INTRODUCTION

Microsampling-coupled bioanalytical techniques are becoming more established in pre-clinical studies, particularly for rodents, to try and avoid or reduce the need for satellite animals for toxicokinetic evaluations. However, the same can apply for large animal species, such as the minipig and dog, when newborns are used in juvenile toxicity studies.

OBJECTIVES

The objectives were to confirm blood sampling sites for each species, test compatibility of the test item with the Microvette sampling tubes and to evaluate if there was any impact of sampling tube size (Microvette® 100 or Microvette® 300) with respect to the target blood sample volume of 60 or 80 µL on bioanalysis.

MATERIAL AND METHODS

In-life Phase

Two litters each of newborn Göttingen Minipig piglets and Marshall Beagle puppies were allocated to 4-week oral (gavage) pilot repeat dose toxicity and toxicokinetic studies with dosing from postnatal day (PND) 1. A series of 5 blood microsamples (60 µL each) was taken at specific time-points after dosing on each of PND’s 1, 8, 15, 22 and 28. Blood beads were drawn by capillary action into Microvette® tubes following needle puncture of the selected vein from each non-anesthetized piglet or puppy. Plasma samples (target of 20 µL) were obtained following direct centrifugation of the Microvette® tubes and stored frozen (between -15 and -25 °C) in cryotubes until shipment, stored frozen, to Janssen Research and Development, a division of Janssen Pharmaceutica N.V. for bioanalysis.

Bioanalysis Phase

For quantifying the test item in the plasma, a qualified LC-MS/MS method was used at the Sponsor. Only 10 µL of plasma was diluted in blank plasma and subjected to a protein precipitation sample preparation. The remaining plasma was retained frozen as a contingency for any reanalysis.

RESULTS

In-life Phase

In total, 25 samples were taken from each animal during the studies, however, on several occasions the target plasma volume of 20 µL was not attained so the blood sample was increased to 80 µL. For the minipig, the saphenous vein was used on PND 1 and PND 8 followed by the ear vein up to PND 28. For the dog, the cephalic vein was used on PND 1 but it proved difficult to obtain the target sample volume so the jugular vein was used thereafter.

Bioanalysis Phase

Bioanalysis of the test item was feasible with microsamples using the described assay setup and provided a precise, accurate and specific measurement with low limits of quantification between 10 and 50 ng/mL. The Microvette® sampling tubes were compatible with the test item evaluated and there was no bioanalytical impact of the size of tube used with respect to blood sample volume.

CONCLUSIONS

We conclude that if a suitable bioanalytical method can be applied, microsampling techniques are possible for neonatal and juvenile minipigs and dogs and provide ethical, scientific and financial advantages with respect to conventional toxicokinetic methodology. Both the saphenous and ear veins are suitable for the minipig depending upon the age of the piglets and the jugular proved to be the preferred vein for puppies.

REFERENCE

1. National Centre for Replacement, Refinement & Reduction of Animals in Research (NC3Rs).

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