Supporting Tests for Non-capillary Microsampling of plasma in rat and mice in-vivo studies: small volumes, containers - it all matters!

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ABSTRACT
Different microsampling techniques have created great interest from toxicokinetic and pharmacokinetic scientists. They offer the potential to reduce the sample volumes for exposure assessment in rodents and, consequently, serial profiles can be obtained. In GLP tox studies, microsampling can remove the use of satellite animals. Although the full potential to reduce the sample volume would be obtained with blood sampling and analysis, the general preference is to keep plasma as the matrix of choice. The current industry trend is to collect a small blood sample in capillaries and after centrifugation transfer plasma to a second, smaller capillary. At Janssen, however, we choose to sample a small volume of blood (60 µL) into an adapted recipient to generate an accurate volume of 10 or 20 µL plasma in a more classical way.

For current GLP studies these procedures were validated before implementation. Accuracy and reproducibility of the pipetting step was confirmed through cross-training of the in-vivo staff with the bioanalytical staff. Two observations were made during the validation of the procedures. The pipetting technique, and type of pipette are critical to obtain accurate results.

In addition, it was observed that for some compounds the recipient and the anti-coagulant influence the recovery. Results clearly showed that volume, temperature and time all can have an impact on the recovery of the analyte from the blood collection tube. No relation with adsorption or instability could be made. Other collection devices did not show similar losses.

METHODS

Figure 1: Traditional sampling with adapted device for small volumes (Microvette CB 300)

Microvette containers show reduced recoveries for 2 out of 3 compounds evaluated. Recovery issues are more pronounced at lower volumes. Blood or plasma matrix in microvette does not change the conclusion. This was consistent across 2 different technicians. For compound 3 no recovery issues were identified.

Table 3: Recovery (% to ref) from microvette tubes as a function of anti-coagulant, collection volume and time

The recovery issues in the Microvette containers were again confirmed. No other type of blood collection device, nor any recipient did show this recovery problems. There are no significant differences in anti-coagulant content between the tubes.

CONCLUSIONS

- Microvette CB300 blood sampling devices have the potential to cause recovery issues which are clearly compound dependent.
- The observed effects do not seem to be related to adsorption potential of compound (compound 3 has shown adsorption issues in method development, while compound 2 has not).
- Also anti-coagulant, time and collection temperature have an impact on the observed effect.
- No other blood collection device or plasma recipient showed these recovery issues.
- The root cause is still unknown.