Experiences with (non-)capillary microsampling in preclinical GLP studies

Pictured above: HIV absorption

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Outline

- Why microsampling
- Microsampling techniques
- GLP-Validated techniques @ Janssen
- CMS or non-CMS ?
- Practical points of attention
- Bioanalytical method validation and sample analysis aspects
- Conclusions

Why Microsampling?

- Reduction
  - Microsample ≤ **±50 µL** compared to 200 – 300 µL for classical sampling
- Refinement
  - **Less** invasive sampling techniques per animal
  - No or **less** warming prior to sampling
  - **Less** stress due to blood loss or restraining
- Scientific
  - Measuring full toxicokinetic profiles from each animal without impacting the animal
  - Possibility to take samples from TOX animals throughout the study and link to TOX parameters

Is Application of Microsampling always possible?

YES

Except:

- Very insensitive compound combined with the need to determine very low concentrations

Microsampling techniques

- Dried Blood Spot (DBS)
- Volumetric absorptive microsample (VAMS, MITRA™)
- Small blood sample
- Small plasma sample
- Capillary blood sample
- Capillary plasma sample
- ….

Plasma or blood?

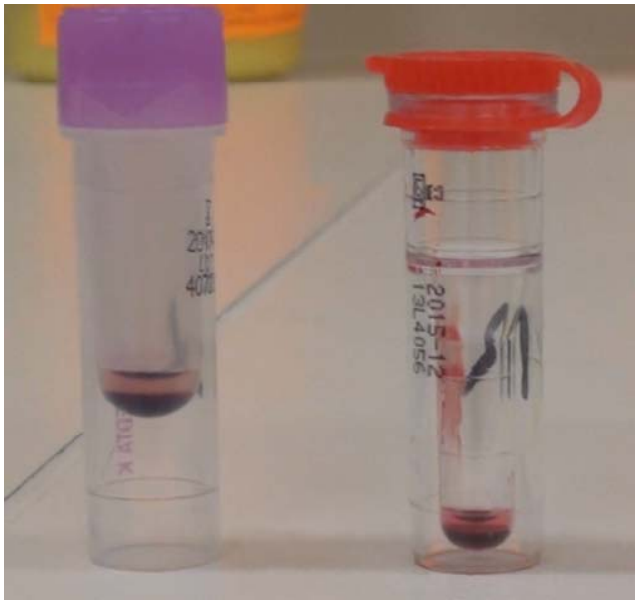
- Plasma: historical data
- Blood: less manipulations: no centrifugation

GLP-Validated techniques @ Janssen R&D

Non-capillary plasma microsampling: 50 – 80 µL blood

- Single aliquot of 10 µL, diluted with 100 µL 2% BSA. Take aliquot for processing.

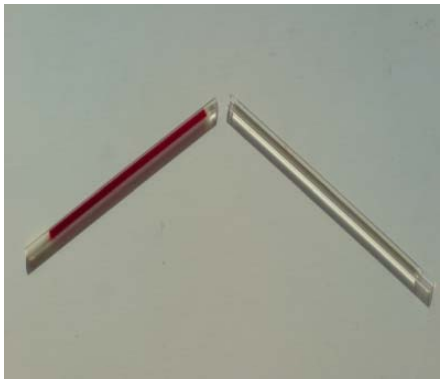
- Microvette 100
- KABE (50, 80 or 100)

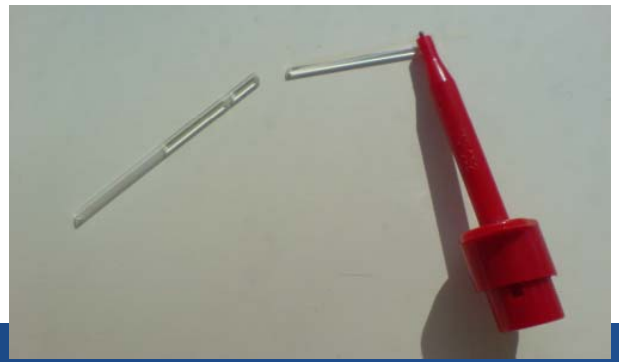


GLP-Validated techniques @ Janssen R&D

Capillary Plasma (GLP and non-GLP)

- Vitrex technique: 32 µL -> 10 µL (or 4 µL) plasma in capillary
- Dilute with 100 µL (or 40 µL) 2% BSA.
- Take aliquot for processing

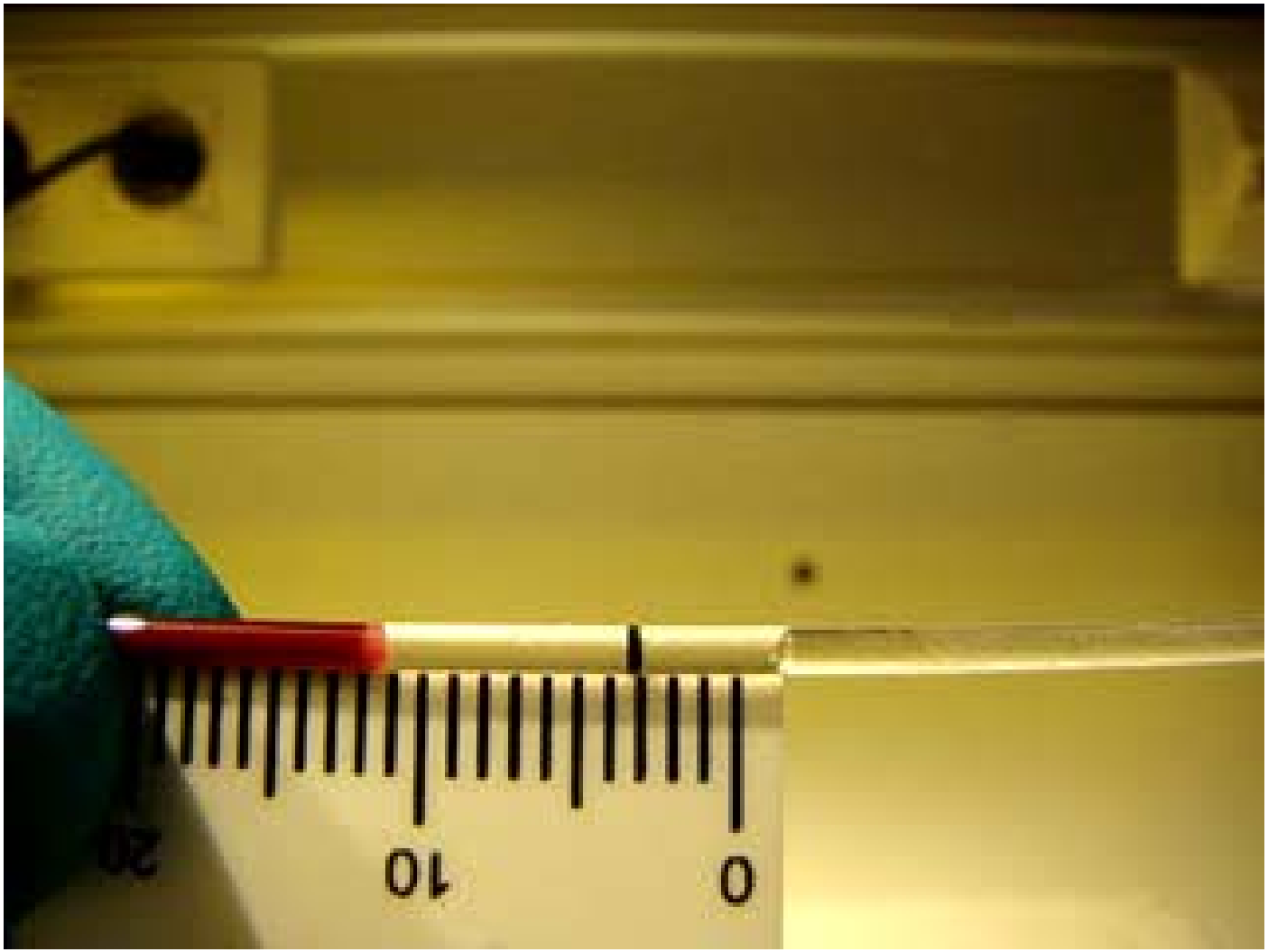




length of capillaries		
volume (µL)	additive	length (mm)
4	none	**15**
8	none	15
8	EDTA	25
10	none	**30**

Primary technique: capillary microsampling: Video blood collection

Primary technique: capillary microsampling: Video plasma harvesting



CMS or non-CMS?

	Capillary	Non-capillary
Total sample volume	32	50 or 80
Sample volume accuracy	Easy	Pipetti volume more c
Sample work-up	Solvent contact with capillary is critical	Easy
Instable compounds	May be challenging to make compatible	Not very different from larger volume sampling
Feedback staff	2011: cumbersome 2015: GREAT!	2011: favourable 2015: less favourable

So CMS it is…

- Implemenation for rabbit studies ongoing (ear vein)
- Plans for implementation for dog, minipig,…(ear vein)

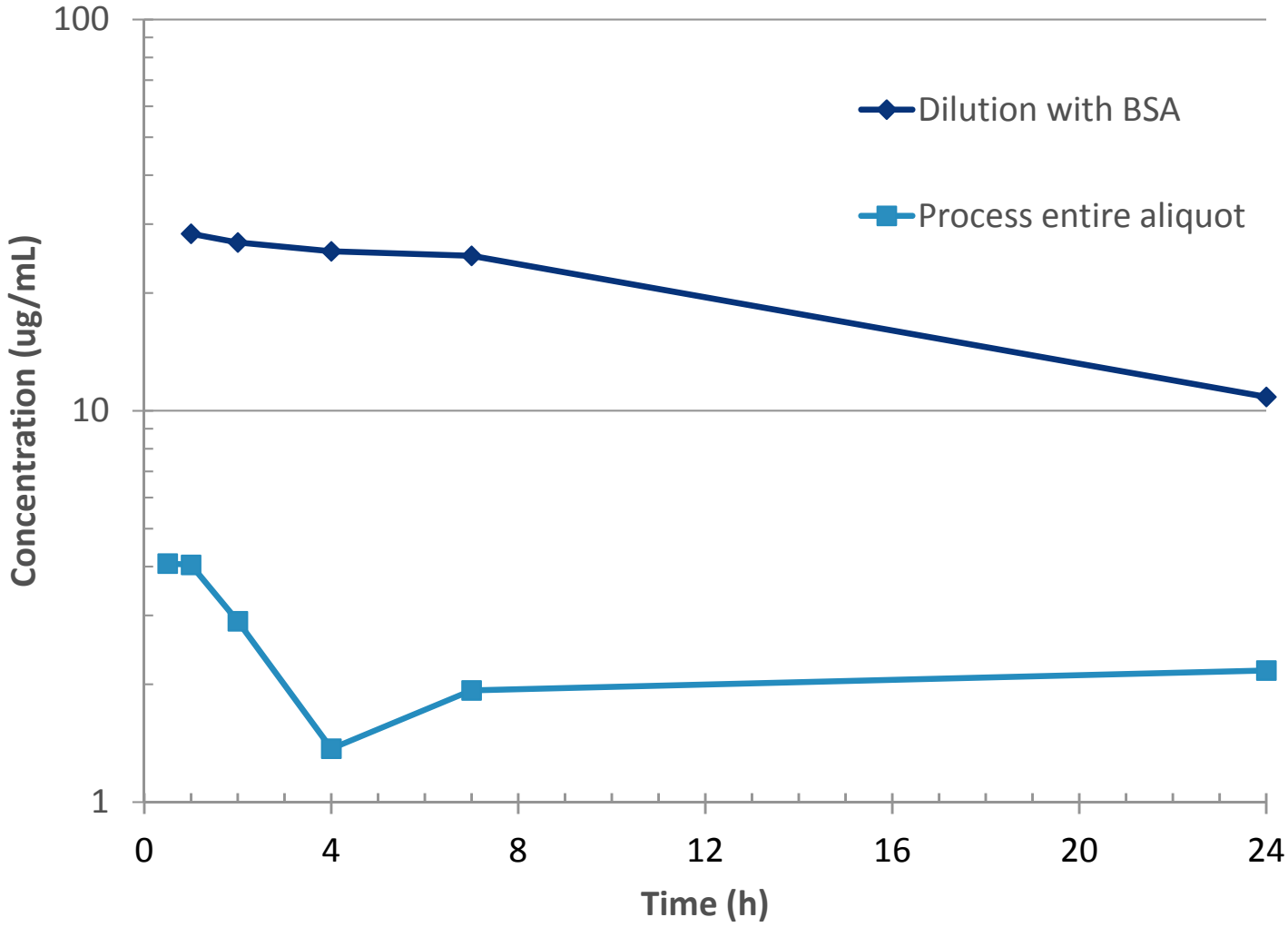
Practical points of attention: Recipient Selection for CMS

Recovery (%) from plasma in 10 µL capillaries after horizontally shaking in presence of 10 volumes 2% BSA solution

Tube and cap	temp.	time (min)	CPD1	CPD2	CPD3	CPD4
Micronic with TPE cap (blue)	RT	5	102.9	90.2	93.7	90.3
	RT	120	101.2	61.3	74.4	53.5
	ice	120	101.7	84.5	89.3	79.6
Micronic with EVA cap	RT	5	96.4	95.0	95.0	97.1
	RT	120	103.3	85.1	85.3	84.1
	ice	120	107.2	93.5	96.7	98.6
FluidX screw cap (no liner)	RT	5	103.2	100.2	98.2	98.9
	RT	120	103.3	104.9	99.9	96.4
	ice	120	109.8	99.9	102.6	106.9

Practical points of attention: Instable Compound

Single dose rat: results with two different sampling methods: effect of dilution with 2% BSA



Conclusions from practical considerations

Compound properties may influence practical details of microsampling technique

- Influence on recovery in combination with recipients
- Stability
- Flexibility of small changes to CMS procedures may help in case of instability, e.g.:
  - CMS with immediate protein crash(esters)
  - CMS with stabilizers already in tube: immediate wash-out of capillary
- Availability of alternative validated microsampling techniques may also help

Influence of CMS on Bioanalytical Method Validation

- Prepare calibration standards as usual, but in **BSA diluted plasma**
- Prepare QC samples in **plasma** (capillaries)
- Dilute QCs with BSA solution

Primary sample changes in composition

- Further sample preparation and LC-MS/MS analysis

- 3 runs, 4 QC concentrations, 6 replicates/run/conc.
- Selectivity, matrix effect, hemolysis evaluated w.o. capillaries

Stability evaluation for GLP Studies using CMS

Double stability program (plasma and BSA diluted plasma):

- Short-term stability
  - Plasma: cover sampling conditions
  - BSA diluted plasma: cover sample conditions for sample preparation
- F/T
  - Plasma: only one required (?)
  - Diluted plasma: 4 F/T
- Long-term stability
  - Plasma and diluted plasma: cover period from sampling until dilution with BSA and from there to last sample analysis

⇒ For simplicity: the same period for plasma and diluted plasma

⇒ Not necessary to evaluate plasma stability in capillaries

⇒ **with CMS, bioanalysis method validation package is larger than with classical sampling techniques**

Influence of CMS on sample analysis for GLP studies with validated assay

First analysis

- calibration standards use **BSA diluted plasma**
- QC samples in **plasma** (capillaries)
- Dilute study and QC samples with BSA solution
- Further sample preparation and LC-MS/MS analysis

Reanalysis: samples are already washed-out of capillary

- ISR, re-analysis for failed run or above ULOQ value
- QC samples
  - Use already diluted QC sample
  - Prepare separate QC samples in BSA diluted plasma
  - use fresh capillary sample

=> Describe in SOP, protocol and/or analytical method

Conclusions

- Smaller volumes: need to carefully review details of sampling procedures and bioanalytical procedures.
- For use in GLP studies: even more detailed review
- There is no such thing as THE microsampling technique
  - @ Janssen, CMS is preferred technique
  - Compound properties
  - Availability of validated alternatives
- CMS in GLP studies impacts BMV and sample analysis
  - QC samples
  - Extended stability program

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