Plasma Protein Binding studies including Bioanalysis in Drug Discovery and Development

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on behalf of EBF (TT-25)

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Plasma Protein Binding (PPB) studies

- Performed in different stages of drug discovery and development.
- Determine the unbound fraction of a drug in plasma.
- Binding of a drug to plasma proteins influences drug ADME.
- Close cooperation between DMPK and bioanalytical scientists.
- Different PPB techniques as well as analytical techniques used.
- No clear Guidance available.

Plasma Protein Binding (PPB) studies:
3 surveys

- PPB studies in Drug Discovery
  - 60 questions
  - 9 companies responded
- In vitro PPB studies in Drug Development
  - 76 questions
  - 10 companies responded
- In vivo PPB studies in Drug development
  - 98 questions
  - 5 companies responded

Summary of survey results

- Techniques used to determine PPB.
- PPB study design.
- Bioanalysis of non-labelled compounds.
- Bioanalysis of radio-labelled compounds.
- Validation/qualification and acceptance criteria.
- Reporting.

Q: Which group in your company is providing responses to this survey?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
DMPK	62.5%	60%	25%
Bioanalytics	12.5%	30%	50%
Shared	25%	10%	25%

Q: Why do you perform PPB studies?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
For compound selection	5	-	-
To define a safety margin	3	5	-
For PK/PD evaluation	6	3	4
Other (please specify)	Allometric scaling	PBPK For PK/PD in clinic Dose prediction Non-linear PK	FDA regulatory requirement

Q: If PPB data are available from the Discovery phase do you repeat in vitro PPB in Drug Development Phase?

Answer Options	in vitro PPB Development phase
Yes	100%
No	0%
Other	0%

Q: For which species do you perform PPB?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Rats	7	10	0
Dogs	5	9	0
Mice	4	7	0
Rabbits	3	6	0
Monkeys	3	5	0
Humans	8	10	4*
Others	2	4	0

*Healthy, renal impaired and hepatic impaired.

Q: Do you have SOPs in place for the experimental part of PPB studies?

Answer Options	Drug discovery phase	in vitro PPB Development phase
Yes	22%	50%
No	78%	50%

Q: Do you have SOPs in place for the analytical part of PPB studies?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Yes	11%	60%	50%
No	89%	40%	50%

Q: What are the PPB techniques you are using?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Ultrafiltration	3	6	4
Equilibrium dialysis	9	10	3
Ultracentrifugation	1	5	2
Charcoal adsorption	0	0	0
Affinity Chrom.	0	0	0
HPFA	0	0	0
Solid phase μ-extraction	0	0	0
In vivo μ-dialysis	0	0	0
Other	Transil COMP-3	Gel filtration Transil	Transil

Q: If you select a technique based on compound characteristics which technique would you select for?

Answers in vitro	Highly lipophilic	Highly hydrophilic	Biologicals	Labile	Sticky
Ultrafiltration	X	X		X	
Equilibrium dialysis	X	X			X
Ultracentrifugation	X				X
Transil Partitioning	X				X
Gel filtration	X				X

Answers in vivo	Highly lipophilic	Highly hydrophilic	Biologicals	Labile	Sticky
Ultrafiltration				X	
Equilibrium dialysis	X	X			
Ultracentrifugation					X
Transil Partitioning	X				X

Q: Do you select a PPB technique based on anticipated protein binding?

Answer Options	in vitro PPB Development phase	in vivo PPB Development phase
Yes	40%	50%
No	60%	50%

Q: If yes,which technique would you select?

Answers *In vitro*	Very high (>98%)	High (85-98%)	Medium to low (<85%)
Ultrafiltration			X
Equilibrium dialysis	X	X	X
Ultra centrifugation	X	X	
Transil Partitioning	X		
Gel filtration	X		

Answers *In vivo*	Very high (>98%)	High (85-98%)	Medium to low (<85%)
Ultrafiltration			X
Equilibrium dialysis	X	X	X
Ultra centrifugation	X	X	
Transil Partitioning	X		

Q: Do you evaluate PPB with radio-labeled or non-labeled compounds?

Answer Options	Drug discovery phase	in vitro PPB Development phase
Radio-labeled compounds	0%	33%
Non-labeled compounds	89%	11%
Both	11%	56%

Questions on Bioanalysis of non-labeled compounds -1

Q: do you first qualify/validate a method for analysis?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
No, we use a generic method	78%	14%	0%
Yes, we qualify a method	22%	72%	75%
Yes, we validate a method	0%	14%	25%

Q: do you use absolute or relative quantification?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Absolute quantification (CAL curve)	6	5	4
Relative quantification*	4	2	0
Other:	Dialysis: relative Transil: absolute UF: absolute	Both Case by case	

*Comparison of peak responses in sample before and after PPB.

Questions on Bioanalysis of non-labeled compounds -2

Q: how do you qualify your bioanalytical method, i.e. what are the pre-study tests to show the method's performance?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Calibration curve	5	7	4
Accuracy	4	6	3
Precision	4	6	3
Selectivity	2	4	3
Stability	3	5	4
Matrix effects	0	5	2
Carry-over	-	5	2
Dilution	2	2	2
Other	Dependent on PPB technique	Recovery	0

Questions on Bioanalysis of non-labeled compounds - 3

Q: do you have batch acceptance criteria cal. samples?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Yes	33.3%	100%	100%
No	44.4%	0%	0%
We do not include calibration samples	22.2%		

Q: please specify.

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
±15%, (± 20% LLOQ)	2	5	3
± 20% at all levels	0	2	1
Other (please specify)	±25%		

Questions on Bioanalysis of non-labeled compounds - 4

Q: do you have batch acceptance criteria for QC samples?

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
Yes	37.5%	100%	100%
No	37.5%	0%	0%
We do not include QC samples	25%		

Q: If yes, please specify

Answer Options	Drug discovery phase	in vitro PPB Development phase	in vivo PPB Development phase
±15%, (±20% at LLOQ)	1	5	3
±20% at all levels	1	2	1
Other (please specify)	±25%		

Q: How do you report in vitro PPB results in the drug dev. phase?

Answer Options	Response Count
Short experimental description of PPB experiment (equipment, materials, conditions, etc).	7
Short experimental description of bioanalytical work.	6
Results of preliminary experiments.	3
Unbound fraction at all concentration levels (individual results); non specific binding taken into account while calculating the unbound fraction.	4
Unbound fraction at all concentration levels (individual results); non specific binding not taken into account while calculating the unbound fraction	6
Average of unbound fraction at each concentration level; non specific binding taken into account while calculating the unbound fraction.	2
Average of unbound fraction at each concentration level; non specific binding not taken into account while calculating the unbound fraction.	6
Non-specific binding results (individual values).	3
Non-specific binding results (average).	4

Towards a Best Practice document

- Provide detailed overview of technologies used by EBF member companies
  - include results obtained from the three surveys
- Comment on level of bioanalytical qualification/validation of methods needed
  - Make use of EBF tiered approach paradigm to use the full range of possibilities of screening and validated assays
- Get further input from
  - All of you today
  - Continued discussions in the Topic team
  - EBF members prior to publication
- Planned: Q2 2013.

Thanks!!

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