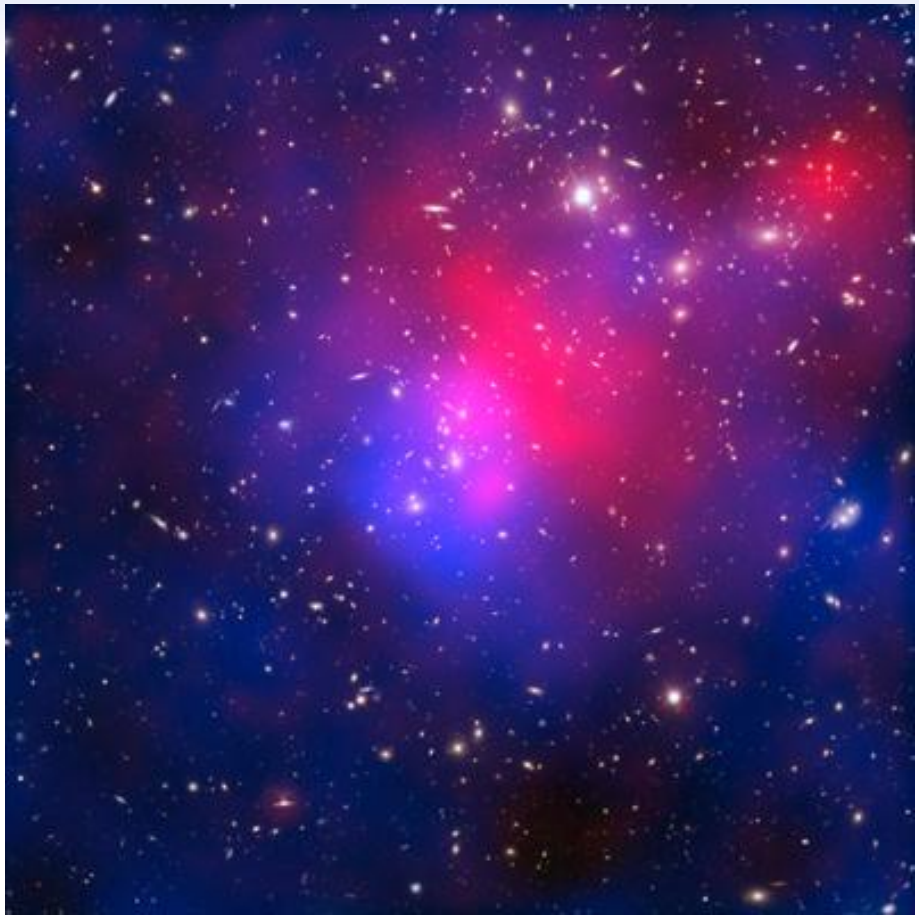
Microdosing and Cold LC-MS/MS : Bioanalysis and It's Evolving Role in Strategic Drug Development

Richard W. Abbott
Biosciences Group,
Shire Pharmaceuticals,
Basingstoke, UK

Shire

Our purpose
We enable people with life-altering conditions to lead better lives

Synopsis



Pandora's Cluster of Galaxies – where less is certainly more!

- Introduction
  - AMS vs LC-MS/MS
  - Sensitivity Improvements in Instrumentation Over Time
  - Changing Role of Bioanalysis in Pharmaceutical R&D
- Shire Case Example
  - Strategic Role of Bioanalysis
  - Assay Validation Strategy
  - Microdosing Study Application
  - Study Outcome
- Conclusions
- Acknowledgements

Introduction to Microdosing

Less is More : The Human Microdosing Concept;

R.C. Garner; *DDT*; 10(7), 449, 2005

Advantages of the Microdosing Approach :

- Only gram quantities of drug are required for safety testing
  - Only 100 µg or 1/100 of the pharmacological dose is administered to the subjects, depending which is less
- A minimal toxicology package is required
  - Extended (14 day) single dose study in a single species
- Enables rapid progression into man
- Cost of microdosing approach is a fraction of FTIH approach
- Enables early decision between several drug candidates with similar pharmacological profiles

Comparison of AMS vs LC-MS/MS

Lappin,G; Wagner,CC; Langer O; van de Merbel, N; *Bioanalysis,* 1(2), 357, 2009

Ings, RMJ; *Bioanalysis*, 1(7), 1293, 2009

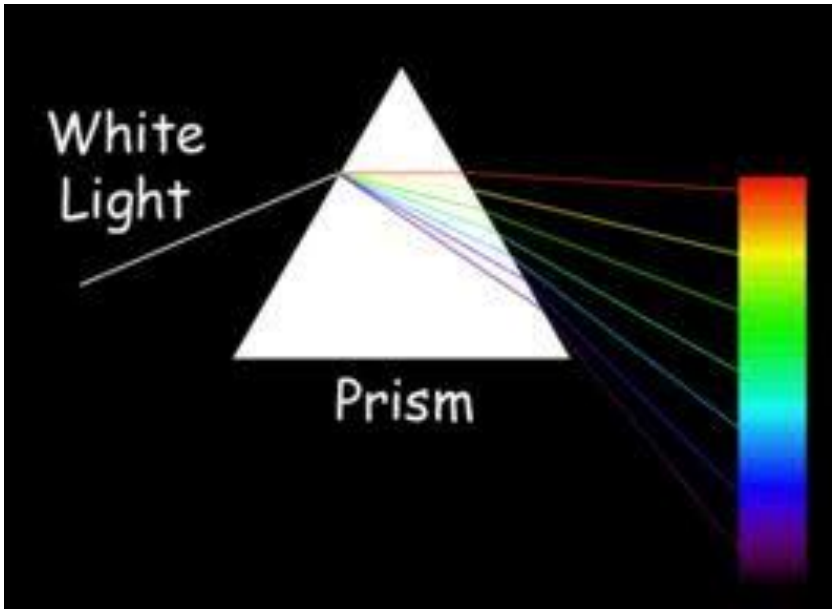
AMS

- No widespread availability
- ^{14}C material required
- ^{14}C synthesis requires time
- Exquisite sensitivity, fg/mL and ag/mL LLOQ's attainable
- Can provide PK information on parent molecule and metabolite
- Metabolism information, as well as absolute bioavailability and clearance

Cold LC-MS/MS

- Already available routinely
- No requirement for ^{14}C-label
- Enables rapid decision making
- Can support studies where low pg/mL LLOQ's required
- Can provide PK information on parent molecule
- No information on metabolism of drug

Evolution of Instrumentation Sensitivity



50s 60s 70s 80s 90s 00s 10s

Time

UV/ GC/ RIA/ HPLC/ ELISA/ X-MS

Instrumentation

White Light : Multi Coloured

µg/mL ng/mL pg/mL

Less is More

Increasing Sensitivity

2011 : Factors Enabling Sensitivity in 21st Century LC-MS/MS

LC-MS/MS bioanalytical challenge : ultra-high sensitivity assays; Aubry,A-F, *Bioanalysis*, 3 (16), 1819-1825, 2011

Sample Preparation

- SLE
- µ SPE
- Column Switching (2D-LC)

Chromatography

- Ultra high pressure LC pumps
- Decreasing particle size, 5 µm → 1.7 µm
- Increasing N and R_s
- Reduce column id

Mass Spectrometry

- Quadrupole MS refinements

e.g. API4000 to API5500, x10 fold sensitivity gain

In Combination

Sub ng/mL LLOQ → Increasing Trend → Single digit pg/mL LLOQ or lower

Traditional Role of Bioanalysis in Pharmaceutical R&D

Routine

- Routine Assay Validation
- Routine Study Sample Analysis
- Study Scheduling
- Enabling Project Progression
- Dependable Study Support and Regulatory Submission Support

‘Back-room’ guys

[Only noticed if an issue arises!]

2011 : Changing Role of Bioanalysis in Pharmaceutical R&D

Strategic

- Enabling fresh thinking regarding sample collection and handling (e.g. DBS)
- Enabling new project strategy (e.g. microdosing)

→ Improvements in instrumentation sensitivity have made this possible

Exciting Times!

Recent paper on applicability of LC-MS/MS to microdosing studies

Clinical Relevance of LC-MS/MS as an Analytical Method in Microdose Clinical Studies; Yamane N et al.; *Pharm. Res*.; 28, 1963-1972, 2011

Objective

Investigation of the sensitivity of the LC-MS/MS approach as a tool in microdose clinical trials

Methods

31 of the 47 top selling drugs with a wide range of physicochemical properties were spiked in human plasma, extracted and analysed by LC-MS/MS

(Exclusions : High mol weight drugs, combination products, drugs requiring derivatisation)

Results

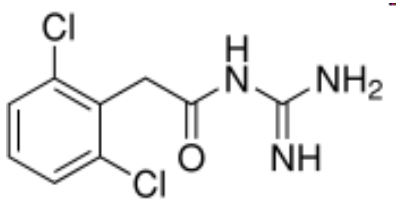
LLOQ's varied from 0.08 to 50 pg/mL – These were < 1/8 of assumed C_{max} at microdose for all drugs except losartan, indicating wide applicability for the generation of full PK profiles following microdosing

Conclusions

LC-MS/MS should be widely applicable for the support of clinical microdosing studies where generation of PK profiles of the parent molecule is the key endpoint.

Shire Case Example

- SSP-1252, Prodrug of Guanfacine (treatment for ADHD)
- Guanfacine



- Objective : Move project into man as rapidly as possible
  - » Verify prodrug absorption and metabolism to guanfacine
- Development Team Goal :
  - Facilitate objective through a microdosing approach

PK Modelling and Bioanalytical Feasibility Study

Initial Plan

(1) Collaboration between Biosciences group and Clinical Pharmacology group to model projected Cmax for SSP-1252 and guanfacine following microdosing

(1) Projected Cmax : 60 pg/mL

(2) Calculated LLOQ required, based on 4 x $t_{1/2}$: 4-5 pg/mL

(2) Bioanalytical feasibility study to check out that LLOQ of 5 pg/mL for each analyte was possible

- As SSP-1252 was a prodrug, sample instability was also an issue, sample collection and handling was an additional complication

Shaping Clinical Development Strategy

- Based on the encouraging results from the bioanalytical feasibility study :
  - Clinical Development Strategy set to include microdosing
  - A first for Shire
  - A first for the bioanalytical group
  - A first experience where bioanalysis had been central in enabling the clinical development strategy
  - NB Shire is a virtual company
  - So this was achieved using an outsourced approach

Assay Validation Strategy

- Extent of validation required?
- Likely to use the assay only once at the extreme sensitivity required
- Regulatory guidance – not for bioanalysis in support of microdosing studies
- No previous discussion within EBF or AAPS regarding commonly accepted approach

- We did not feel comfortable taking anything other than the Full validation approach

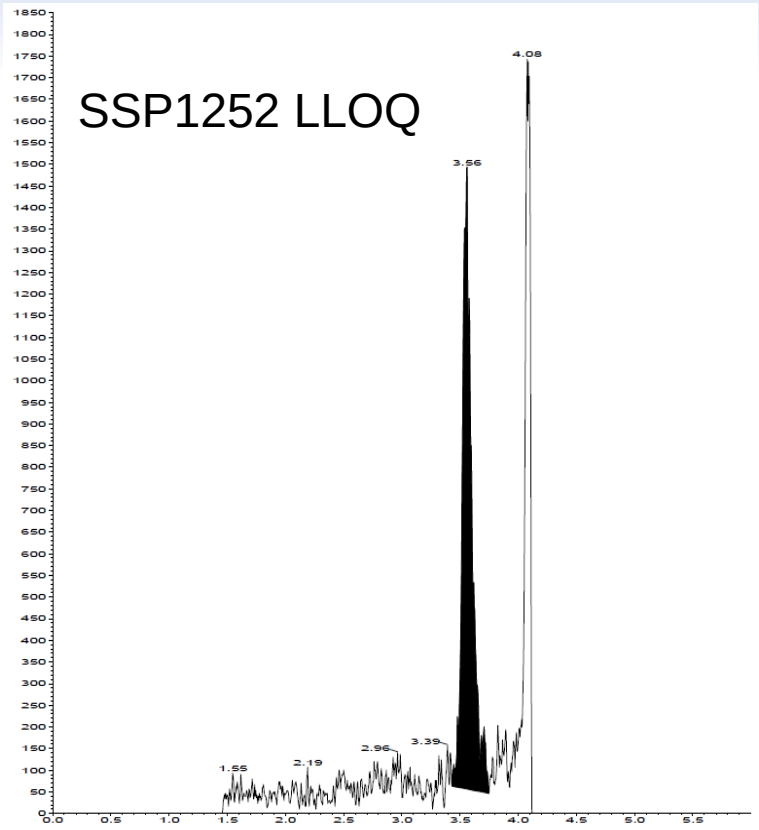
- So we adopted this strategy and moved forward with full validation packages for both SSP-1252 and guanfacine

Method Summary

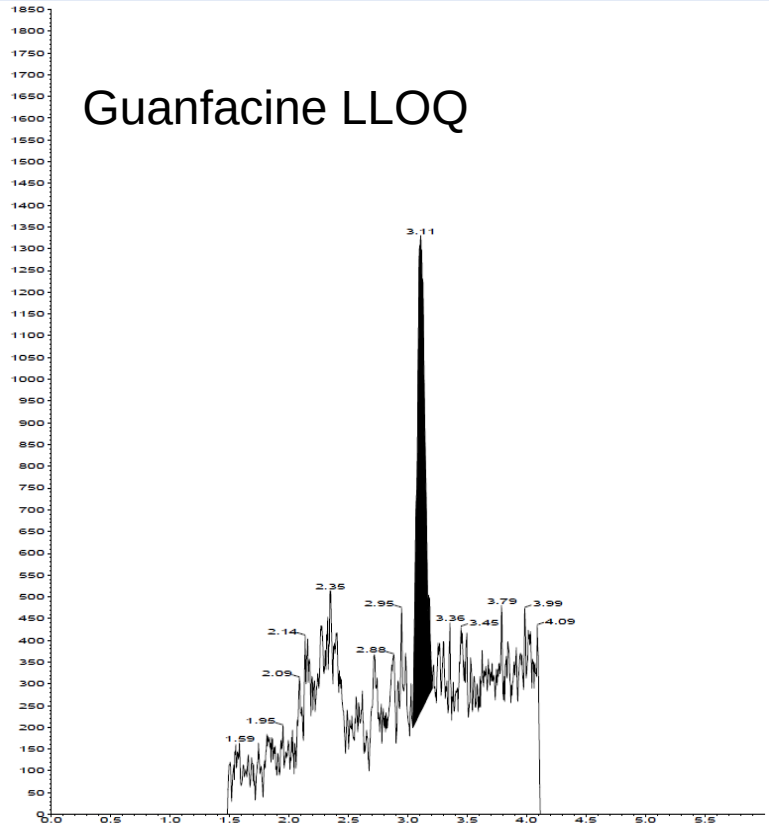
- Matrix
  - 220 µL acidified (10% formic acid) K_2EDTA/NaF human plasma
- Internal Standard
  - Analogue, Guanabenz
- Sample Preparation
  - LLE with MTBE coupled with PP (MeCN)
- HPLC
  - HyperClone BDS C_{18} 130A, 5 µm, 150 x 2 mm with gradient elution
- Mass Spectrometer
  - Sciex API-5500
  - Turbo IonSpray, positive ion mode
- Calibration
  - Separate lines for guanfacine and SSP-1252, 5 to 2500 pg/mL

Example Chromatograms

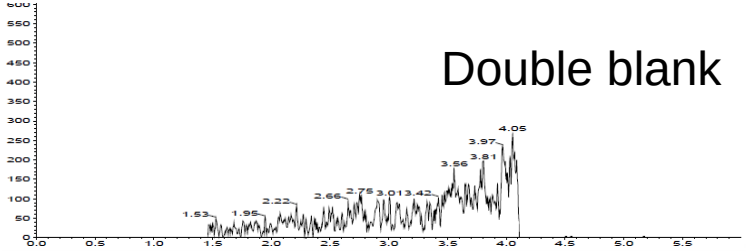
SSP1252 LLOQ



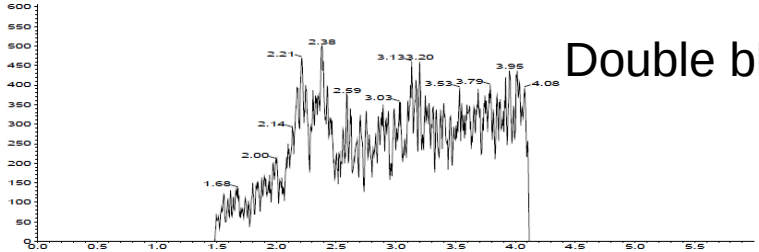
Guanfacine LLOQ



Double blank



Double blank



Headline Assay Validation Summary

	Intra-Day Data		Inter-Day Data	
	LLOQ	Other QCs	LLOQ	Other QCs
1024 (%cv)	9.5 to 14.5	1.3 to 10.5	12.8	2.7 to 8.2
1024 (% bias)	-8.6 to 8.2	-12.5 to -2.7	1.0	-10.5 to -3.5
1252 (%cv)	6.0 to 9.2	1.4 to 14.3	9.2	5.0 to 11.5
1252 (% bias)	-0.6 to 12.0	-11.6 to 5.2	3.8	-10.0 to -1.2

Stability

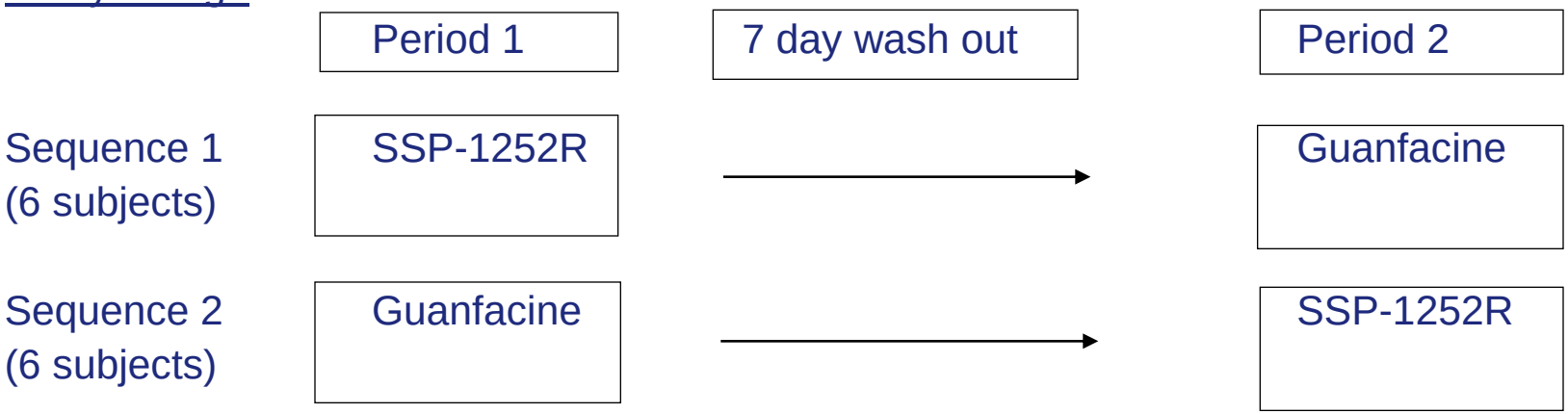
- At least 4h bench top acidified plasma stability (ice/water bath)
- At least 3 F/T cycles
- At least 31 days long term frozen acidified plasma stability (-70ºC)
- At least 72 h extract reproducibility (4ºC)

Clinical Microdosing Study Details

Primary Objective

Determination of the relative oral bioavailability of guanfacine from SSP-1252R compared to guanfacine HCl after a dose of 100 µg in healthy male volunteers

Study Design

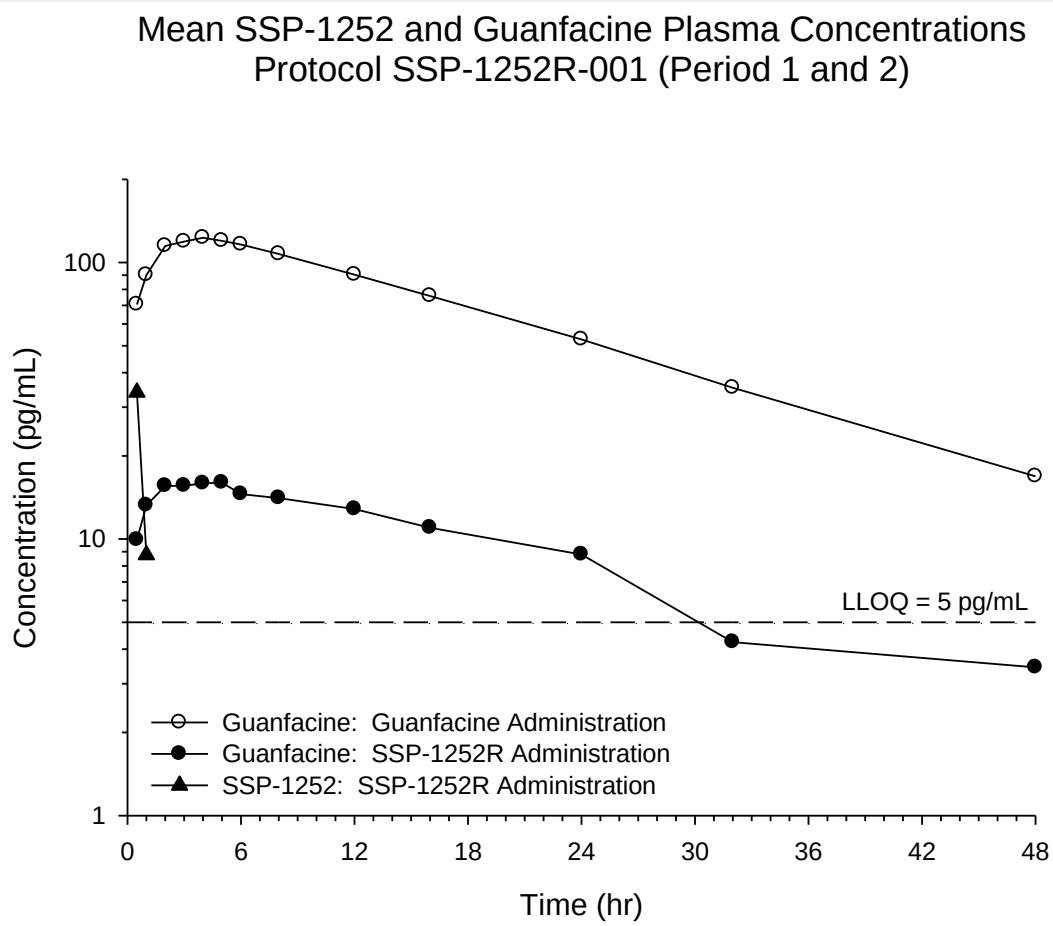


Sample Collection

- Blood samples (3 mL) collected into Vacutainers (2 mg NaF / 1.75 mg K_2EDTA)
- Place in crushed ice bath immediately and centrifuge within 30 minutes at 4ºC
- Aliquot 0.6 mL plasma into tube containing 60 µL 10% formic acid, mix & freeze (-70ºC)

NB – Training provided in pipetting by Bioanalytical CRO staff to clinical site staff

Summary of Microdose Clinical PK Study Output



- Guanfacine concentrations measured to 24h (1252R administration) and 48h (guanfacine HCl administration)
- SSP-1252 rapidly absorbed and eliminated with measurable concentrations for 1h after administration

- Taking into account the different molar doses, relative F of guanfacine from SSP-1252R after oral dose of 100 µg was 28.3%

Bioanalytical Study Performance Details

- All 5 bioanalytical batches were acceptable
- QC data demonstrate acceptability of assay performance

	Quality Control Samples		
	Low 15 pg/mL	Mid 200 pg/mL	High 2,000 pg/mL
1024 (% cv)	10.3%	5.8%	4.1%
1024 (% bias)	-3.9%	-0.6%	4.3%
1252 (% cv)	9.7%	4.1%	10.2%
1252 (% bias)	0.8%	6.2%	4.4%

- 8 days from final sample receipt to availability of preliminary QC'd data
- Rapid sample analysis enabled rapid study data review and decisions

Conclusions

- The improvement in LC-MS/MS sensitivity is enabling the technique to be used as a tool in microdosing studies
- The introduction of low pg/mL assay sensitivity is allowing bioanalysis to play a strategic role in drug development
- Recent literature suggests the use of cold LC-MS/MS in microdosing may be widely applicable across therapeutic areas
- Shire studies over the past year have verified these findings (4 cold microdosing studies completed)
- Wider discussion across industry should enable assay validation for microdosing studies to be appropriately tailored

Acknowledgements

- Phillip Wang, Shire Bioanalysis
- Steven Troy, Shire Clinical Pharmacology
- Shire R&D Management

- Yong Xi-Li and the team at Medpace Bioanalytical Laboratories

- EBF Microdosing Team for stimulating discussions
- You, for your attention