Merck global bioanalytical strategy to ensure data quality in the discovery space and successful LC-MS/MS methods transfer to preclinical GLP and clinical bioanalytical groups

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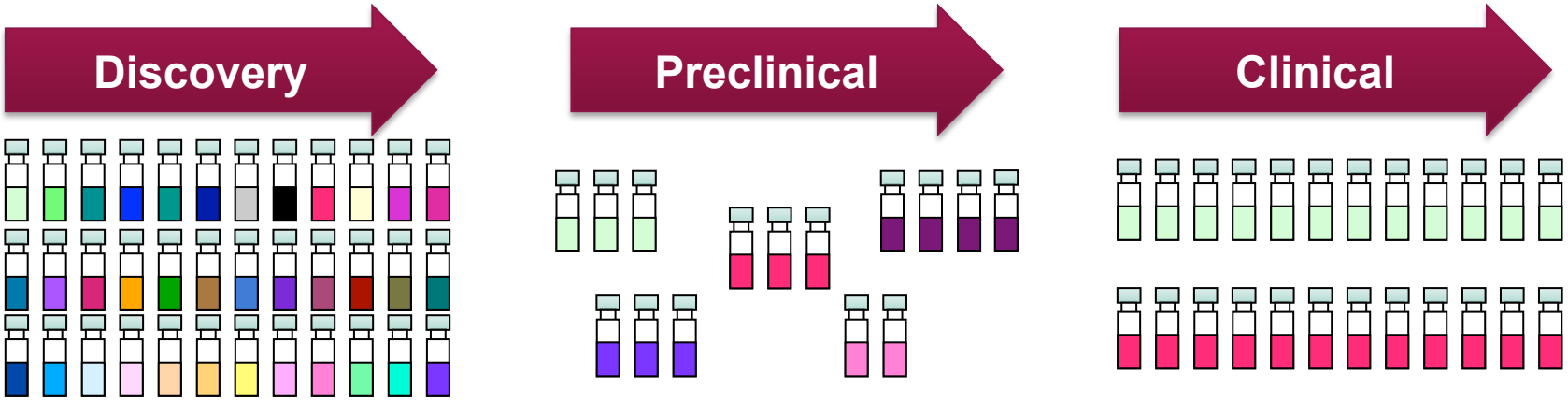
Outline

- Role of Global Bioanalytics in PPDM
  - Drug discovery and assay deployment
- High Data Quality Maintaining High Throughput
  - Standardized routine analytical platforms
  - MS performance check
  - Minimizing errors
  - Merck bioanalytical acceptance criteria
- From Discovery to Regulated Bioanalysis
  - Assay validation components
  - Regulated method development
  - Method development experiments

Role of Global Bioanalytics in PPDM



DMPK Screening Assays

Pharmacokinetics Studies

Bio/Efficacy/Engagement Markers

Metabolite Identification

Tissue Analysis

Program-Specific Assays

Throughput → Reproducibility → Accuracy

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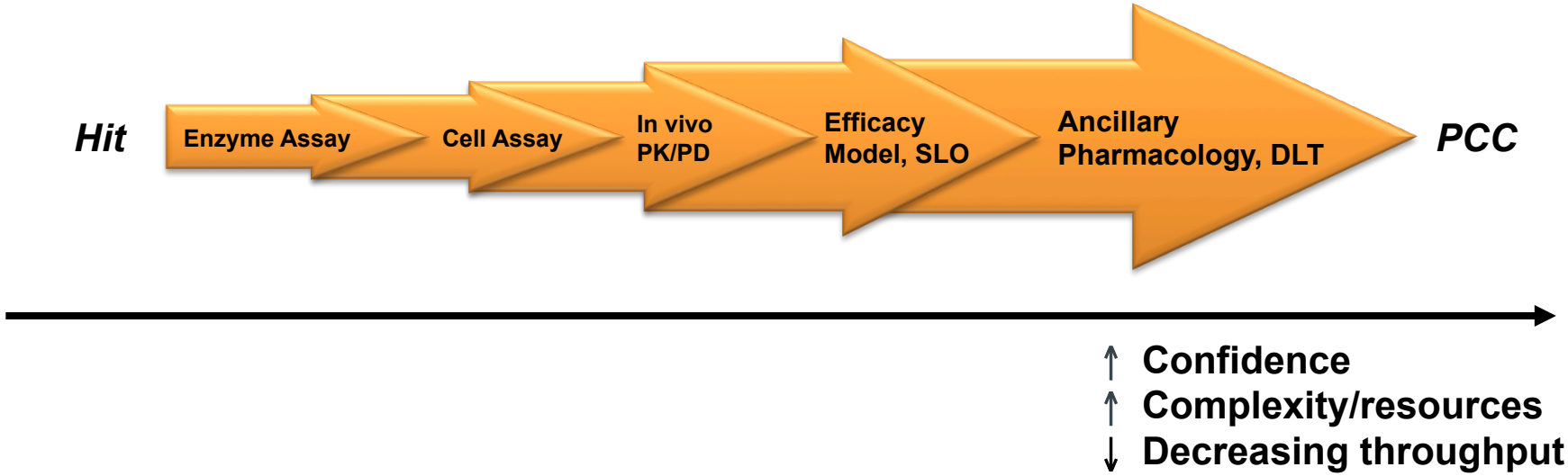
Drug Discovery Assay Deployment and Consideration



PK/BioA	**Screening PK** Insight into challenges	**Expanded PK support** PK incl. metabolites in add't species/matrices	**Tox./ancillary pharm. enabling PK** Multidose/Formulation
In vitro/ADME	**MSIC and MetID** In microsomes or S9 (liver/GI/ lung), hepatocytes	**DDI:** TDI CYP (k_{obs} or KI, k_{inact}), hep. induction, UGT inhib., add't transporter	**Disposition** Quantitative in vivo assessment of ADME
	Pgp, P_{eff} PXR* CYP inhib.* / **PPB, B/P and Plas. Stab.**	**Quant./qual. metabolism** In vitro/in vivo met. profiles and ID	

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High Data Quality Maintaining High Throughput

"Today's standardization is the necessary foundation on which tomorrow's improvement will be based"

Henry Ford

- Standard process designs and platforms reduce data variability and subsequently create greater flexibility and more predictable outcomes
- Standardization and in turn increased data confidence are the foundation of speed

Harmonization of Routine Analytical Platforms

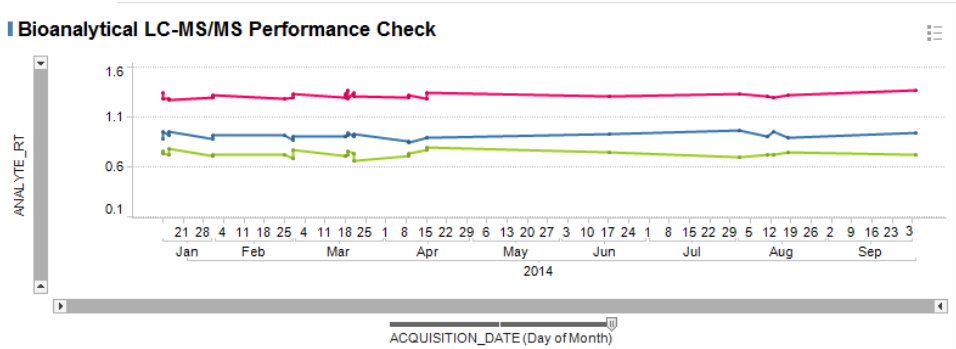
- Standard platform for routine quantitative assays to ensure data quality and high throughput
  - MS/MS Method Development
    - Nanomate/FIA based compound tuning platform with global database of MRM parameters (DiscoveryQuant)
  - LC-MS/MS Sample Analysis
    - Sciex triple quadrupole mass spectrometer
    - Thermo LX-2 parallel UHPLC system
    - Standardized UHPLC conditions for discovery quantitative assays
    - Generic internal standard mix
  - Sample Preparation: Automation/Optimization
    - Hamilton Liquid Handler Platform
  - Acceptance Criteria for Discovery Assays

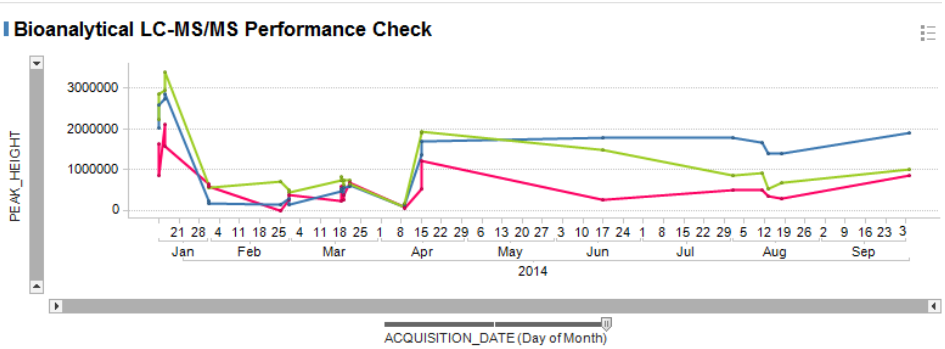
Scientifically Qualified and Properly Maintained Instruments

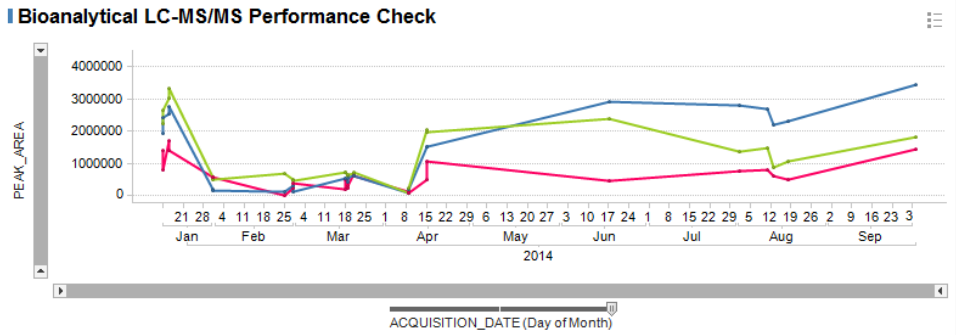
- System suitability tests are recommended to ensure success
- Performance of mass spectrometry systems are checked periodically to ensure that the system is operating properly
- MS performance checks are routinely conducted for our standard chromatographic methods to ensure that the system is sufficiently sensitive, specific, and reproducible
- The MS performance tests do not replace the required run acceptance criteria with calibration standards and QC samples

LC-MS/MS Performance Check

- Performance check data is displayed in three plots for analyte retention time, peak height, and peak area, as well as a summary table







Summary Table

Column	INSTRUMENT_SN	PRODUCT	Min	Max	Avg	StdDev
ANALYTE_RT	AG20300703	Diclofenac	1.30	1.37	1.32	0.02
		Imipramine	0.86	0.94	0.90	0.02
		Labetalol	0.70	0.76	0.73	0.02
PEAK_AREA	AG20300703	Diclofenac	41500	1780000	658712.00	456630.58
		Imipramine	27800	3370000	1298672.00	1136754.84
		Labetalol	180000	3260000	1274040.00	892470.80
PEAK_HEIGHT	AG20300703	Diclofenac	42200	2160000	644732.00	517618.73
		Imipramine	28100	2820000	1068464.00	894659.45
		Labetalol	166000	3310000	1137560.00	866417.82

Minimizing Errors: Matrix Effect

- PEG400 and PEG300 are common vehicles used in drug discovery known to cause matrix effect
- For studies with PEG based vehicles, QCs containing PEG (0.1, 1%) are incorporated in analytical runs
- Use of APCI as an alternative to ESI as well as different columns or chromatographic method (e.g. HILIC)
- Sample dilution (e.g., 1x, 10x and 100x)
- Selection a new internal standard (i.e. structural analog)
- Alternative sample extraction methods (e.g. LLE, SPE, SCX)
- When PEG suppression is difficult to solve analytically, alternative vehicles should be evaluated

Minimizing Errors: Carryover

- Ad hoc MultiQuant queries highlights carryover as well as other acceptance criteria failures
- Autosamplers and washing solutions were optimized in order to reduce carryover
  - DLW autosamplers
  - Sol 1: Water/ACN/Formic Acid
  - Sol 2: ACN/IPA/Acetone/Formic Acid
- For analytes that show significant carryover with the above conditions, analysts may increase the number of wash cycles or rinse times, or an alternative wash method using acidic/basic solutions
  - Sol 1: 2% ammonium hydroxide in 5 mM aqueous ammonium acetate/Acetone/Isopropanol/Acetonitrile/Methanol
  - Sol 2: Methanol/Water/Formic Acid, 10/89/1 (v/v/v)
- Assessment and solution flowchart (column and autosampler carryover)

Merck Acceptance Criteria for Discovery Assays

Study Type	Level Criteria			
	Level 1	Level 2	Level 3	Level 4
PGP, PPB, Non-definitive PK/PD	X			
Single and Cassette PK, Definitive PK/PD, Cold-BDC Studies		X		
Definitive PK and PK/PD Studies, TK, CV, DLT			X	
Definitive Formulation – Safety Formulation, Post-PCC PK Studies				X

Level 3/4 – Calibration Standard and Quality Control Acceptance Criteria

- Powder for stock solutions preparation
- Calibration curve must have a minimum of 6 standards
- Calibration Standards and QC sample accuracy of 25%
- Minimum of two QC sets (n=3)
  - Low = 1 or 5-fold LLOQ
  - High = 75-100% ULOQ
- 2/3 of the QC samples must be within ± 25% of nominal concentration
- Extrapolation below the LLOQ and above ULOQ is not allowed
- Dilution integrity QCs for samples > ULOQ at nominal concentration ≥ expected. Dilution integrity QCs samples should be frozen for at least 8 h and then analyzed. The mean (n≥3) accuracy should be within 25% of the nominal concentration.
- The LLOQ must be greater than three times the mean of the back calculated value of the zero standards
- When the assay range is not known, several QC levels should be prepared at or near the expected LLOQ and ULOQ
- If the calibration standard criteria specified are not met, the analysis will be repeated

Level 3/4 – Blank Matrix Samples, Carryover Calculations and Interference

Carryover Test

- Carryover test must be ran by assaying one Single Blank after the highest standard
  - Carryover < LLOQ no further checking is needed
  - Carryover ≥ LLOQ then it needs to be converted to percent carryover
  - Percent Carryover = [Carryover] / [High STD] • 100
- Re-assay is required when percent carryover is >25%

Analyte interference test

- The peak area Single Blank <3 peak area of LLOQ
- The Double Blank will be used to monitor internal standard interference

Single Blank (SB) is made from the calibration curve matrix with no analyte added

Double Blank (DB) is made from the calibration curve matrix with no analyte or internal standard added

Level 3/4 – Internal Standard Criteria

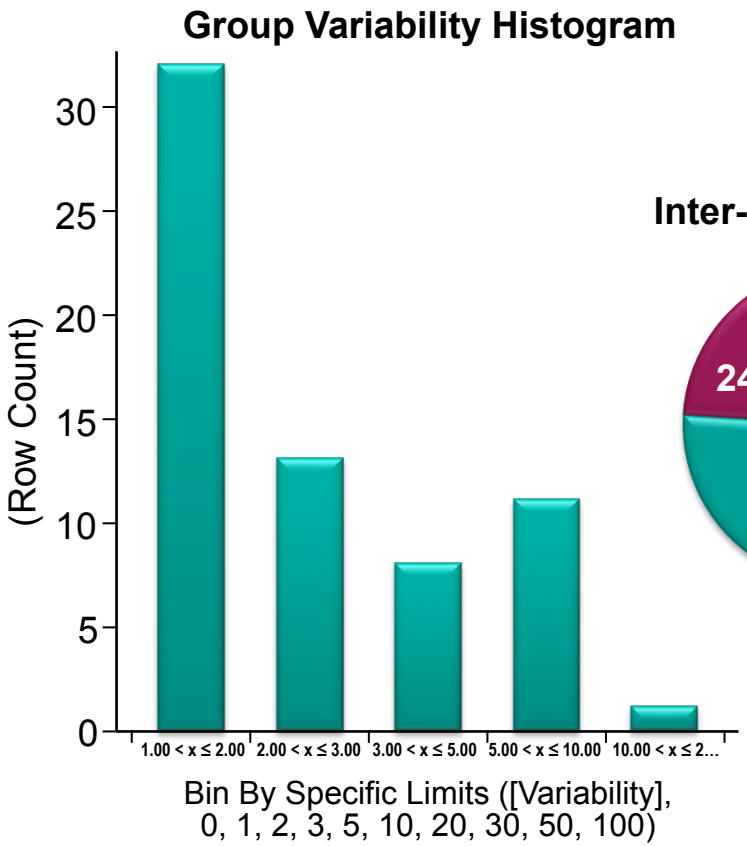
- Scientific judgment should be used when monitoring the internal standard response during an analytical run.
  - IS response unknown ≤ 1.5 IS response STD and QC

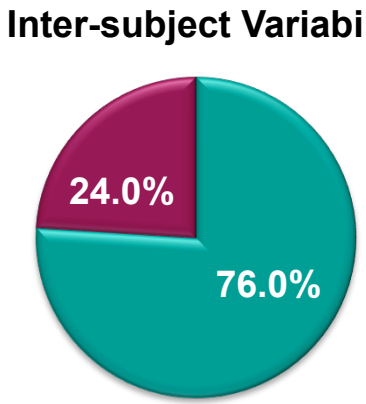
  - Level 3
    - The internal standard can be a combination (cocktail) of generic internal standards (e.g. labetalol, imipramine and diclofenac) unless a structural analog or stable isotope label is chosen for a particular structural class/program
  - Level 4
    - The internal standard (IS) should be a structural analog or stable isotope labeled IS, when available

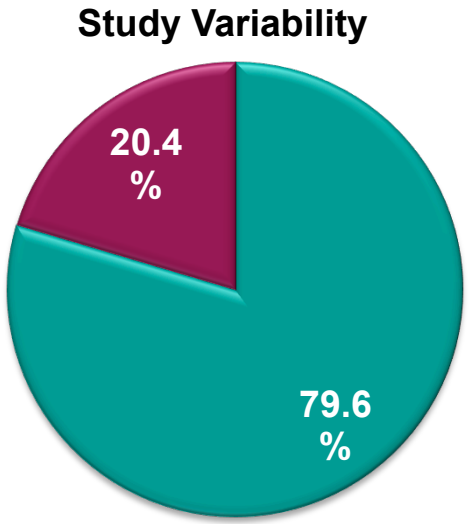
Level 4 – Analyte Matrix Stability Testing

- Analyte matrix stability testing will be conducted using QCs at low and high concentrations (n≥3)
- Room Temperature Analyte Matrix Stability
  - 3 aliquots of low and high QCs for at least 2 hours
- Freeze/Thaw Analyte Matrix Stability
  - A minimum of one freeze and thaw cycle
  - 3 aliquots of low and high QCs frozen for at least 8h
- Long-Term Storage Analyte Matrix Stability
  - Equal or higher the period of time between sample collection and sample analysis
  - 3 aliquots of low and high QCs should be stored under the same storage conditions as the study samples

Preclinical Studies Variability Analysis – Dog







Statistical analysis of 25 compounds in 54 Dog Studies (n=3, 65 treatment groups 2-10 mg/kg oral dosing). Variable and Variability: AUC and metric cutoff of 3

Analyses does not take into account difference in formulations and possible non-linear PK

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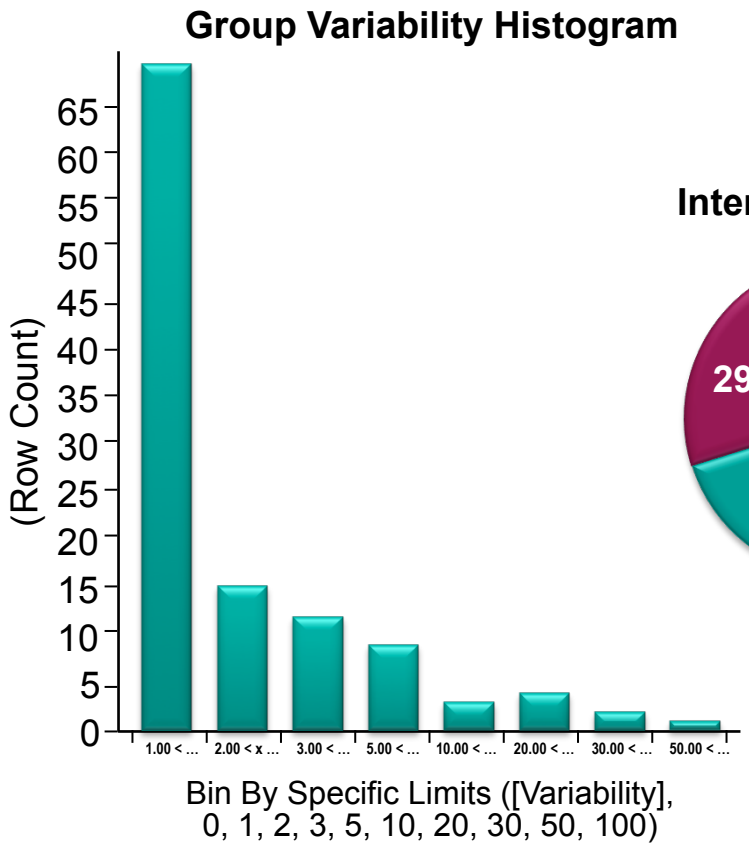
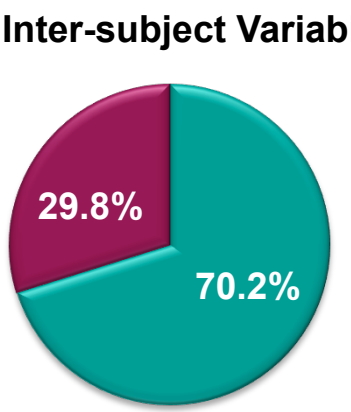
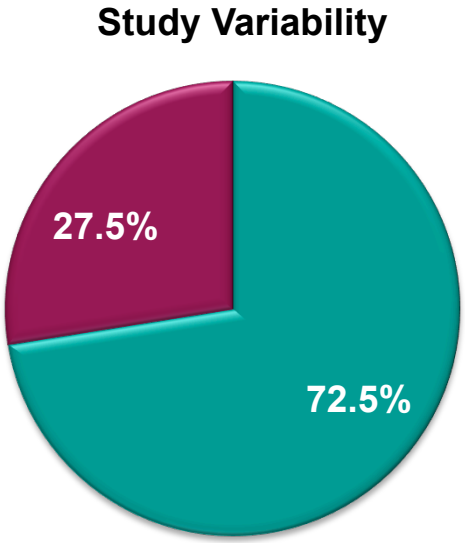
Preclinical Studies Variability Analysis – Rat





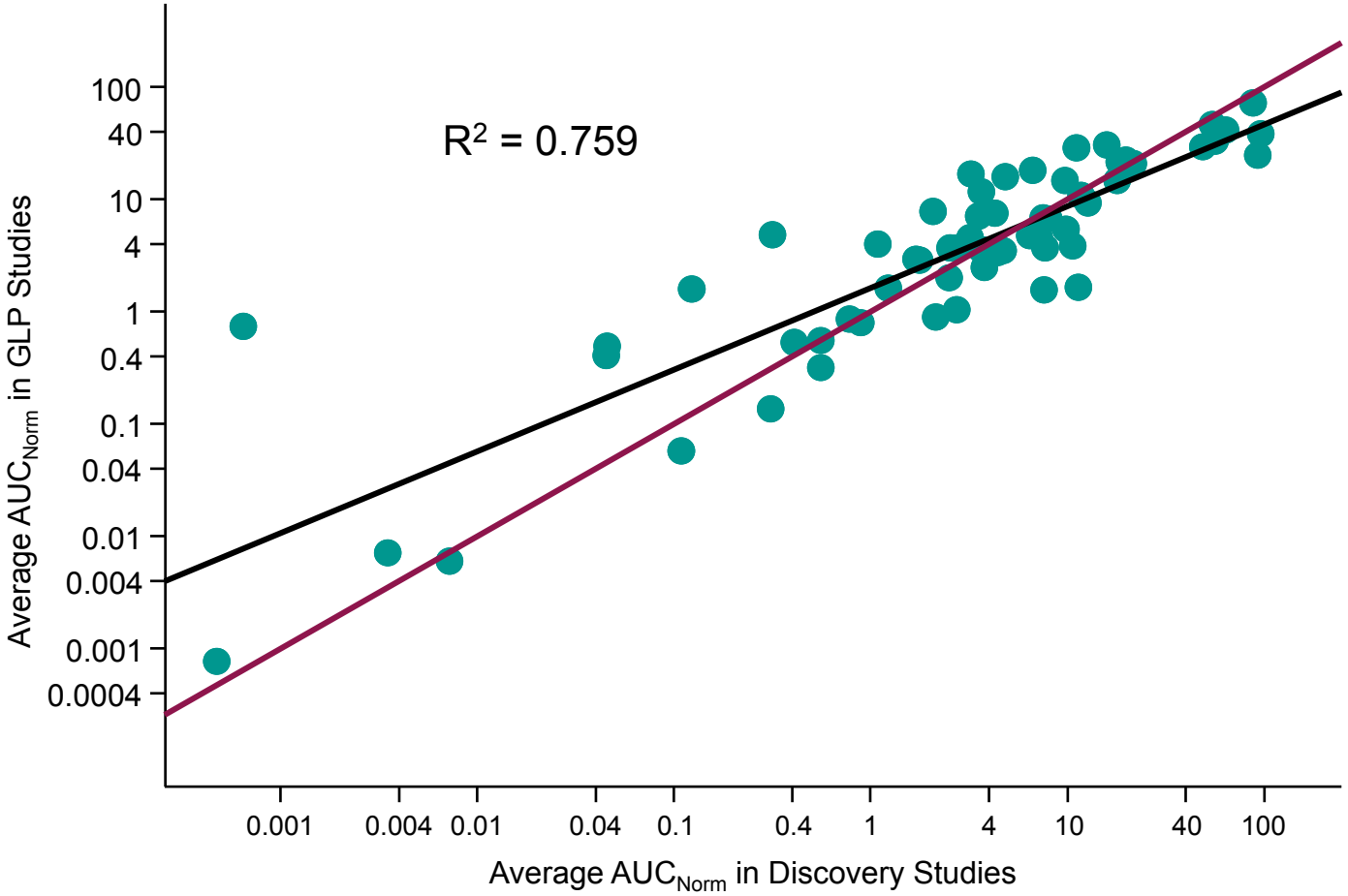


Statistical analysis of 40 compounds in 94 Rat Studies (n=3, 115 treatment groups 2-10 mg/kg oral dosing). Variable and Variability: AUC and metric cutoff of 3

Analyses does not take into account difference in formulations and possible non-linear PK

Discovery PK and GLP TK Data

- PK data comparison shows good agreement between discovery and GLP TK studies



From Discovery to Regulated Bioanalysis

- Bioanalytical information is passed across different groups across the organization

- Discovery Acceptance Criteria evaluates essential parameters such as accuracy, precision, selectivity, sensitivity, reproducibility, limit of detection, and stability
- Information such as compound instability, plasma stabilization, presence of metabolites and possible interference with parent, method conditions (e.g. HILIC, SPE, LLE) move from Discovery to Regulated Method Development

Assay Requirements for all SA Studies

Assay Validation Components	Discovery	Exploratory	GLP
Acceptance Criteria Level	3	4	SOP
Accuracy	± 25%	± 25%	± 15% (± 20% LLOQ)
Precision	± 25%	± 25%	± 15% (± 20% LLOQ)
Analytical Standard	DMSO solution or solid for PCC	Solid	Solid Analytical Reference Standard
Solution Stability	No, prepared daily	Yes ± 7%	Yes ± 7%
Stability Check	Not Typical	Yes, some ± 25%	Yes (exhaustive) ± 15%
Dilution	Recommended	Yes ± 25%	Yes ± 15%
Validation (pass/fail)	P&A same day	Pre-study validation	Pre-study validation
Pre-study Validation Investment	None/Ad Hoc	5-7 business days	~ 20 business days

Comparison of Assay Validation Components

Assay Validation Components	Exploratory	GLP
3 Days Precision and Accuracy		√
Carryover	√	√
Specificity	√	√
Selectivity		√
≥ 1 Freeze Thaw Cycle in Matrix	√	√
≥ 2h Short-Term Stability in Matrix	√	√
Long-Term Freezer Stability in Matrix	√	√
≥ 6 h @ RT & Long Term Stock & Working Standard Stability	√	√
Dilution	√	√
Post-Preparative Stability		√
Recovery		√
Matrix Effect		√

Regulated Method Development Strategic Intent

- Develops robust regulated analytical methods
  - Leverages information from assays developed for discovery programs
- Ensures the effective implementation of these methods for GLP and Clinical sample analysis
  - Assists with resolution of issues during both validation and sample analysis
- Focuses on developing new techniques and approaches for new modalities, expanding BA science into innovative areas

Regulated Method Development: Principles

- Apply a standardized approach as much as possible
  - Intended as starting point for routine small molecules, capturing ~70% of work in this space
- Do enough to ensure that the method won't fail during validation
- Transfer method 3-4 weeks prior to sample collection
- Communication with PK, Regulated GLP BioA and Clinical BioA groups is a key

Regulated Method Development: When?

- First method is for support of GLP Tox studies
  - md initiated ~2-3 weeks from API 1st delivery
  - LLOQ
    - 2-5 ng/ml - small molecules
    - 0.1-1 ng/ml - large molecules
- Once FIH is scheduled, clinical method development is planned
  - md initiated ~2 month before FIH
  - LLOQ
    - 0.1-1 ng/ml - small molecules
    - 0.05-0.5ng/ml - large molecules (has been a challenge)

Method Development Experiments

- Optimize MS/MS conditions for analytes/IS
- Evaluate standard HPLC conditions
  - Columns, mobile phase and gradient profiles
  - Cross talk and/or metabolite interference as appropriate
- Standards and QCs preparation methods
  - Solvents, % organic
- Sample Preparation
  - Volumes, methods (protein precipitation, liquid-liquid extraction etc.)
- Assay evaluation
  - Linearity and STD curve reproducibility (n=5)
  - Intraday precision and accuracy using freshly spiked QCs
  - Selectivity
  - Carryover
  - Stability QCs
  - Dilution Integrity
  - Ruggedness

Conclusions

- Standard platforms and protocols reduce variability
- Work can be easily shared across groups and sites
- Judgment calls allow discovery groups to work in a high-throughput environment with minimal analyst oversight
- Essential parameters for bioanalytical method validation are evaluated at early discovery stage
- Documentation and interactions for successful LC-MS/MS methods transfer to preclinical GLP and clinical bioanalytical groups

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