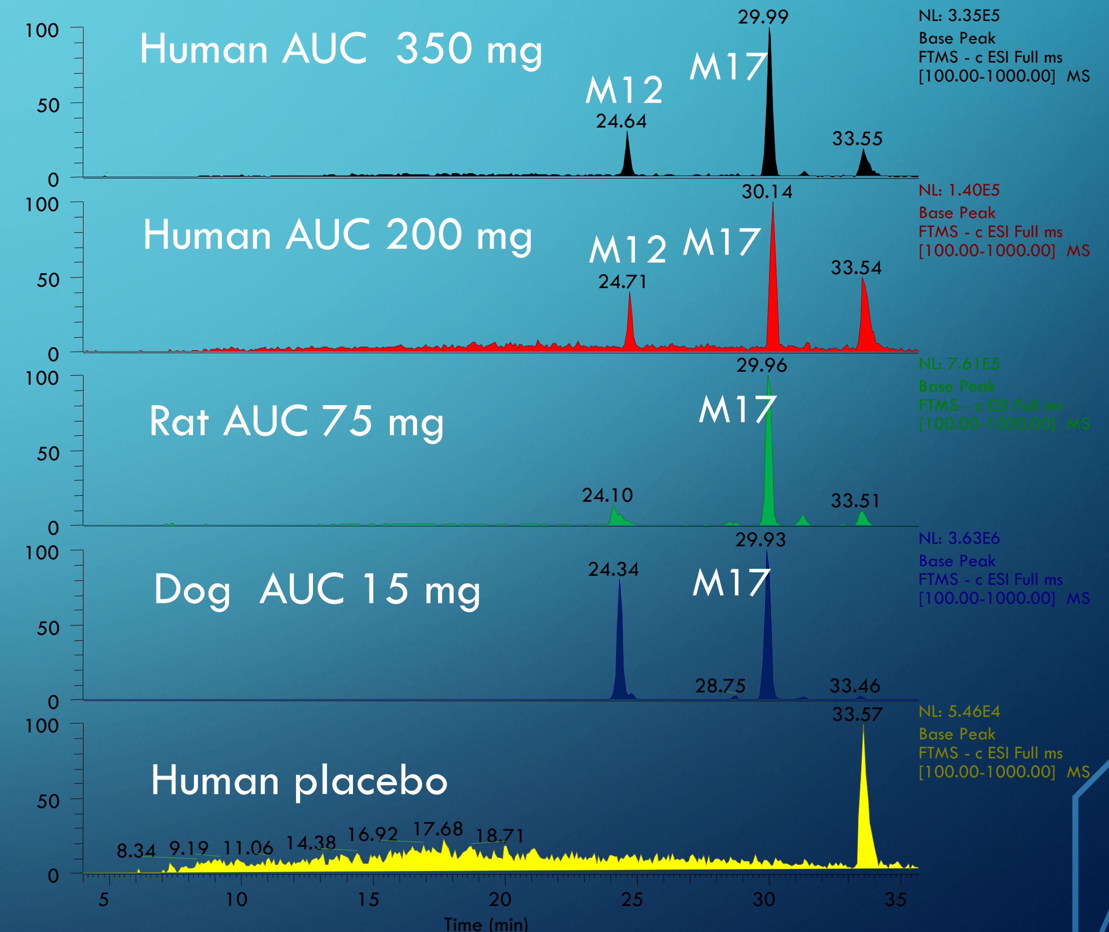
Peak Area Ratios of M12 in tox species against human

MS Fragment	H_200 mg	H_350 mg
Rat_75mgk	0.4	0.2
Dog_15mgk	0.6	0.3

Ratios of M17 in tox species against human

MS Molecular ion	H_200 mg	H_350 mg
Rat_75 mgk	5.4	2.0
Dog_15 mgk	39.6	15.0

Results showed that M17 was covered and M12 was borderline-covered in tox species



CASE 1: COMPOUND-A

Major human metabolites from metabolite profiling

- ❑ M12 (borderline covered in dog) >10% in SAD but <10% in MAD
- ❑ M17 (covered in animal) >10% in SAD and MAD

Qualified LC-MS/MS Assay

- ❑ M12 and M17 were chemically and enzymatically synthesized
- ❑ Separation and isolation of M12 and M17 from the synthetic mixtures
- ❑ Structures of the synthetic M12 and M17 were determined by NMR
- ❑ Quantitation of M12 and M17 in human plasma from MAD

The future studies for M17

- ❑ Pharmacological activity test.
- ❑ Inhibition of major CYP and transporters.
- ❑ Quantitation of M17 by qualified LC-MS/MS assays
  - in selected clinical studies
  - in GLP and other tox studies

LC-MS/MS quantitation of MAD	
	% of total exposure
Cpd-A	68.4
M12 (Oxidation)	7.8
M17 (Oxidation)	21.6
M11 (Des-methyl)	2.1
Total	100

QUANTITATION OF METABOLITES UNDER NEW BMV GUIDANCE

- **Bioanalytical Method Validation Guidance for Industry, FDA, May 2018**

7. Stability (page 9): For drugs administered as fixed combination, or part of a specific drug regimen, the stability of the analyte should be assessed in the presence of the other drug. The sponsor should also consider the stability of the analyte in the presence of other co-medications that are known to be regularly administered to patients for the indication of the drug under development.

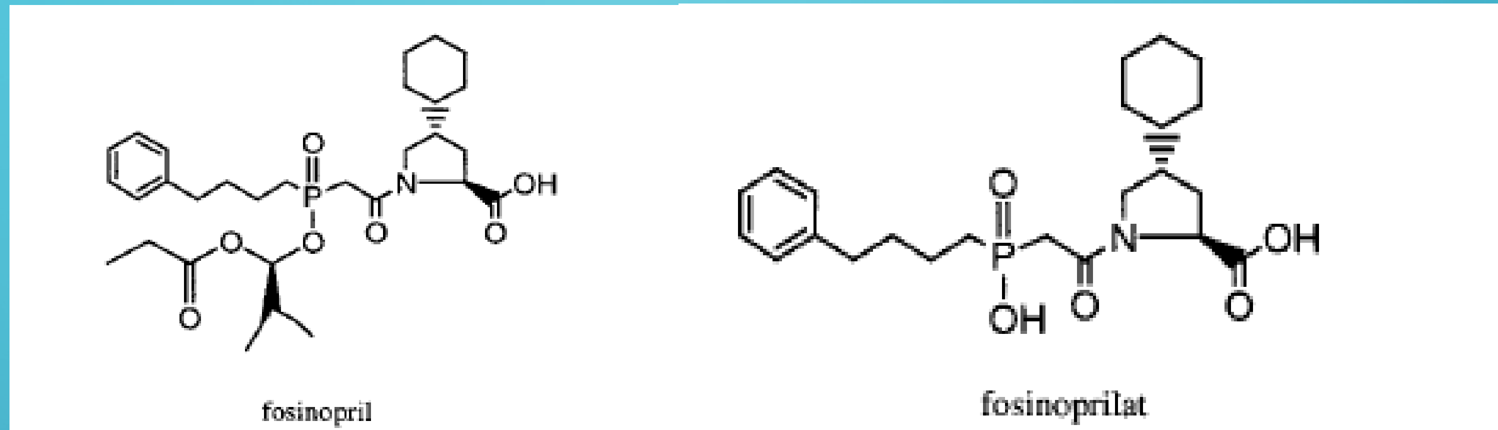
- **Bioanalytical Method Validation M10, (draft) ICH, February 2019**

3.2.8 Stability (page 14) Line 403-405: If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific regimen) the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes.

CASE 2: VALIDATION AND QUALIFICATION OF COMPOUND-B AND ITS N-GLUCURONIDE (N-GLU) METABOLITE IN PLASMA

- ❑ Cpd-B: Validated methods; N-Glu: Qualified methods; two separate methods in pre-clinical species and human
- ❑ Potential conversion of N-Glu to Cpd-B
- ❑ Options in stability evaluation of Cpd-B in validation
  - ❑ Co-spiked QCs
  - ❑ Consider to test BMS-B only QCs as well
  - ❑ Conduct co-spiked QCs for all stability evaluations? Or only for “major” ones: bench-top, freeze/thaw (F/T) and long term storage (LTS) stabilities?
- ❑ Options in stability evaluation of N-Glu in qualification
  - ❑ Co-spiked QCs (bench-top, F/T, LTS, and WB)
- ❑ Seek industry experience and regulatory recommendations

CASE 3: FOSINOPRILAT VALIDATION IN HUMAN PLASMA FOR BE STUDY



- ❑ Fosinoprilat: validated method in human plasma
- ❑ Fosinopril: qualified method for method development and stability evaluation during blood sample collection and processing
- ❑ Options of stability evaluation of Fosinoprilat in validation
  - ❑Co-spiked QCs and Fosinoprilat only QCs in all stability evaluation experiments
  - ❑Is this excessive?
- ❑ Seek industry experience and regulatory recommendations

CONCLUSIONS

- Metabolites monitoring has been following MIST and ICH M3 (R2) guidance
- The strategy and work flow have evolved over the past several years
- Decisions are made amongst drug safety evaluation (DSE), biotransformation, clinical pharmacology and bioanalytical groups
- Notable changes include:
  - DSE group prefers to monitor “important” animal metabolites in toxicology studies to evaluate their contribution to the potential toxicity, even though these metabolites may not be major human metabolites.
  - The current practice is that the metabolite needs to be monitored in one repeated-dose GLP study for each species.
  - Significant or active metabolites may need to be monitored in additional or selected toxicology studies.
- Bioanalytical method development and validation has been following FDA and ICH M10 (draft) bioanalytical method validation (BMV) Guidance
- Planning and logistic issues add complexity to metabolite monitoring
  - earlier bioanalytical outsourcing start at IND toxicology studies
  - earlier initiation of Chronic toxicology studies during rather than post FIH
  - availability of metabolite reference standards

Q/A FOR INTERACTIVE ACTIVITIES POST PRESENTATION

- ❑ What is the ratio of your bioanalytical work in-house vs. outsourced?
- ❑ What is your experience and typical metabolite monitoring workflow?
  - ❑ How to decide metabolites monitoring at IND tox?
  - ❑ How to decide metabolites monitoring at FIH?
  - ❑ How to decide metabolites monitoring post FIH?
- ❑ What is your strategy in metabolite assay development and qualification?
  - ❑ What experiments are included in the qualification?
  - ❑ How do you deal with metabolite reference standard and IS?
- ❑ What is your opinion on stability evaluation with parent and metabolite co-spiked QCs?