TT24: Tissue analysis

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CONTINUATION FROM ADJACENT POSTER

Survey results, continued

Q30: In Drug Development, what does your generic approach look like?

Answer Options	Response Percent	Response
I can not share	7,7%	1
We use the method which is in place for plasma and just apply it for tissues, by making only small changes to the sample preparation	92,3%	12
We use a generic process which looks like this: please try to summarize approach in 4 lines in the box below for sample homogenization and extraction and for Chromatography (if LC-based) and/or detection	0,0%	0
Sample homogenization and extraction / Chromatography and or detection		0

Q31: In Drug Development, if you don't use a generic approach, how do you establish your assays?

Answer Options	Response Percent	Response Count
A different assay is established per tissue and per compound	31%	4
A different assay is established per compound, but we don't consider different tissue types when developing the assay	54%	7
Other (please specify)	15%	2
Total responders		13

Q32: Across all Drug Development phases, do you use method validation or qualification? (more than one option can apply)

Answer Options	Drug Discovery	Drug Development non GLP	Drug development GLP	Response
screening methods..i.e. look at ratios between tissue levels or plasma/tissue levels	6	0	0	6
qual. methods (i.e. absolute concentrations) using widened acceptance criteria	12	11	4	16
qual. methods (i.e. absolute concentrations) using acceptance criteria in line with BMV	3	9	3	10
validated (i.e. absolute concentrations) using widened acceptance criteria	0	2	8	9
validated (i.e. absolute concentrations) using acceptance criteria in line with BMV	1	2	8	8

Method specific questions related to method establishment (ME)

Q33: Do you assess following parameters mentioned in FDA/ EMA guidance principles for method establishment?

Answer Options	Response Percent
Yes, all key parameters are assessed from Q34	45%
No, go to Q34	55%

Q34 - No, following parameters are not established or assessed

Answer Options	Response Percent
Calibration curve (incl. LLOQ and ULOQ)	43%
Selectivity	50%
Accuracy and precision	50%
Dilution integrity	57%
Matrix effect	50%
Carry-Over	71%
Stability (short term/long term)	21%
Hook effect (for large molecules)	64%
ISR	
Other (please specify)	

Q35: Do you apply acceptance criteria according to FDA/EMA guidance principles?

Answer Options	Response Percent
Yes, usually	55%
No, not always	45%

Q36: Calibration curves : a priori assessment of linearity

Answer Options	Response Percent
Yes	81,%
No	19%

Q37: Calibration curves : a priori assessment of LLOQ

Answer Options	Response Percent
Yes	76%
No	24%

Q38: Calibration curves : acceptance criteria 4-6-15/20

Answer Options	Response Percent
Yes	65%
No	35%

Q39: Selectivity/specificity

Answer Options	Response Percent
Yes, a priori	55%
Yes, but in study (e.g. extra blanks, zero)	45%

Q40: Assessment of accuracy and precision using independent QCs, number of QC levels

Answer Options	Response Percent
Three = LLOQ, MQC, HQC	32%
Four = LLOQ, LQC, MQC, HQC	58%
Other (please specify)	11%

Q41: Assessment of accuracy and precision using independent QCs, acceptance criteria, 4-6-15/20

Answer Options	Response Percent
Yes	63%
No	37%

Q42: Assessment of accuracy and precision using independent QCs, accuracy and precision: Intra-day and inter-day assessment

Answer Options	Response Percent
Only Intra-day	39%
Intra-day and inter-day	61%

Q43: Stability assessment: do you perform stability assessment on tissue homogenate?

Answer Options	Yes, a priori	No	No, we rely on plasma data	Cfr. FDA/EMA criteria for assays	We apply other criteria	Response Count
Short term	8	6	3	4	0	18
(< 1 week)	4	5	3	3	0	14
Long term	9	6	3	4	1	19
(> 1 week)	3	6	3	1	0	12
F/T	9	5	3	4	0	18
Processed sample	9	4	3	4	1	18
Other criteria (please specify)						2

Q44: Do you assess Dilution integrity?

Answer Options	Response Percent
Yes, a priori	42%
Yes, if needed during sample analysis	37%
No	21%

Q45: Do you assess Matrix Effect?

Answer Options	Response Percent
Yes, a priori	53%
No	47%

Q46: Do you assess carry-over?

Answer Options	Response Percent
Yes, a priori	53%
Yes, if needed in during sample analysis	32%
No	16%

Q47: Do you assess ISR as part of sample analysis?

Answer Options	Response Percent
Yes and same acceptance criteria than in FDA/EMA guidance	28%
Yes but with different criteria than in the FDA/EMA guidance	6%
No	67%

Q48: Which parameters do you assess in case of change of matrix or species?

Answer Options	Revalidation due to change in matrix (i.e.: liver to kidney)	Revalidation due to change in species (i.e.: rat liver to dog liver)	Response Count
Calibration curve (incl. LLOQ and ULOQ)	13	13	15
Selectivity	11	11	13
Accuracy and precision	13	13	15
Dilution integrity	8	9	10
Matrix effect	8	9	10
Carry-Over	8	7	9
Stability (short term/long term)	10	11	12
ISR	5	7	7
Other (please specify)			4

Q49: What is your preferred study design to analyze single tissue samples in a study?

Answer Options	Response Percent
CAL and QCs prepared only in homogenate from target tissue	52,4%
CAL and QCs prepared in plasma, CAL and QCs spiked with blank target tissue. Unknowns spiked with equal amount of plasma (for matrix matching)	19,0%
Other (please specify)	28,6%

Q50: What is your preferred study design to analyze multiple tissue samples in a study?

Answer Options	Response Percent
CAL and QCs prepared in homogenate from each target tissue	76%
CAL prepared in homogenate from one tissue, QC in each target tissue. Back calculate QCs (all tissues) and unknowns on curve from CAL (one tissue)	8%
CAL and QC prepared in pooled homogenate (all tissues from study), dilute unknowns with pooled tissue homogenate for matrix matching.	8%
CAL and QCs prepared in plasma, CAL and QCs spiked with blank target tissue. Unknowns spiked with equal amount of plasma (for matrix matching)	8%

Q51: Do you 'archive/store' tissue homogenates after analysis?

Answer Options	Response Percent
Yes, as for plasma	48%
Yes, but duration independent from plasma	19%
Yes, on sponsor request	24%
No	10%

Questions related to protocol and reporting

Q52: Does your lab have a specific SOP for tissue analysis?

Answer Options	Response Percent
Yes	19%
Yes, but as a non-regulated (i.e. non-GLP) procedure	10%
No, no SOP in place.	62%
Other (please specify)	10%

Q53: Does your lab include tissue analysis in the protocol of the main study (Tox/TK study,…)?

Answer Options	Response Percent
Yes, both for GLP and non-GLP studies	67%
Yes, but only if GLP	0%
Yes, but only if non-GLP	0%
Yes, but always as an amendment	0%
No, tissue analysis is always requested as a separate study	33%

Q54: If tissue analysis is part of a study, does your lab have a specific section on tissue analysis within the protocol of the main study (Tox/TK study,…)?

Answer Options	Response Percent
Yes	50%
No, it is part of general statement on bioanalysis or part of a separate document	50%

Q55: Does your lab specify the status GLP - non-GLP of the tissue analysis in the protocol?

Answer Options	Response Percent
Yes	71%
No	29%

Q56: Does your lab provide description/guidance of tissue collection in the protocol?

Answer Options	Yes	No
Size of tissue sample to be collected	13	7
(sub)Section of tissue sample to be collected	9	9
Description of tissue pre-processing/handling	15	3

Q57: How does your lab report data of tissue analysis?

Answer Options	non-GLP studies	GLP studies Analysis using qualified assay	GLP studies Analysis using validated assay	Response
Separate 'contributing scientist report (CSR)	7	6	3	10
Part of main study report	4	2	4	6
As part of toxicokinetic CSR	4	3	4	5
As part of bioanalytical CSR	9	8	6	12
Other (please specify)				1

Recommendation on validation: starting form the critical questions driving quality

Document exposure of dosed drug (or metabolites) in tissue (homogenates)	Recommended level of Bioanalytical rigour for tissue homogenate analysis
1. as unique endpoint of PK/safety/PD after topical dosing (e.g. skin, lung,..)	Consider validated assay. Consider a priori widening of the acc. & prec. acceptance criteria (e.g. from 4-6-15 to 4-6-20)
2. in relation to *a priori* identified safety assessment in a GLP study	
3. in a PK study, mechanistic/GLP tox./PD study	Use a qualified assay . Use widened acceptance criteria
4. in relation to understanding relative tissue distribution	Use alternative simplified bioanalytical processes Use widened acceptance criteria. (Evaluate need of absolute conc. above relative ratios).

Future plans

Get further input from continued discussions in the Topic Team, EBF members and international bioanalytical prior to publication.

Publication strategy:

- Publish the survey results as a separate paper
- Publish a recommendation on EBF's view on challenges and bioanalytical qualification or validation required for tissue homogenate analysis in the same issue of Bioanalysis *(including GLP vs. nonGLP question)*