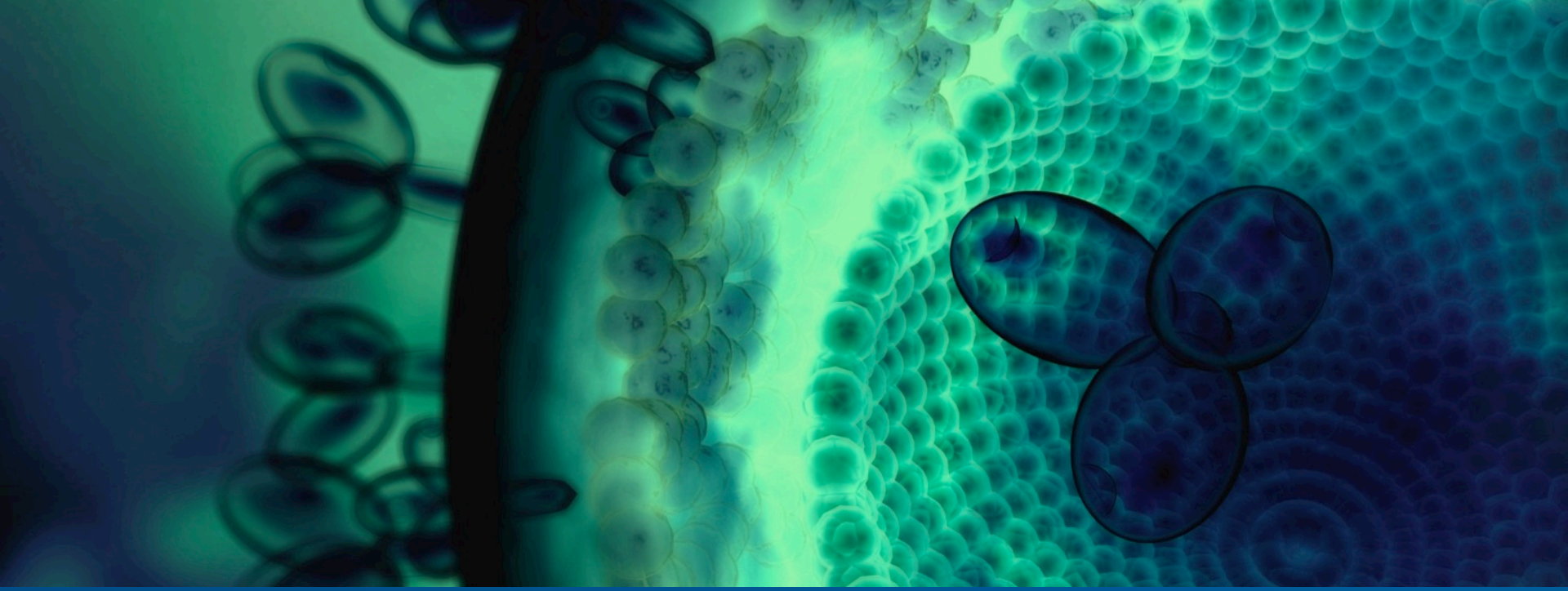


Assessment of capillary microsampling of blood in a healthy volunteer study

Pictured above: The structure of HIV.

Vera Hillewaert | EBF Meeting Barcelona | 21 November 2014

janssen | PHARMACEUTICAL COMPANIES OF Johnson&Johnson

Background

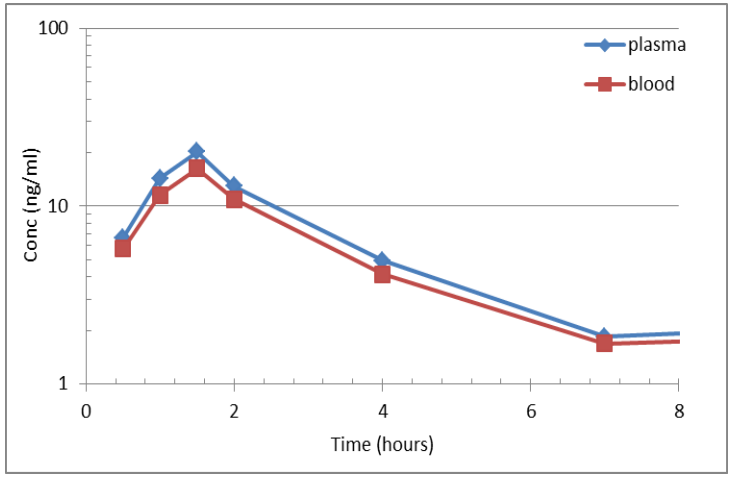
- Compound for treatment of adults and children
- Available as regular tablet, only to be used for children above the age of 5 years
- New tablet was developed to be given to younger children

The project

- A phase III trial in children is planned in developing countries, using the new tablet
- PK sampling will be done, in children 1 – 16 years of age, at sites in remote areas
- Sampling needs to be done in a way that all participating sites can comply → team has decided to use capillary micro sampling and working with blood samples
- A method needed to be developed and validated to measure the expected concentrations in the 15µL blood samples that will be present in the capillaries

Analytical methods

- Validated method for human plasma for parent drug (normal sample volumes) is available at CRO
- Additional requirement to establish method for human blood via capillary microsampling in-house
  - To gain some experience with this type of samples, satellite capillary blood samples were taken in dog study
  - Good correlation between plasma and capillary blood



Analytical methods

- Overview of analytical method:
  - Sampling in capillaries (15µl)
  - The capillaries are stored in Nunc™ tubes
  - BSA/Buffer is added in the tubes, tubes are shaken vigorously and centrifuged.
  - 100 µL of this solution is processed: IS is added, the aliquot is buffered and extracted with TBME over an Isolute fixed well plate
  - Range 1.00 – 1000 ng/mL
  - UPLC
  - API-4000

Method validation

- Carefully consider what to include in validation
- Extra testing of the diluted sample generated in the first step
  - F/T stability
  - Short term (4h on melting ice, 8h at RT)
  - Long term (up to 29 days)
- Reanalysis and ISR is done on the diluted sample, since the original (micro)sample is completely consumed in step 1 of sample processing

Comparison conventional – CMS - DBS

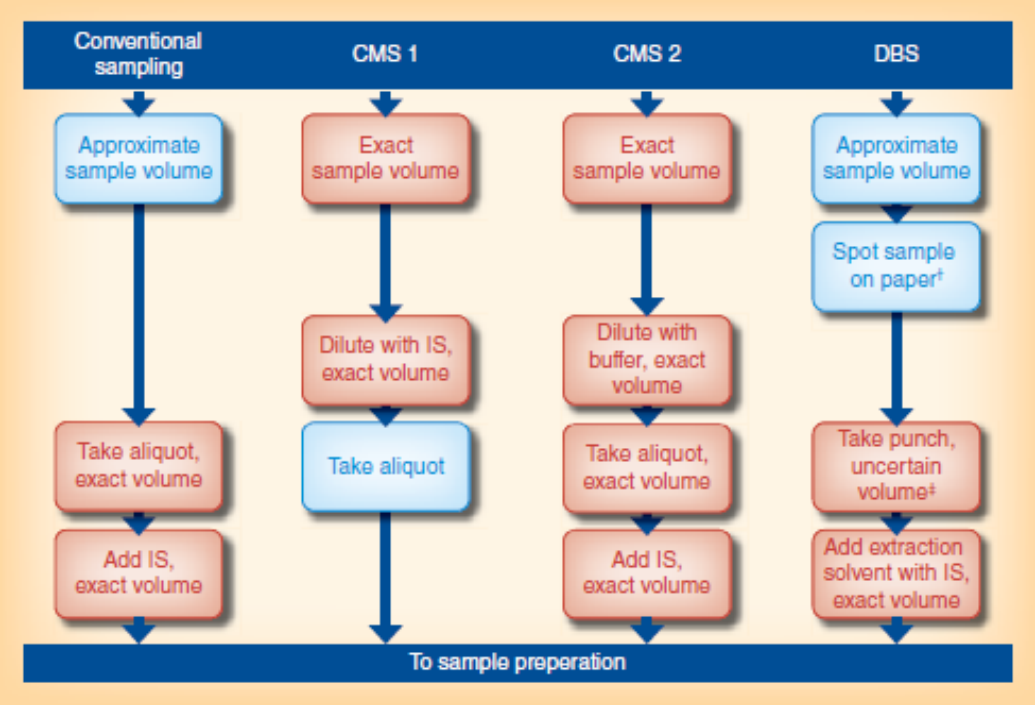


Figure 1. Sample transfer steps between sampling and sample preparation.
Volume critical steps in red.
†Incorrect spotting might influence the results.
‡The actual blood volume in the punch will depend on the hematocrit and other factors influencing the viscosity of the blood.
CMS: Capillary microsampling; DBS: Dried blood spot; IS: Internal standard.

Lars B Nilsson, Martin Ahnoff, Ove Jonsson, Capillary microsampling in the regulatory environment: validation and use of bioanalytical capillary microsampling methods, Bioanalysis (2013) 5(6), 731–738

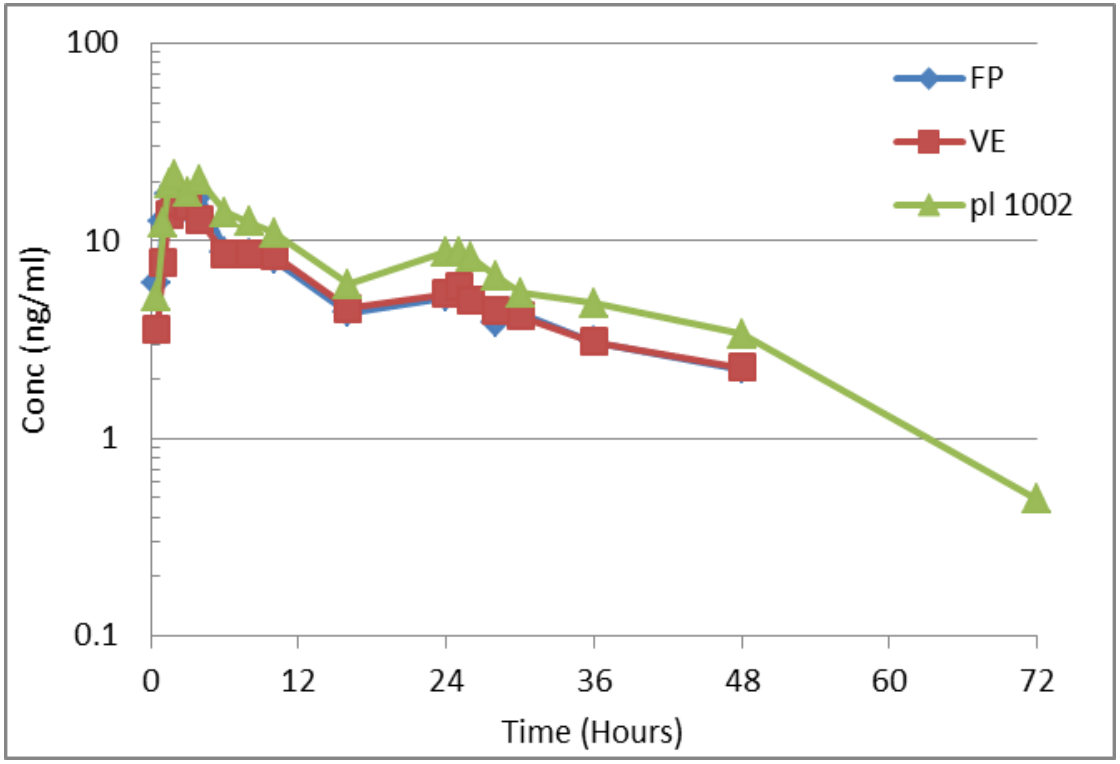
Preparing for the Phase III study: Application in Phase I study as a pilot study

- In a phase I food effect study, extra capillary sampling was added to get experience with the technology to be used in the upcoming phase III study
  - At each timepoint of the fasted arm, a capillary was filled out of the venous blood draw
  - At each timepoint of the fasted arm, a capillary was also filled via fingerprick

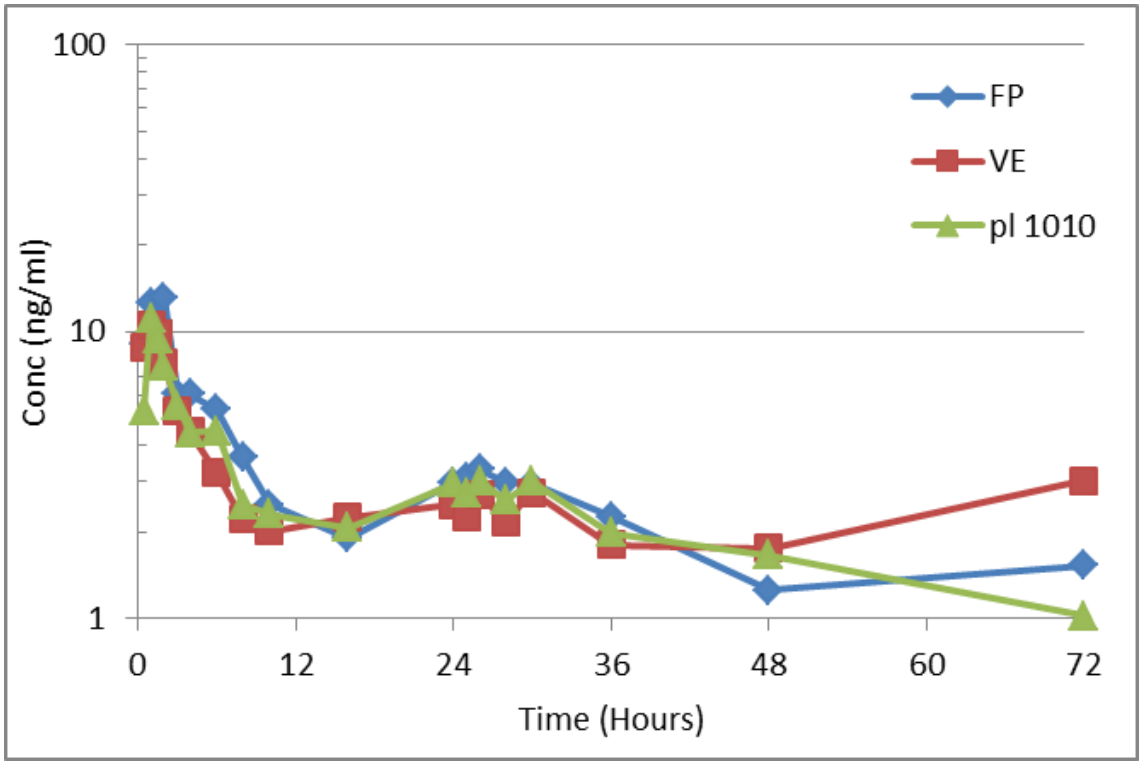
Preparing for the Phase III study: Application in Phase I study as a pilot study

- The results for the capillary blood samples taken out of the venous blood draw correlate well with the plasma results
- The capillary blood via fingerprick:
  - For about half of the subjects, the results correlate well with the venous capillary blood
  - For the other subjects, results of the fingerprick blood are much higher for some timepoints, with occasional outliers

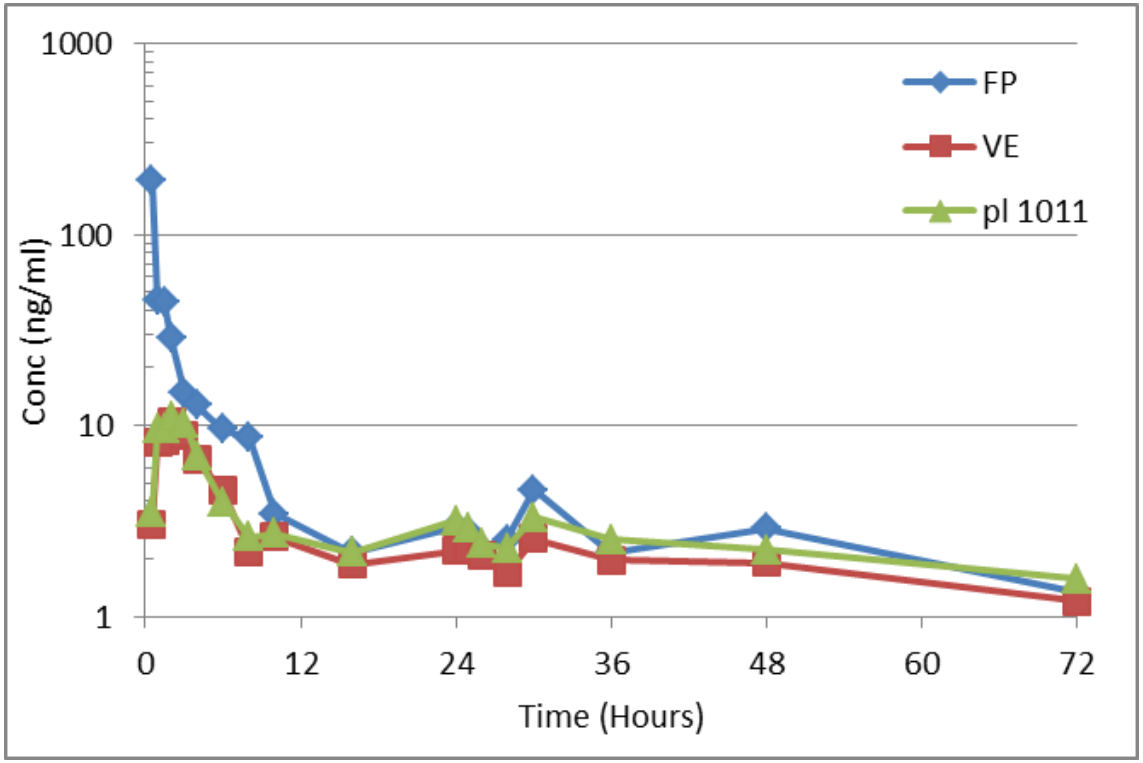
Some examples



Some examples



Some examples



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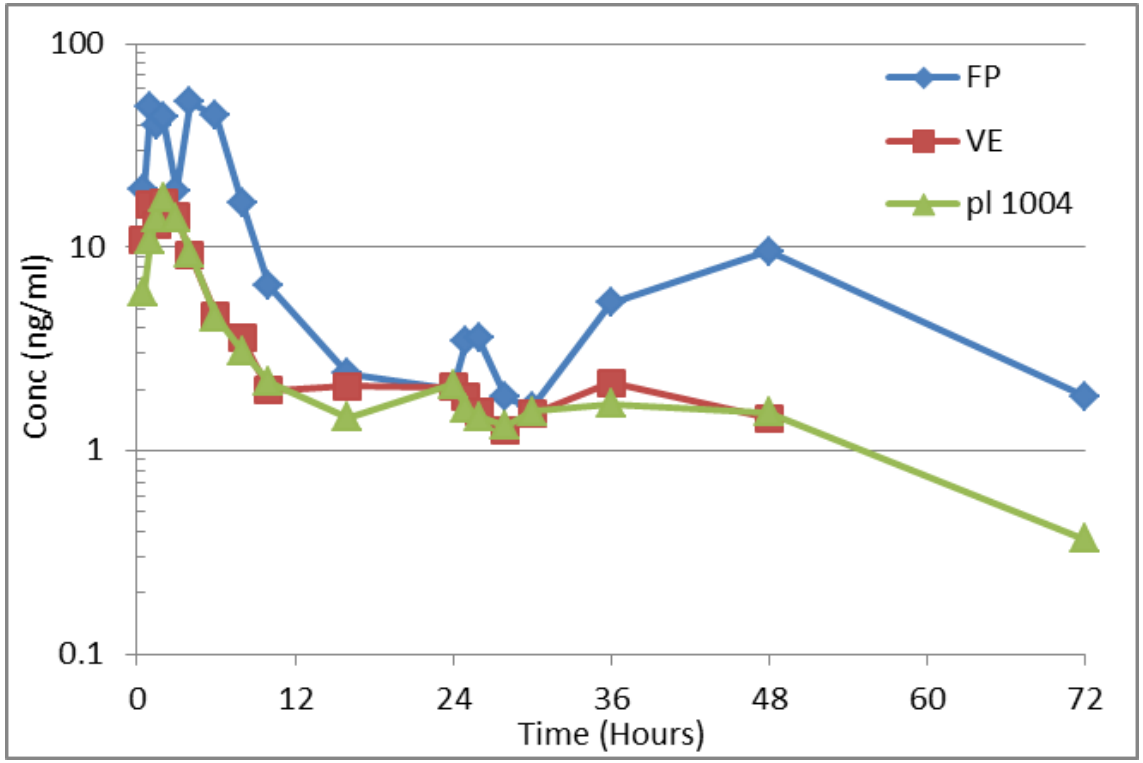
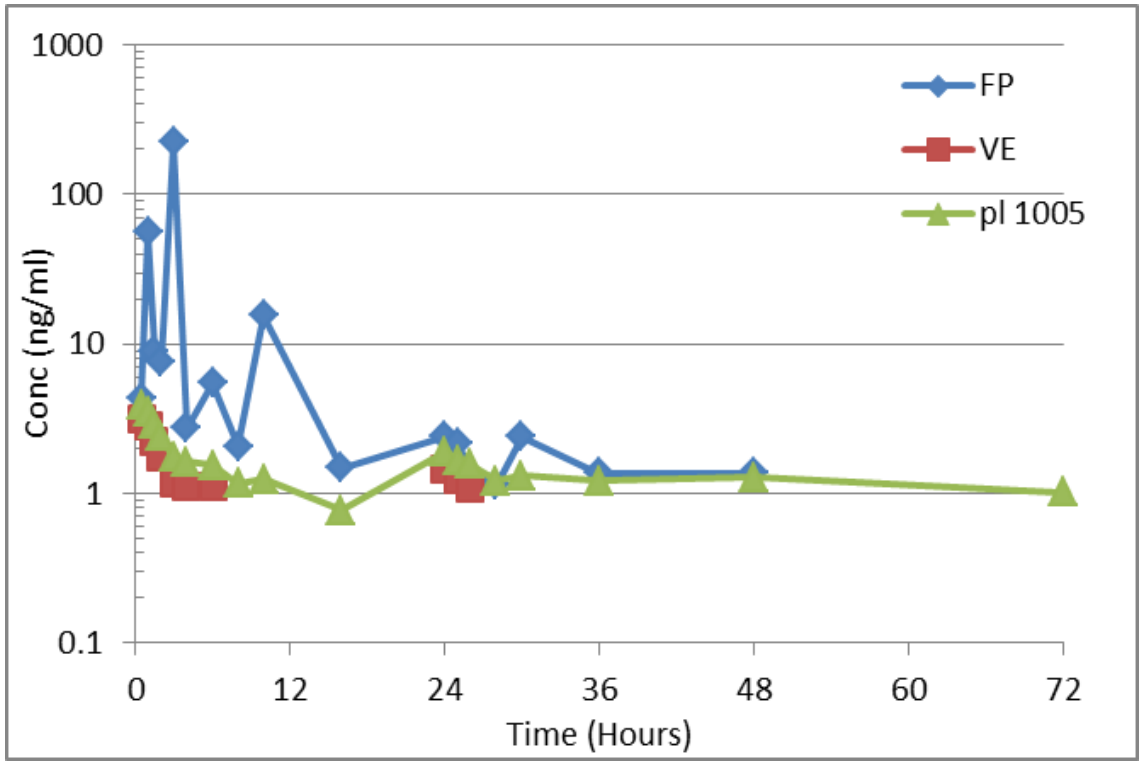


Some examples



Understanding the issue: root cause analysis

- In the bioanalytical lab:
  - All capillaries looked the same during analysis
  - They were all emptied during step 1 of processing
  - Precautions were taken to avoid contamination
  - ISR confirmed high concentrations found
  - Chromatography was fine for all samples

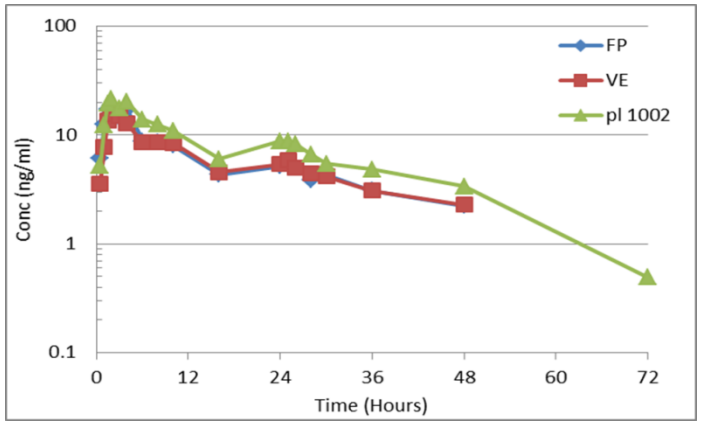
Understanding the issue: root cause analysis

- At the clinical site:
  - How were the capillaries filled?
  - Did the same person fill the fingerprick capillary and the venous blood capillary?
  - Did the volunteers touch the medication?
  - Was the finger adequately cleaned?
  - Was it possible to touch the mouth after medication was taken? So in general, was contamination of the finger possible through touching, sneezing, …?

Understanding the issue: root cause analysis

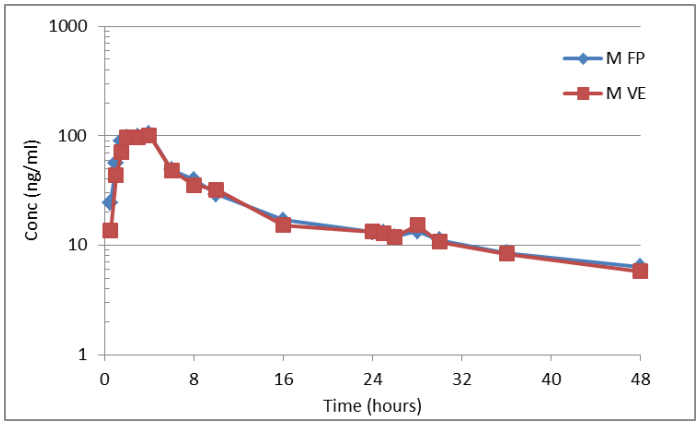
- Metabolism
  - Exploratory analysis of the presence of metabolites (hydrolyzed and reduced metabolite) in the samples
  - PK profile of the metabolites was similar in subjects with and without unexpected parent drug results

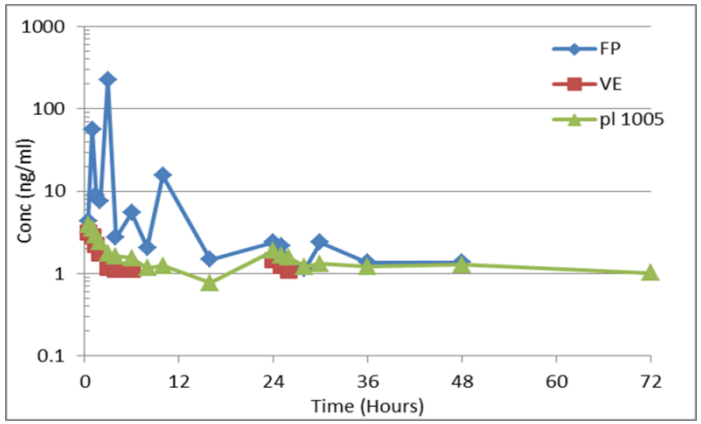
Understanding the issue: root cause analysis



Parent subject without outliers

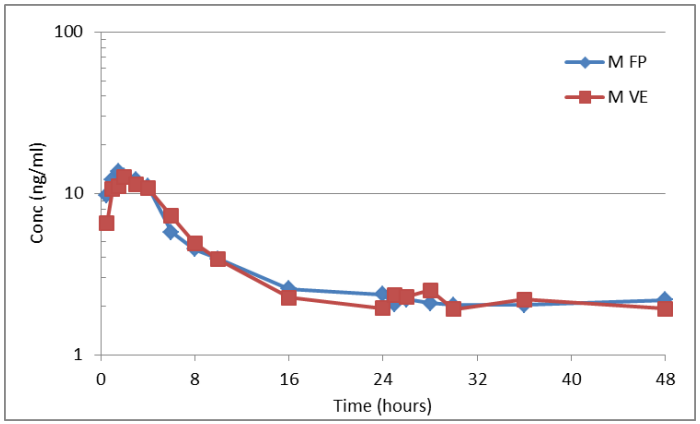
Metabolite subject without outliers





Parent subject with outliers

Metabolite subject with outliers



Conclusions of investigations

- No unexpected profile for the metabolites, this points in the direction of contamination
- Check in the bioanalysis lab suggests no contamination during sample handling
- Possibly contamination during sampling

Conclusions from the pilot study

- Capillary micro sampling technique as such worked very well in the study
- Volunteers found the technique not to be a burden
- The capillary blood subsamples taken from the venous blood were in line with the plasma samples, so technique looks applicable
- The unexpected high results for some of the fingerprick samples seem to point to contamination which needs to be further controlled

Way forward in the Phase III study

- How to avoid contamination in upcoming phase III study?
  - Fingerprick is the only possibility for sampling, heal prick is not possible in view of age and in view of the fact of walking barefoot
  - Extra precautions have been implemented
    - More thorough cleaning of the fingers before sampling
    - Changing of gloves
    - Making sure mouth is empty and cleaned after chewing the tablet
    - Prevent fingers touching the mouth

Way forward in the Phase III study

- How to deal with possible contamination?
  - Interim analysis will be performed after 5 subjects
  - Method will be established (scientific validation) to quantify the reduced metabolite, with the aim of disqualifying potential PK outliers for the parent drug
  - A priori criteria will be defined to allow decision if sample is contaminated and if we have a valid reportable concentration value.

Acknowledgements

Marc Verhemeldonck

Ann Vroman

Jelke Backeljau

Tom Verhaeghe

Philip Timmerman

Questions??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