A complex problem: what (and how) should we quantify? – Tiered approach for clinical studies

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Vera Hillewaert, Janssen Research & Development

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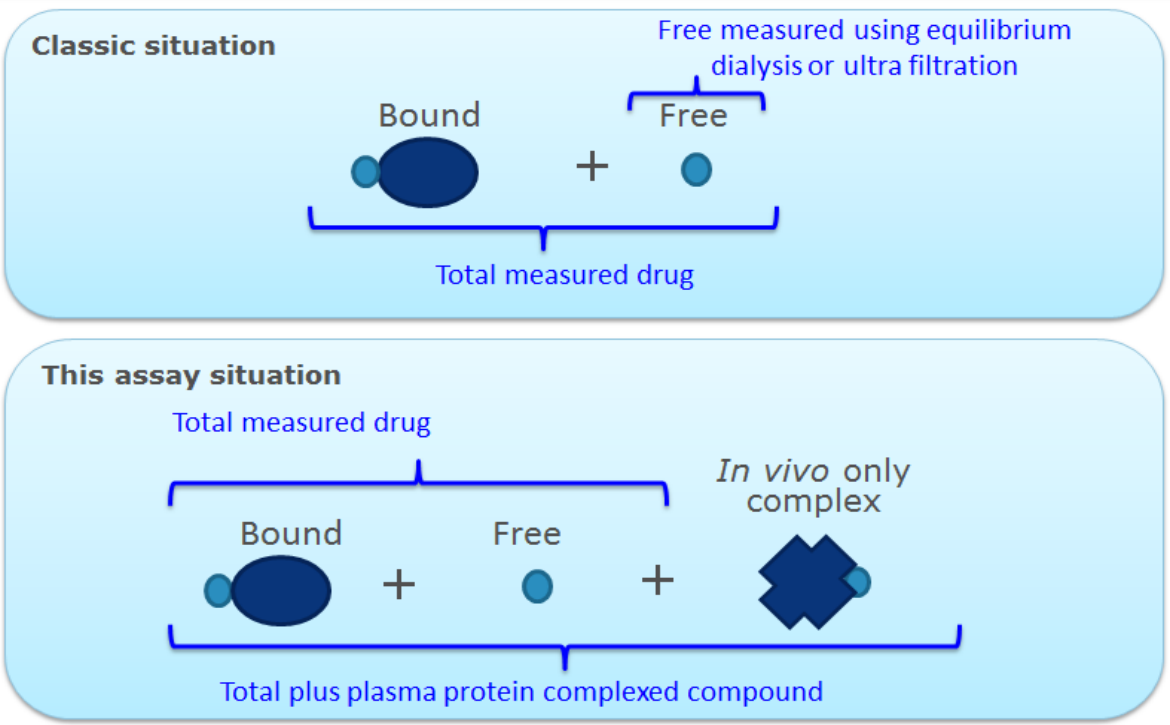
Background

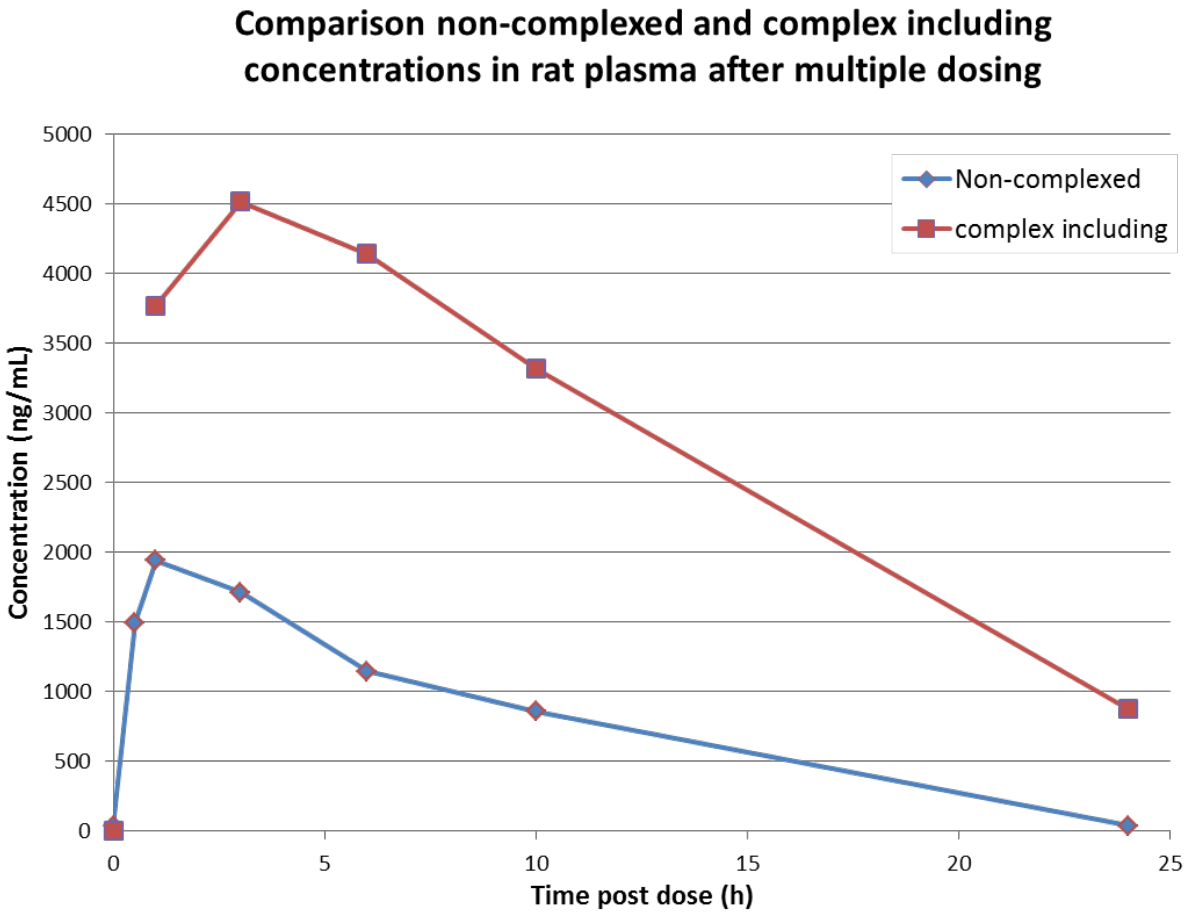
- In-licensed compound
- From mass balance studies: Presence of protein complex in rat and mouse plasma samples



Complex

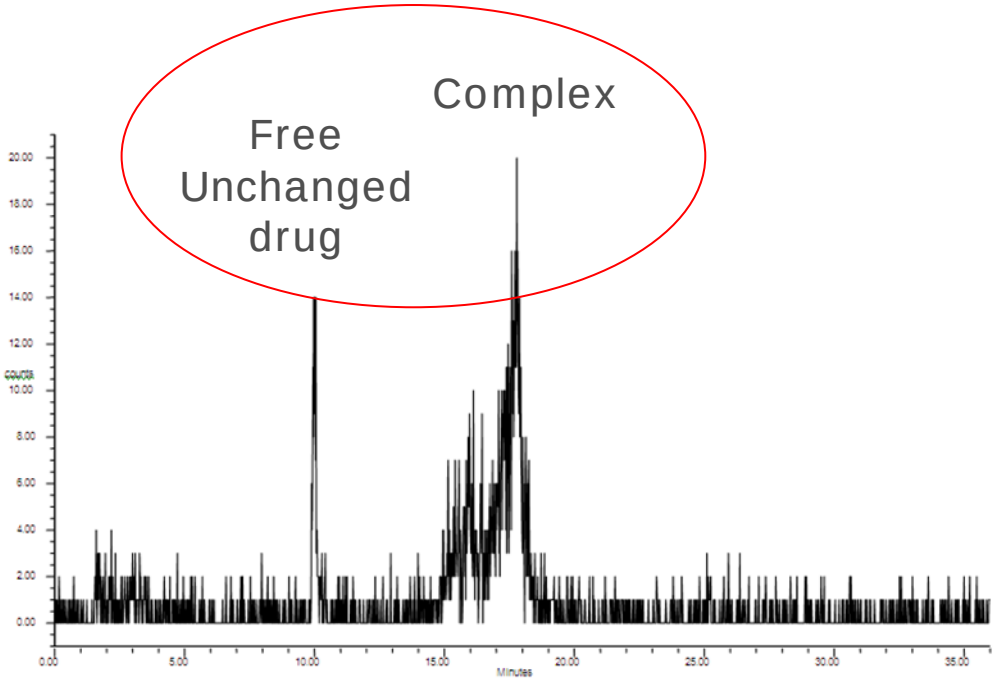
- The complex is only seen in-vivo in rat and mouse, cannot be reproduced ex-vivo/in-vitro
- Not seen in dog plasma, what about human plasma?
- Instable under basic sample preparation conditions
- Formation of parent: factor 3-4 or even up to factor 10 higher concentrations compared to acidic/neutral sample preparation

Profile in rat



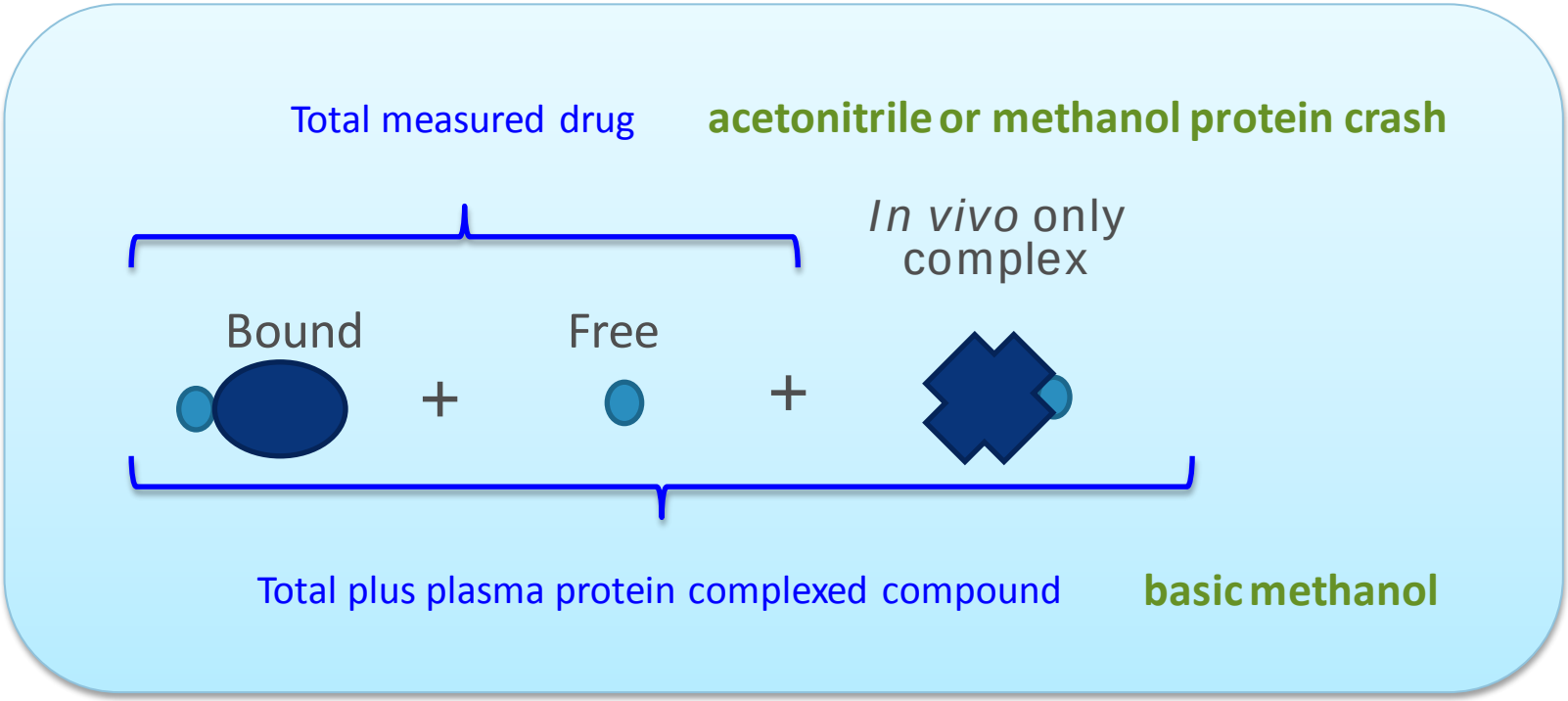
Complex

- In-house radio-HPLC chromatogram confirms presence of the complex in rat plasma samples



- Reversible: proven with isolated complex

Sample preparation



Sample preparation

- Choice of sample preparation will determine what is measured
  - Thorough assessment of nature of protein complex required before correct method can be selected
  - Relevance for human assay not yet known

Analytical methods

- Quantitative methods in plasma of rat, dog, mouse (were available upon inlicencing)
  - Protein precipitation with (acidic) acetonitrile, so measuring without complexed
  - Chiral separation for analysis of (-) enantiomer
  - LC-MS/MS analysis using stable isotope labeled internal standard
  - Validated for parent and metabolite

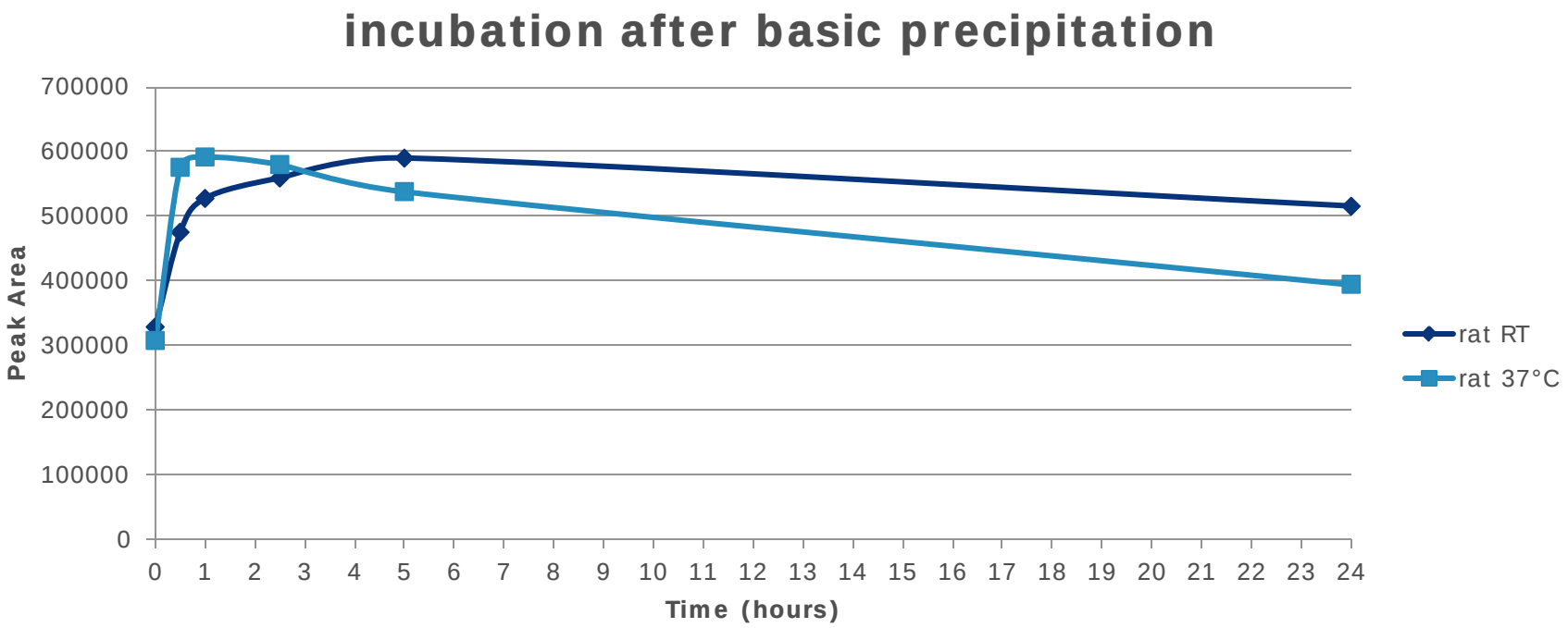
Analytical methods

- At Janssen for preclinical studies:
  - Protein precipitation basic
  - Parent: validated non-chiral
  - Metabolite: qualified non-chiral
    - Exception: for mouse, possible tox issue, so method validated

Problem

- Are we sure what we are measuring?
  - ISR failed in rat (passed in dog)
  - ISR results were more than 20% lower than first results
  - Investigation learned this was dependent on duration of sample preparation (large batch vs small batch)

Sample preparation



– Conclusion: incubation time is important, so for measurement of total + complexed: incubation at 37° C for 1h

Next: FIH

- What about complex in human samples?
  - Will the complex be present in human plasma?
  - What incubation time is needed to set all the complexed analyte free?
  - Can only be tested in samples, but of course no samples available
  - No use validating a method

Tiered approach

- Ongoing exercise on tiered approach
  - Use tiered approach to drive the best scientific decision and compliance to provide cost-effective bioanalytical support for various types of studies
  - Global alignment needed
    - Compare practices at different sites
    - Decide on way forward
  - Validated – qualified – research - screening

Qualified assay

- Undergoes limited characterization with calibration standards and QC samples
- Using a non-certified standard is allowed
- The method provides absolute analyte concentration
- Criteria 4 – 6 – 20 allowed
- One accuracy and precision run
- Minimum 5 STD levels
- Minimum 2 QC levels

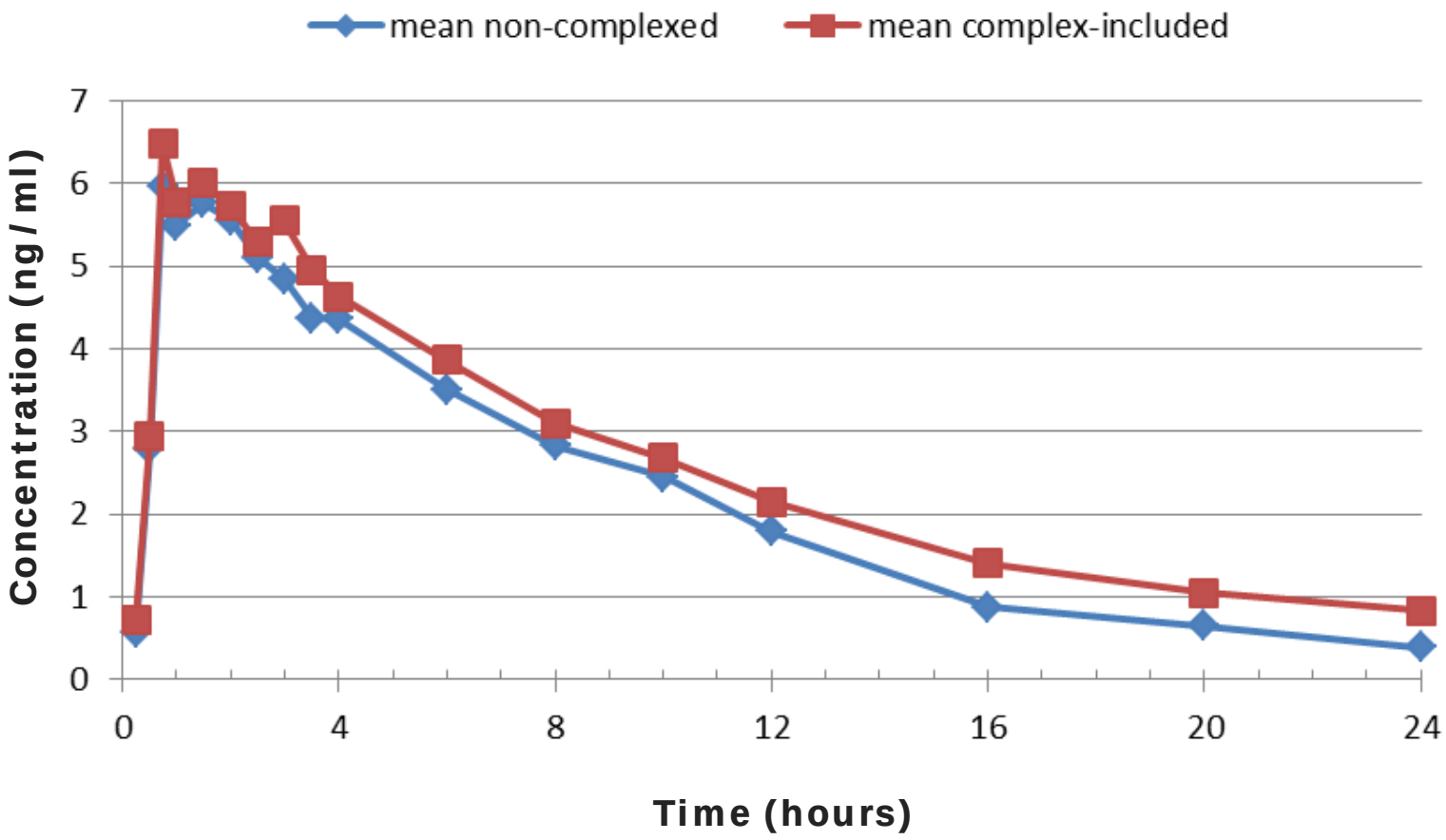
Qualified assay

- Report: depends on type of study
  - Biomarker clinical studies
  - Human matrix other than plasma/serum
  - MIST TOX and clinical
  - Metabolites pre-MIST clinical
  - Non-GLP part of a GLP study
  - Chiral support
  - FIH: SAD / MAD

FIH: validated versus qualified?

- Qualified assay for FIH SAD
  - No info on complex behavior in human plasma
  - Total plus plasma protein complexed compound on all plasma samples
  - Total measured drug without plasma protein complexed compound on a selection of cohorts
  - With these results decide on what to validate for further studies
- Results: The results with and without complexed compound were comparable

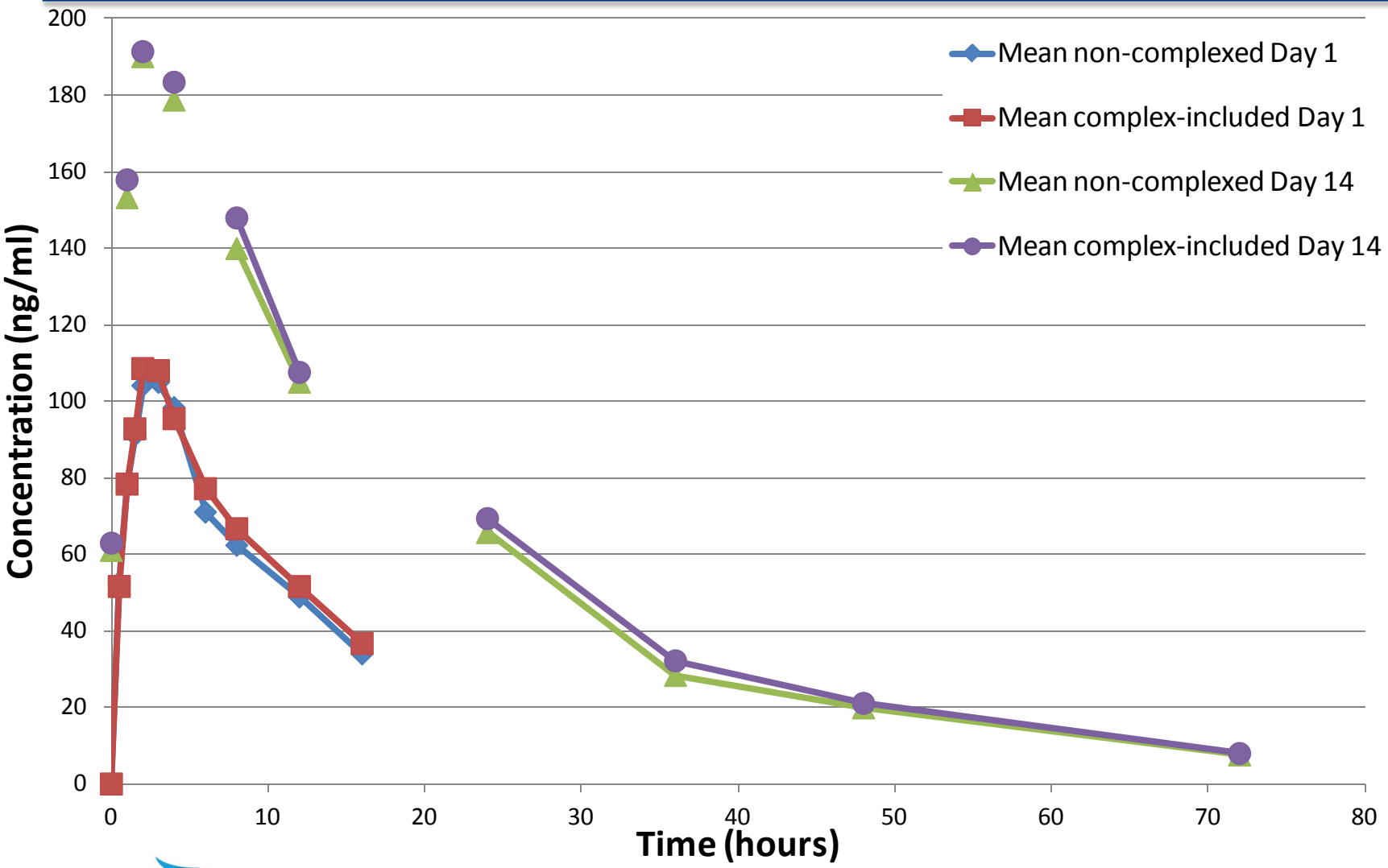
SAD: With and without complexed



MAD: validated

- Validated assay for MAD
  - The clinical team is not used to this approach
  - Method validated with basic precipitation, so measuring total plus complexed compound
  - Also in this study measure total drug without complexed compound on a selection of cohorts

MAD: With and without complexed



Conclusion

Tiered approach allowed for increased scientific focus in an early stage of development without jeopardizing timelines or reconstructability of the assay

Acknowledgments

- Hans Stieltjes
- Tinne Huybrechts
- Tom Verhaeghe
- Philip Timmerman

Questions? Comments?