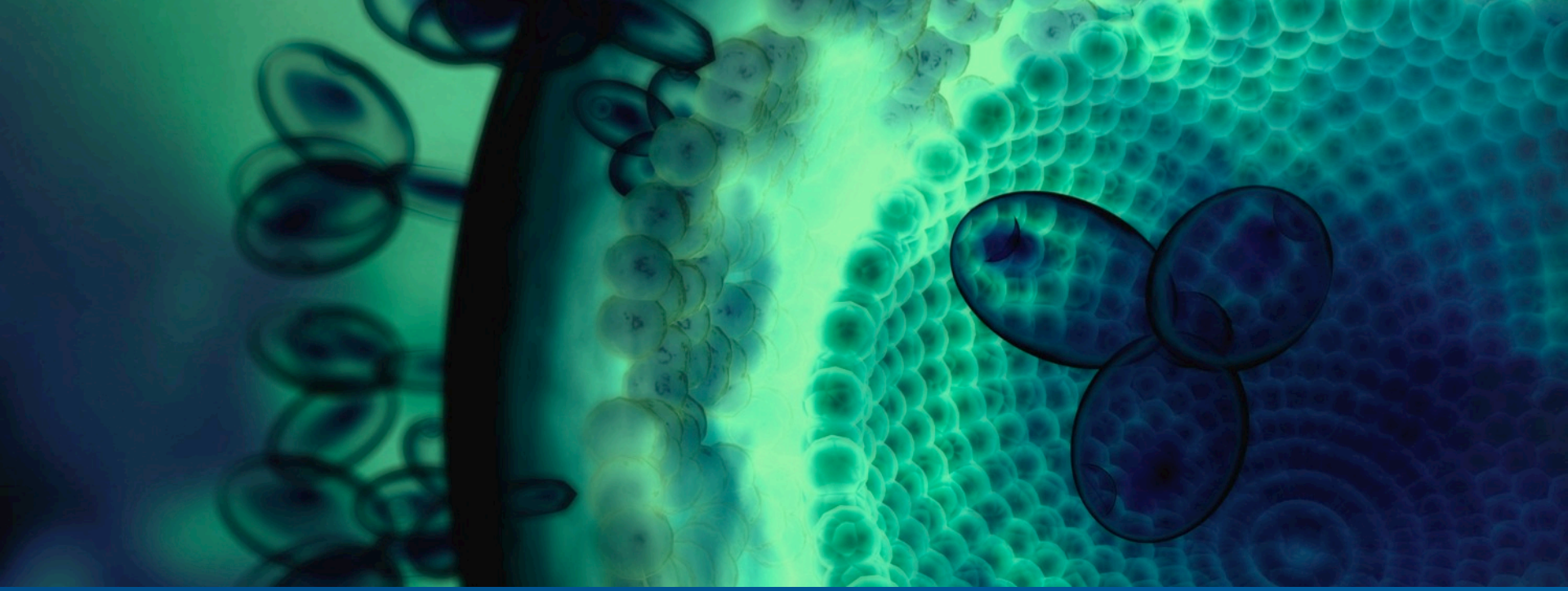


LC-MS/MS as an enabler for a broader application of microdose studies in drug development; the Janssen experience

Pictured above: The structure of HIV.

Tom Verhaeghe| EBF Meeting Barcelona | 19-21 november 2014

Introduction

- Microdose = 1/100th of therapeutic dose (≤ 100 µg)
- Benefits of applying a human microdose:
  1. lower toxicological risk to subjects
  2. limited TOX package to go into man
  3. requires less drug substance
- High sensitivity required of bioanalytical assay (~ pg/ml level) to allow generating full PK profile.
- In the past these levels were only achievable by AMS after dosing ^{14}C-labelled drug
- AMS remains unbeatable in sensitivity but also expensive, demanding sample processing, low throughput, number of labs with AMS expertise is limited
- With evolution of technology (more sensitive mass specs, advances in LC equipment) LC-MS/MS is becoming a viable alternative

Introduction

- 2 case studies of LC-MS/MS in support of microdosing studies at Janssen
- Case #1: 100 µg oral dose of unlabelled drug, comparing 3 compounds to select candidate with optimal human PK profile
- Case #2: 100 µg IV dose of stable isotope labelled drug combined with therapeutic oral dose (>100 mg) of unlabelled drug to determine absolute bioavailability

Microdose to facilitate early candidate selection

Background

- Lead compound showed suboptimal human PK profile (rapid clearance, short half-life)
- Investigate PK in man of 2 back-ups (one metabolite and one structural analog) which showed lower *in vitro* clearance
- To limit resources and lead time conduct a microdose study comparing PK of 3 compounds: lead + 2 back-ups
- Required limited TOX package, limited amount of drug substance and limited formulation efforts
- 18 healthy volunteers randomized to receive one of the 3 compounds as a single oral microdose of 100 µg
- 19 PK samples per subject over a 72 hour period

Assay Development Strategy

- Technically not too challenging:
  - analytes are intrinsically MS sensitive (each contains 4 N-atoms)
  - 2 stable isotope labelled IS available, 1 for lead and 1 for metabolite
- Given structural similarity develop combi-assay (even though compounds are dosed separately)
- Sample volume: 200 µl plasma
- Anticipated calibration range: 1-500 pg/ml
- Approach:
  - optimize sample prep
  - Use UPLC
  - Use most sensitive mass spec.

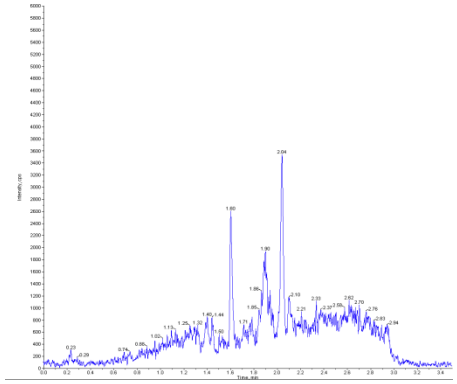
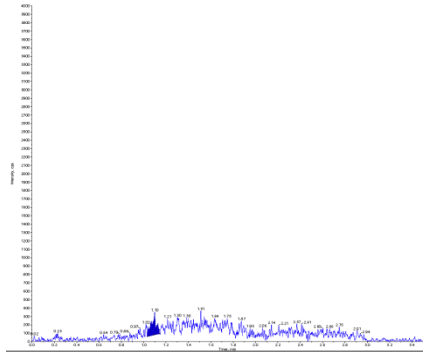
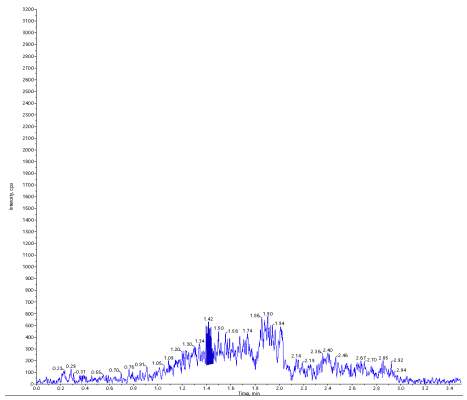
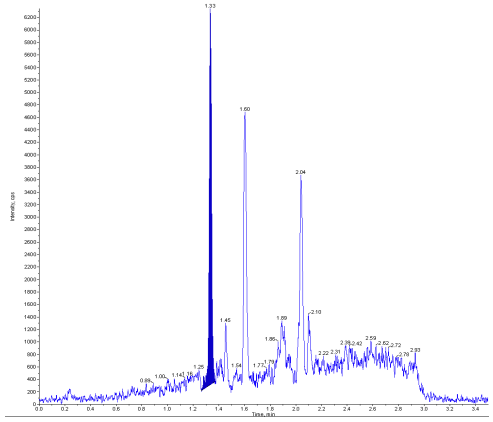
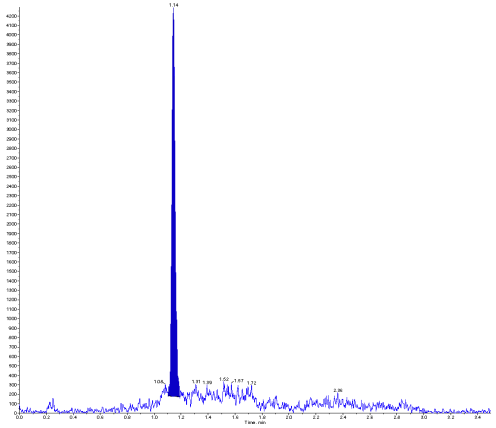
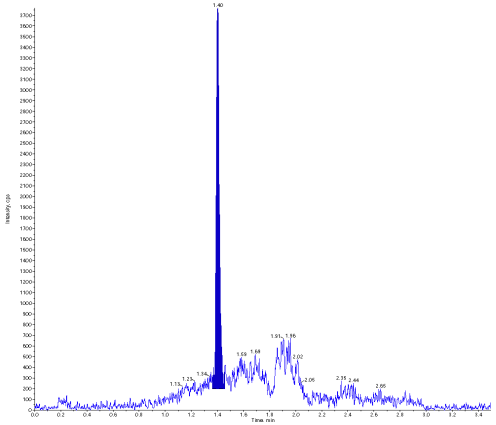
Assay highlights

- 200 µl plasma aliquot
- Liquid-liquid extraction TBME (Isolute 96-well plate)
- Nexera UPLC: 5 cm x 2.1 mm x 1.7 µm BEH phenyl hexyl; 700 µl/min;55 °C
- High pH mobile phase ($(NH_4)_2CO_3$/MeOH) for increased MS response
- MS: API5500

To validate or not to validate ?

- Study was conducted for internal decision making: select compound with optimal human PK properties
- Only occasion that this assay will be used
- No formal “regulatory” validation was performed, instead a qualification (aka scientific validation):
  - Ran a large batch as part of method development to test robustness of the assay
  - No stability testing; stability data were available for lead and no issues
  - Acc. & prec. OK for all 3 compounds; ready to support clinical study

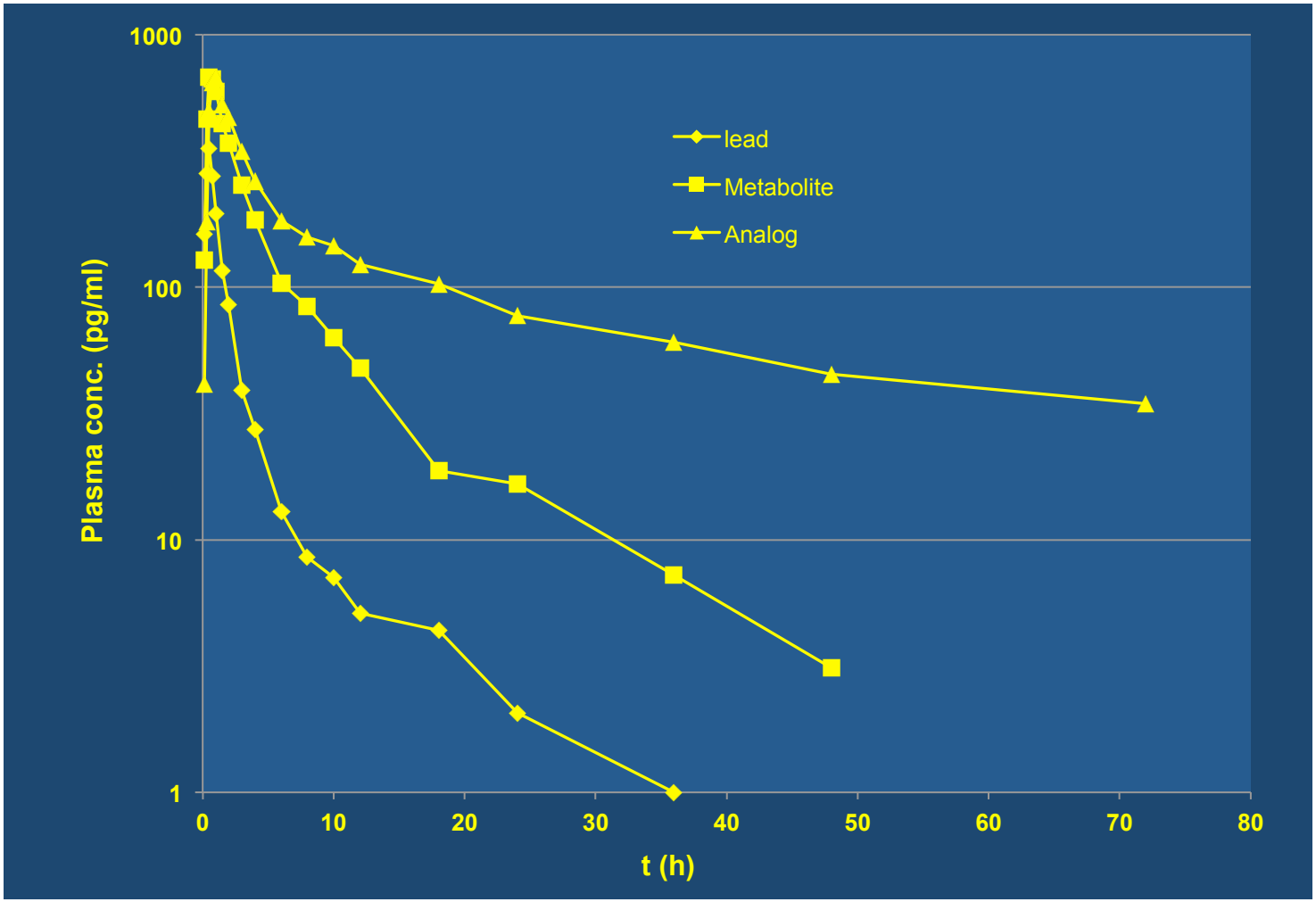
Chromatograms blank and 1 pg/ml standard for lead, metabolite and analog

	Cmpd #1: lead	Cmpd #2: metabolite	Cmpd #3: analog
blank			
1 pg/ml			

Study sample analysis

- At lower levels of calibration curves some standards rejected, mainly overestimated
- One batch rejected due to contamination of blank plasma
- All QCs well within 15% of nominal
- LC-MS/MS assay was sensitive enough to draw valid conclusion from the study
- Microdose study enabled selection of drug for further development, comparing the human PK across 3 candidates.
- Both metabolite and structural analog showed improved human PK profile over lead

PK plots for different compounds (average of 6 subjects)

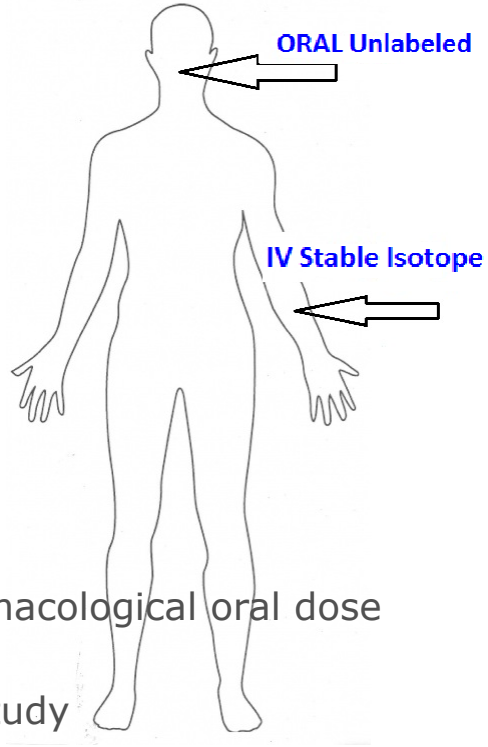


Application of microdose in absolute bioavailability study

Background

- Request from FDA to determine absolute bioavailability of orally dosed drug
- Traditional design compares AUC after oral and IV dosing using pharmacological dose in cross over design, but:
  - Requires development of IV formulation
  - Requires IV TOX studies
  - Cross over design introduces between-period variability thus need to include sufficient number of subjetcs
  - Cross over design means study takes longer to complete

Background



- Team decided to look into microdose approach
- Administer 100 µg stable isotope labeled drug IV on top of pharmacological oral dose of unlabeled drug
- No cross over design, less subjects needed (8), less expensive study
- Using mass spec fate of IV and oral drug can be discriminated
- Limited amount of drug substance (isotope labeled)
- No issues with developing IV formulation, no solubility issues anticipated
- Co-dosing of IV microdose and pharmacological oral dose eliminate risk of non-linearity (IV dose given at t_{max} of oral dose)

Assay development strategy

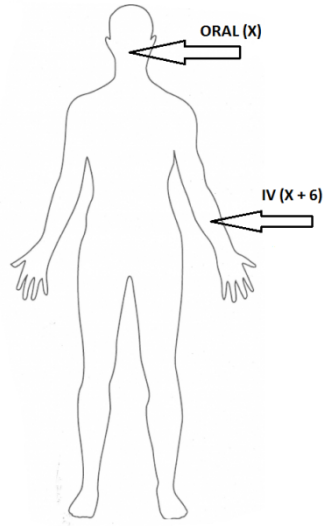
- High levels op oral drug in presence of low levels of IV drug
- Potential for isotopic interference and suppression (oral dose on microdose compound and on IS)
- From simulations LLOQ below 10 pg/ml required for IV drug
- Need 2 labelled compounds; one for IV dose and one as the IS
- Need to carefully consider how many labels are required for each and where to build in the labels
- Avoid metabolic soft spots or labeling that could affect PK (eg. preference for ^{13}C or ^{15}N over ^{2}H)
- Thorough theoretical assessment[1] before commencing synthesis; avoid unpleasant surprises
- Separate assays for oral drug and iv drug

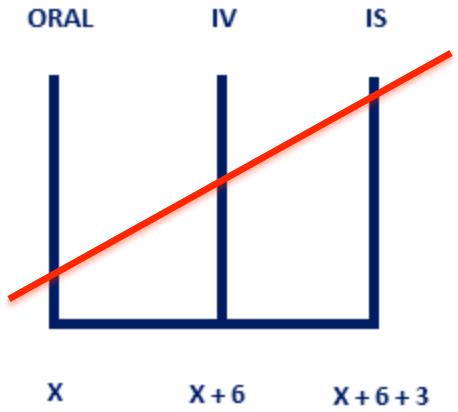
[1]: Huidong Gu, Jian Wang, Anne-Françoise Aubry *et al.*, Anal. Chem. 2012, 84, 4844-4850

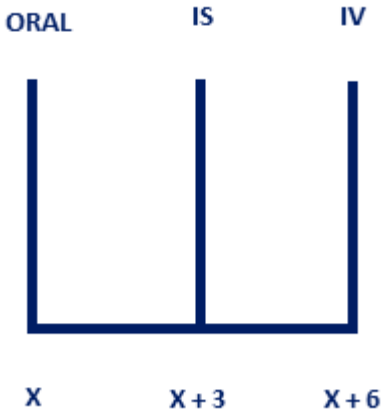
Selection of labeled compounds

- Based on calculations need at least 4 labels for microdose compound
- D5 labelled was available (IS for unlabelled assay) but not preferred because at site of metabolism (hydroxylation)
- $^{13}C_6$ could be synthesised fairly easily and was selected
- Synthesizing an IS with 9 labels complicated and expensive
- Based on calculations using only 3 labels ($^{13}C_3$) for the IS would work as well

Selecting the appropriate labels







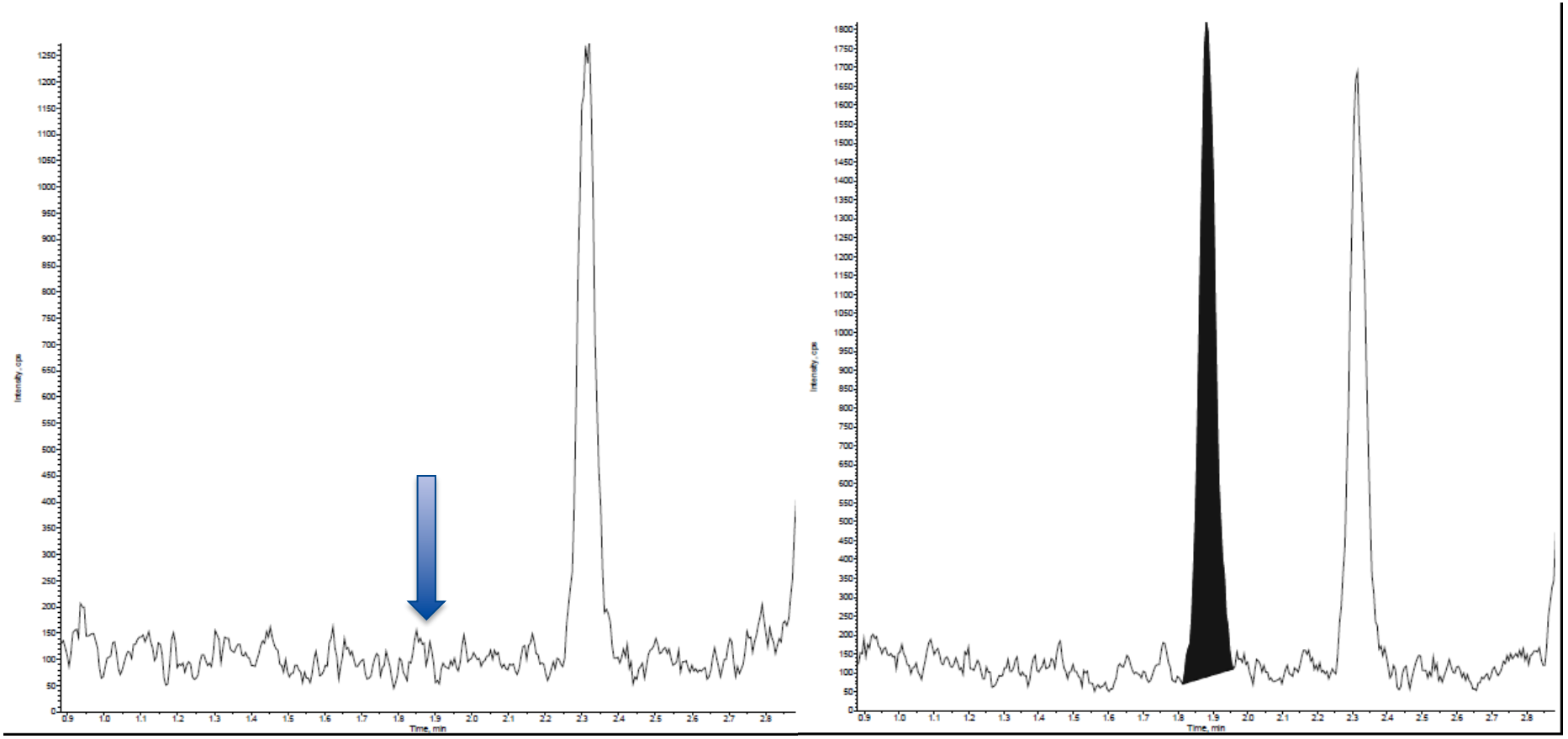
Assay highlights

- Assay range: 2-1000 pg/ml; IS conc. 1000 pg/ml
- 200 µl plasma; liquid-liquid extraction TBME (Isolute 96-well plate)
- Nexera UPLC: 5 cm x 2.1 mm x 3.5 µm BEH C18;600 µl/min;50 °C
- High pH mobile phase ($(NH4)_2CO_3$/MeOH) for increased MS response
- MS: API5500

To validate or not to validate?

- Since question came from FDA opted for validated assay with some adaptations
- Matrix stability experiments were not repeated; assumed stability identical to unlabelled analyte
- QCs co-spiked with high conc. of unlabelled analyte (20 or 50 ng/ml) to confirm no suppression or isotopic interference impact on accuracy of microdose assay
- Blanks without IS, spiked with 10, 20 or 50 ng/ml of unlabelled analyte to assess contribution in IS channel; at 50 ng/ml signal is 1.5% of IS peak area
- Currently considering if full validation is necessary for future studies

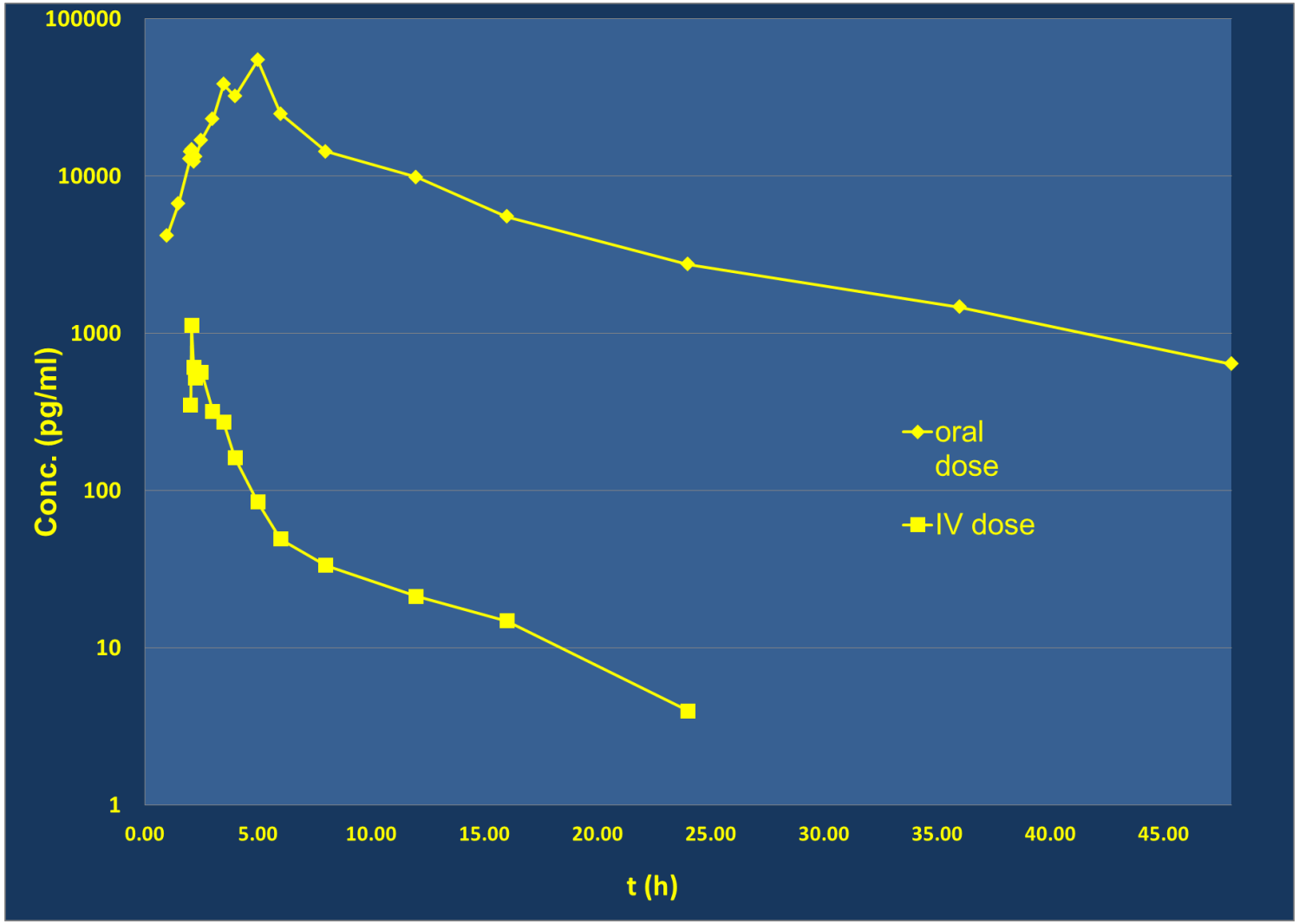
Chromatograms blank and 2 pg/ml standard



Study sample analysis

- 432 samples analyzed in 5 batches; all batches passed
- In second batch interference at rt of analyte; LLOQ was rejected
- Interference coming from extraction plates and lot dependant
- Issue solved by washing and drying plates before use
- LLOQ of 2 pg/ml allowed quantifying levels up to 24h post dose in all subjects

PK plots oral and IV dose (average of 8 subjects)



Conclusions

- LC-MS/MS can be a viable alternative for AMS in conducting microdose studies but need to consider case by case.
- In case of absolute BA study selection of the appropriate number and location of labels for the IV drug and the IS is vital for a successful study outcome.
- Measuring at these low concentrations requires attention in the lab to avoid contamination and unexpected interferences.
- Because LC-MS/MS is a more readily available technology, a broader application of microdose studies in drug development is to be expected.
- Need to carefully consider if full validation of the assay per guidelines is a requirement.

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