Blood microsampling for itraconazole pharmacokinetic study on individual rodent in the context of drug formulation optimization

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Nowadays in animal studies, it is important to comply with the so-called Three Rs rule by replacing or reducing the number of tested animals. Volumetric absorptive microsampling (VAMS) can be used to collect small quantities (10 or 20 µL) of whole blood, thereby limiting the amount of animals needed. In this study, a quantitative method was developed and subsequently validated for the poorly soluble drug itraconazole (ITZ) using VAMS and ultra-performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS). A developed and validated LC-MS/MS method enabled the quantification and the construction of pharmacokinetic profiles of itraconazole and its main metabolite [1]. We paid particular attention to the sample preparation. Ideally, the extraction solvent should provide a maximum recovery of the analytes and a low recovery of other compounds to minimize matrix effects. The stability of ITZ in dried samples of whole rat blood was also investigated. We showed that ITZ stability cannot be guaranteed for more than 24 hrs in VAMS when stored at room temperature. Therefore, precautions have to be taken in long-term bioavailability studies. Storage of the samples at -80°C after extraction and evaporation have shown to be an effective approach.

Then, we demonstrated the applicability of VAMS to study the bioavailability of drug formulations. Four itraconazole-containing formulations were administered to rats. VAMS were used to collect 14 whole blood samples of only 10 µL each within a time frame of 48 hours. Significant differences in pharmacokinetics were obtained and compared to *in vitro* dissolution rates of the various formulations [2].

Using VAMS, substantially lower blood volumes can be taken per sampling point (10-20 µL instead of the conventional 0.2-0.5 mL) avoiding the sacrifice of animals. Moreover, the same rats can be used to compare different drug formulations, which strengthens the validity of the results and helps to suppress the inter-individual variability.

[1] Thiry J. et al., *J. Chromatogr. A.* **2017**, *1479, 161-168.*
[2] Thiry J. et al., *Eur. J. Pharm. Sci.* **2017**, *99, 1-8.*